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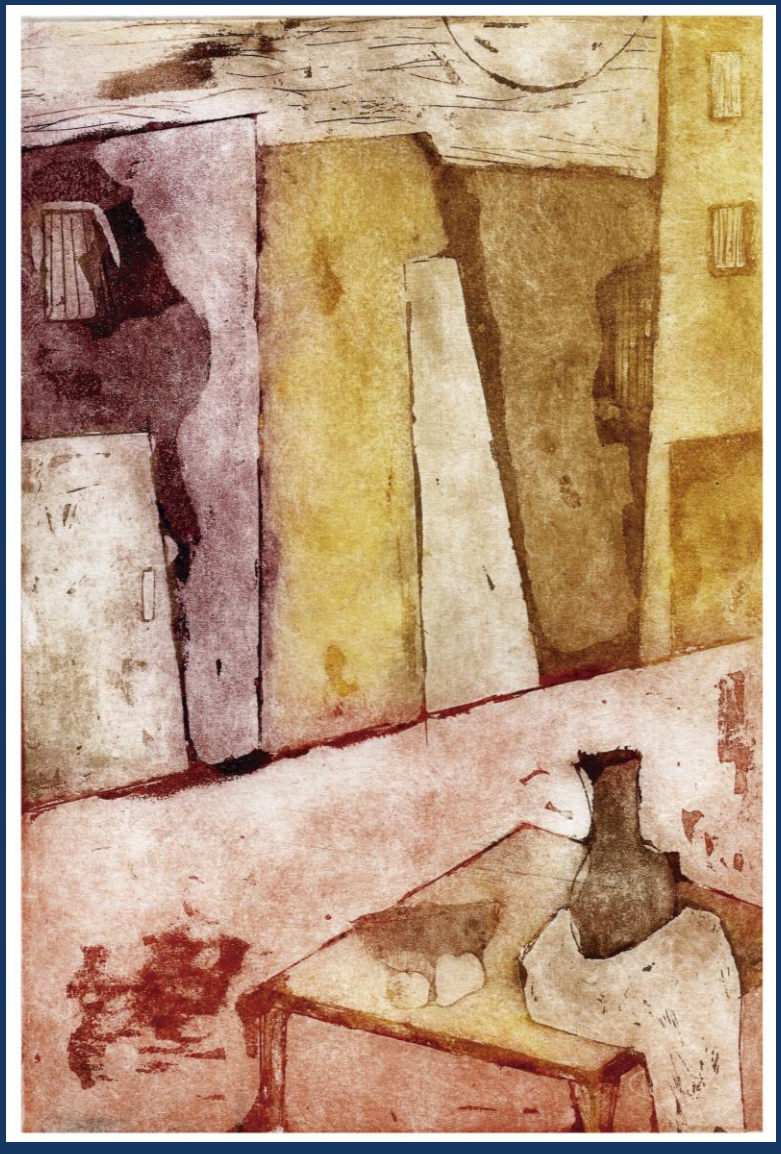

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La Tapa (*Ver* pág. 4)
Atardecer en la tarde
Antonella Ricagni

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REUNIÓN ANUAL DE SOCIEDADES DE BIOCIENCIA 2019

LXIV Reunión Anual de la
Sociedad Argentina de Investigación Clínica (SAIC)

LI Reunión Anual de la
Asociación Argentina de Farmacología Experimental (SAFE)

XXI Reunión Anual de la
Sociedad Argentina de Biología (SAB)

XXXI Reunión Anual de la
Sociedad Argentina de Protozoología (SAP)

IX Reunión Anual de la
Asociación Argentina de Nanomedicinas
(NANOMED-ar)

VI Reunión Científica Regional de la Asociación Argentina
de Ciencia y Tecnología de Animales de Laboratorio
(AACyTAL)

con la participación de
The Histochemical Society

13 - 16 de noviembre de 2019
Hotel 13 de Julio - Mar del Plata

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ANNUAL MEETING OF BIOSCIENCE SOCIETIES 2019

LXIV Annual Meeting of
Sociedad Argentina de Investigación Clínica (SAIC)

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VI Regional Scientific Meeting of Asociación Argentina
de Ciencia y Tecnología de Animales de Laboratorio
(AACyTAL)

with the participation of
The Histochemical Society

November 13th – 16th, 2019
Hotel 13 de Julio - Mar del Plata

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Dra. Mónica Costas
Dra. Gabriela Marino
Dr. Pablo Azurmendi

LA TAPA

Antonella Ricagni. **Atardecer en la calle**

Técnica: Aguatinta /aguafuerte. Año 2011. Medidas: 21 x 29 cm. Gentileza del autor.

Antonella Ricagni es Licenciada en Artes Visuales, con orientación en Grabado. Ha ejercido la docencia en Artes Plásticas en el nivel primario. Trabajó en varios museos como orientadora de sala y tallerista. Es escenógrafa egresada de la Escuela Metropolitana de Arte Dramático (EMAD). Ha realizado una residencia artística en México especializada en Xilografía.

Actualmente es docente en la materia Ilustración, en la carrera de Diseño Gráfico en la Facultad de Arquitectura, Diseño y Urbanismo, Universidad de Buenos Aires, y en Plástica y Tecnología en varias instituciones educativas en la ciudad de Buenos Aires.

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- Fundación Gador por su contribución al premio "Mejor trabajo sobre necesidades médicas insatisfechas" de la SAIC

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Queridos amigos, amigas, compañeros y compañeras,

Tengo el enorme honor de darles la bienvenida a la inauguración de la sexagésima cuarta reunión anual de la SAIC, junto a las autoridades de otras seis sociedades científicas: Dra. Fernanda Parborell de la SAB, Dra. Ana Genaro de SAFE, Dra. Hebe Durán de Nanomed.AR, Dra. Adelina Riarte de la SAP, Dr. Ernesto Gulin de AACYTAL y el Dr. Alejandro Adam en representación de la HCS. Junto a todos ellos hemos organizado esta Reunión Anual de Sociedades Biocientíficas.
Tendremos 25 simposios, 16 minicursos, 20 conferencias, 3 mesas redondas en torno a distintas temáticas: para discutir políticas científicas, de género y ética en la utilización de animales de laboratorio, más de 800 trabajos en formato póster en distintas áreas temáticas, varios tipos de premio para estos trabajos y otros de exposición oral preseleccionados por prestigiosos jurados.
Contaremos con la participación de más de 140 disertantes con una relevante trayectoria académica y reconocimiento internacional.
Con el objetivo de promover iniciativas de proyectos de investigación clínica vinculados a demandas concretas en salud en Argentina, hemos convocado a investigadores de distintas disciplinas e incluso a expertos del área asistencial y de gestión en salud.

Quiero agradecer a todos los miembros de SAIC que han dado su aprobación para mi desempeño como presidente. Cargo que me honra, conmueve y enorgullece profundamente, dado que fue la sociedad científica donde presenté mi primer trabajo, allá por el año 1988. Fue justo aquí, en el Atlantic, coordinaba la sesión el inolvidable, querido y respetado Dr. Martín Isturiz.
Un especial y afectivo agradecimiento a mis 3 manos derechas: secretarias Dra. Gabriela Marino e Ivana Rossetto y tesorero Dr. Pablo Azurmendi.
Un equipo maravilloso y de una capacidad de trabajo y compromiso increíble, que junto a todos los miembros de la Comisión Directiva de SAIC hicieron posible todas las actividades de gestión anual, así como la organización de esta reunión conjunta, en un clima fraterno de cordialidad, respeto y compromiso. ¡Gracias! A todos ellos por sus iniciativas, paciencia y eficiencia de respuesta a mis demandas.
También quiero agradecer a las autoridades de las otras sociedades científicas por su participación y prestigiosa contribución para la realización de este evento.

Un agradecimiento especial, a aquellos que realizaron donaciones, también a los organismos CONICET, Agencia Nacional de Promoción Científica y Tecnológica, Ministerio de Educación, Cultura, Ciencia y Tecnología de la Nación, todas las fundaciones que aportaron ayuda financiera para el evento y premios, empresas, a G2 por su gestión en la organización de la reunión y al hotel 13 de Julio.
Finalmente, gracias a todos los participantes y también a asistentes que amablemente aceptaron actividades como jurados de premio y coordinación de sesiones.

Hoy, bastante más madura, que en los 80, en un contexto de cambio de vientos políticos, pero luego de altos grados de agitación, confrontación y violencia, quiero inaugurar este evento, convocando a todas y todos a un diálogo superador de cualquier grieta, dejando de lado lo banal para comenzar a construir de una vez por todas un modelo de país, donde vivir dignamente, sea un hábito para todos.
Desde 1810 a la fecha, hemos recorrido un duro camino de confrontaciones y grietas intentando proyectar un modelo de país que reiteradamente se dirime entre polos opuestos.
Las líneas argumentales en ambos polos suelen utilizar a otros países como modelos referentes.
Aunque la globalización nos imponga una inserción a cualquier precio, tal vez, sea hora de construir nuestra propia identidad, con más estrategia que urgencia, no intentando asimilarnos o compararnos con el mundo y haciendo lo que otros hacen, en el afán desesperado de "pertenecer", sino haciendo nuestro propio camino, logrando la mejor versión de nosotros mismos y por qué no, haciendo precisamente lo que otros no hacen, seamos innovadores también en esto. Ningún país quiere desaparecer del mapa, pero la humanidad debe plantearse seriamente cómo participa en el juego y quienes marcan el rumbo del planeta, porque ese mundo globalizado, está dejando a mucha gente fuera del mapa o abandonada en balsas en el océano.
Los individuos necesitan construir identidad para crecer y desarrollarse, los países también.
Podemos bailar al ritmo del mundo, pero no debemos dejar de producir nuestra propia música.
Que no nos engañen, crecimiento y desarrollo, no son lo mismo. Los países pueden crecer económicamente y aun así, vivir en forma subdesarrollada, sin industrias, con altos índices de pobreza, desocupación, mortalidad infantil e ignorancia.
En pleno siglo XXI, carece de sentido que nos permitamos confrontaciones y falsas dicotomías en la distribución de presupuestos entre salud o educación, trabajo o tecnología, ciencia o salud, ayudas sociales o desarrollo. Las mismas falsas dicotomías venimos planteando entre ciencia útil y no útil, ciencia básica o aplicada, como si la financiación de algunos proyectos debiera costearse sacrificando otros. Es un absurdo y es porque hemos naturalizado el "no hay para todos".
Tan arraigado tenemos el concepto que hasta invertimos una enorme cantidad de horas en evaluar desde el mismo estado varias veces la misma cosa, e incluso llegando a dictámenes contradictorios. Primero ocupamos

una silla en comisiones donde habilitamos a investigadores para ejercer como tales y aprobamos sus proyectos. Luego cambiamos de silla y desde otro organismo del estado, volvemos a evaluar al mismo investigador y su proyecto para resolver si es mejor o más interesante que los que evaluaron otros investigadores y así decidimos, en definitiva, si va a resultar financiado y podrá trabajar. No se trata de un premio, sino de una financiación básica y muy elemental que, en definitiva, nos diferencia entre pobres e indigentes académicos, según se obtenga o no el subsidio.

No conforme con esto, al año siguiente o en dos, volvemos a evaluar al mismo investigador, ahora indigente, y el informe de su proyecto, exigiéndole una producción equiparable a la del pobre y lo castigamos si no publicó en revistas de primer cuartil a razón de U$ 3000 el paper. Una sucesión de absurdos que hemos naturalizado. Los científicos no deberíamos naturalizar ninguna afirmación no comprobable científicamente.
Mientras tanto, somos testigos de una fiesta de lebacs, letes, botes, lelics, a la que muchos de nosotros no hemos sido invitados. Bonos de deuda a 100 años y muchos probablemente comprados por los mismos que los emiten, generando una nueva tanda de fondos buitre. Se genera una deuda de 57.000 millones de dólares, contraída por pocos, pero en nombre de todos, sin nuestra firma ni habilitación, sin mejoras en la calidad de vida de la mayoría, sino generando más pobreza y retroceso.
No se trata de condenar al especulador, sino de preguntarnos, ¿cómo es posible que generemos o permitamos modelos de organización política y social que hagan más redituable una especulación financiera que una inversión genuina en bienes y servicios que mejoren la calidad de vida de todos, promoviendo el progreso?
El país no va a desarrollarse a través de falsas inversiones especulativas en una bicicleta financiera. Tenemos la responsabilidad, de una vez y para siempre, de sentarnos a pensar y debatir, poniendo todo nuestro ingenio, como lo hacemos con nuestros proyectos académicos, cuáles serán nuestras políticas en ciencia y tecnología, si vamos a repetir los viejos modelos, copiar los de otros, o a generar uno nuevo, uno nuestro. Uno que se ajuste a nuestras necesidades y objetivos como país, uno que en vez de responder a demandas de intereses particulares donde se prioriza lo redituable por sobre lo necesario para el país, logre atraer esos capitales hacia proyectos que cubran demandas sociales e impliquen desarrollo.

Los científicos no reclamamos presupuesto con objetivos mezquinos o sectarios, es hora de reaccionar, que el país necesita de nuestro trabajo para crecer y desarrollarse. Las decisiones políticas las toma quien lidera, pero los líderes, son construcciones sociales. Los buenos, responden a esas demandas sociales. Cuando la sociedad en su conjunto toma consciencia, pues no hay movimiento político que pueda poner cimientos antagónicos a estos objetivos. Se construye desde abajo. Todo se construye desde abajo.
El mundo nos demuestra que nuestra inserción como país exclusivamente agrícola-ganadero ha quedado obsoleta y no es suficiente para un ingreso de divisas que garantice la sustentabilidad y trabajo de todos los argentinos.
Las billeteras y estómagos en el mundo tienen un límite y no nos van a comprar más soja porque cambiemos el régimen de retenciones.

El país nos necesita para construir conocimiento, producir bienes y servicios que mejoren la calidad de vida de todos, acorde a nuestras propias necesidades, para producir nuestras propias vacunas, nuestros insumos básicos en salud e investigación, que nos eviten gastar divisas que no emitimos, en importaciones, y otros que se puedan exportar y generen más ingreso de divisas al país y trabajo. No hay modelo de país posible sin ciencia y tecnología.
Esto no se contrapone a los objetivos personales de carreras científicas exitosas, obtención de premios y prestigio internacional.
No son las políticas que contemplan lo colectivo las que frustran nuestros sueños, u opacan nuestros objetivos personales, por el contrario, es la ambición desmedida de unos pocos, el pensamiento corporativo o monopólico del bienestar, la que nos enfrenta a falsas confrontaciones u obstáculos.

¿Por qué nosotros? porque somos los mejores capacitados para quebrar dogmas, nos entrenamos para eso en nuestro quehacer cotidiano. Estamos para poner el mundo de cabeza y cuestionarlo todo, para comprobar científicamente cómo funciona, para demostrar que existen otras formas de concebir el mundo, de interpretar sus reglas, de cambiarlas.
Porque todo lo que podamos demostrar científicamente puede quebrar pensamientos dogmáticos que imponen antiguos acervos culturales y construir nuevos rumbos a través del conocimiento y el pensamiento crítico.
Porque la historia del mundo nos muestra que con la Fe no alcanza, apenas apacigua la angustia de sabernos mortales, pero no logra una convivencia pacífica en un justo equilibrio de derechos y bienestar en todos los rincones del mundo.
En sintonía con estas ideas, porque creemos que el saber debe ser patrimonio de todos, desde SAIC, hemos realizado actividades de divulgación a la comunidad a través de notas escritas por científicos (proyecto SAIC y La Comunidad), dirigidas a público en general y también actividades participativas en escuelas donde los chicos realizaron trabajos prácticos (proyecto SAIC va a la escuela) y pudieron conversar con jóvenes científicos.

Junto a mis compañeros de las otras sociedades científicas, inauguramos un foro de Biosociedades para discutir propuestas de política científica. Emitimos varias declaraciones, asistimos a medios de comunicación y a reuniones de comisiones parlamentarias en el Congreso de la Nación. Algunos, además, estuvimos en la calle, en las numerosas manifestaciones de científicos ocurridas en estos 4 años.

Para finalizar, el modelo de país lo hacemos entre todos y sin ciencia, tecnología, salud y educación, no hay modelo de país posible. En Argentina, con enormes riquezas naturales, 46 millones de habitantes y capital humano de excelencia hay lugar para todos. Endeudados o no, tenemos la oportunidad de pensar, conciliar, acordar objetivos, de una vez y para siempre emprender un camino hacia el desarrollo real de nuestro país. Corrijamos el trayecto en ese rumbo todas las veces que sea necesario, pero no volvamos a admitir retrocesos.

Otra forma de hacer ciencia es posible, otra forma de vivir es posible.
Damos comienzo a la reunión de Biosociedades 2019.

Dra. Mónica Costas
Presidente SAIC 2019

LECTURES

OPENING LECTURE

SAIC OPENING LECTURE

Chairs: Alejandro Curino/María Marta Facchinetti

HOW BAD IS THE HEDGEHOG? GLI-DEPENDENT, HEDGEHOG INDEPENDENT CANCERS - ON THE IMPORTANCE OF BIOMARKERS FOR PROPER PATIENTS' SELECTION

ALAIN MAUVIEL

Institute Curie. INSERM. Paris, France

Hedgehog (HH) signaling plays an important role both during embryonic development and adult life. It is involved in the regulation of cell differentiation, cell proliferation and tissue polarity, as well as in the maintenance of stem cells, tissue repair, and regeneration. HH ligand binding to the 12-transmembrane receptor PATCHED-1 (PTCH1) activates the 7-transmembrane G-coupled receptor Smoothened (SMO), and HH signal transduction proceeds towards activation and nuclear accumulation of GLI transcription factors. The direct role of the HH signaling pathway in tumorigenesis was first established through the identification of loss-of-function mutations in the *PTCH* gene in patients with familial and sporadic basal cell carcinomas of the skin and in medulloblastoma in children. HH pathway activation, often estimated as elevated *GLI1* expression, has since been described in a number of tumor types, yet efficacy of HH inhibitors is essentially limited to tumors bearing activating mutations of the pathway. GLI2, a critical HH effector, is necessary for *GLI1* expression and is a direct transcriptional target of TGF-ß/SMAD signaling. We hypothesized that the lack of efficacy of SMO antagonists in numerous tumors occurs because high *GLI1* expression and activity may not be linked to HH, but rather to TGF-ß, ligand expression, taken as surrogates for HH and TGF-ß signaling in tumors. We compiled data from publicly available gene expression datasets from over 23,500 cancer patients, of which over 15,000 with survival annotations. While *GLI1/2* expression is correlated with both *HH* and *TGFB* expression, their prognostic value is tightly correlated with that of *TGFB*, not *HH*. High *GLI1/2* and *TGFB* expression, associated with a mesenchymal/EMT signature, often represent parallel markers of poor clinical outcome. Inversely, high expression of *HH* is mostly associated with increased survival. Our results provide a likely explanation for the frequent failure of anti-HH therapies in tumors, as they suggest a key role for TGF-ß, not HH, ligands, in tumors with elevated *GLI1/2*-expression. Patients' selection based upon an inadequate marker of HH pathway activation may therefore contribute to the lack of clinical efficacy of SMO antagonists in various neoplasms.

HONOR LECTURES

SAIC HONOR LECTURE I TO DR. SAMUEL FINKIELMAN

Chair: Guillermo Semeniuk

NEUROENDOCRINE TUMORS: A LONG JOURNEY FROM BENCH TO BED

EDUARDO ARZT

Biomedicine Research Institute of Buenos Aires (IBioBA) - CONICET- Partner Institute of the Max Planck Society. Buenos Aires, Argentina

During the last years we have described several novel pathways and mechanisms involved in the control and onset of abundant neuroendocrine tumors.

Pituitary tumors are mostly benign, non-metastatic and monoclonal neoplasms. Cellular senescence is a state of permanent and stable proliferative arrest. Several lines of evidence have implicated Oncogene-induced senescence (OIS) as a vital cause of arrest of benign neoplasms. IL-6 plays a key role in OIS induction indicating that IL-6 can function as an autocrine or paracrine tumorigenic factor. IL-6 maintains pituitary tumoral senescence by its autocrine action, which explains the benign nature of these abundant adenomas.

We have cloned RSUME, the product of the *RWDD3* gene, which is induced by hypoxia and its expression is increased in pituitary and in Von Hippel Lindau (VHL) tumors. RSUME stabilizes HIF-1, leading to increased

HIF-1 activity and VEGF expression. RSUME inhibits the oncogene pVHL function, thus suppressing HIF-1 and 2a ubiquitination and degradation. By this mechanism, it promotes the establishment and development of VHL-tumors.

Pituitary tumor transforming gene 1 (*PTTG*) has an increased expression in a wide variety of human solid tumors. Its degradation by the ubiquitin proteasome system has been shown. Overexpression of human securin leads to transformation and tumor induction. No mutations, epigenetic modifications or other mechanisms that deregulate and explain its overexpression and action as an oncogene had been found so far. Increased stability of PTTG caused by RSUME diminishes its degradation by the ubiquitin proteasome system and increases PTTG steady state levels providing a novel mechanism for PTTG oncogenic action.

In the experimental cellular model of pituitary corticotroph adenomas, AtT-20, we have shown that retinoic acid (RA) inhibits ACTH secreted by the tumor cells and POMC transcription. RA inhibits also corticosterone-secreting cells proliferation. In the tumoral cells RA induces the production of the cytokine BMP-4, which participates in its anti-proliferative effects. In RA-treated AtT-20 xenographed nude mice, an *in vivo* model of cortocotrophinoma, RA reduces tumor growth and reverses endocrine alterations, decreases plasma ACTH and cortisol, together with a reduction in adrenal hyperplasia. A 6-to-12-month treatment with RA in dogs with Cushing's disease not only normalized cortisol but prevented the pituitary adenomas from recurring. Based on these results, clinical trials with RA in patients with Cushing disease are in course.

SAIC HONOR LECTURE II TO DR. MARTÍN ISTURIZ

Chair: Jorge Manni

PROTONS, DAMPs AND REGULATION OF THE IMMUNE RESPONSE

JORGE GEFFNER

[INBIRS] Institute of Biomedical research in retrovirus and AIDS - Faculty of Medicine, University of Buenos Aires. Buenos Aires, Argentina

Low extracellular pH is a hallmark of a variety of inflammatory processes and solid tumors. In this talk I will give an overview of our contributions directed to clarify the immunomodulatory actions induced by high concentrations of protons on the course of the innate and adaptive immune response. We have focused our studies in the phenotype and function of neutrophils, monocytes, macrophages and conventional dendritic cells. Overall, our observations suggest that high concentrations of protons act as a danger-associated molecular pattern (DAMP) alerting the immune system to the presence of an insult in order to mount the most appropriate response. Characterization of the mechanisms through which extracellular acidosis modulates the function of immune cells has revealed novel potential targets able to either suppress or enhance the immune response.

SAIC HONOR LECTURE III TO DR. EDUARDO CHARREAU

Chair: Claudia Pérez Leirós

FROM TESTICULAR FUNCTION TO BREAST CANCER. TWO AREAS JOINED BY A BRIDGE CALLED EDUARDO CHARREAU

JUAN CARLOS CALVO

IBYME, CONICET - Department of Biological Chemistry, FCEyN, UBA. Buenos Aires, Argentina

It is an honor for me to remember Dr Charreau's labor in both Academy and Research. If I had to choose a single word to summarize his activity, that word would be pioneer. As poet Antonio Machado said, he was tracing paths for someone else to follow. Even though the title of the lecture comprises both testicular function and mammary cancer, I will focus my talk on the reproductive function of the testis because there will be two posters on mammary cancer from our laboratory. In this respect, testicular function depends on hormonal stimulation and, when characterizing the receptor for luteinizing hormone, Dr Charreau was close to follow the path towards a Nobel Prize, as Lefkowitz and Kobilka did with their study on G protein and the 7-transmembrane receptors. In his PhD thesis, Dr Charreau indicated that a prior labelling of the receptor with radioactive hCG and posterior solubilization of the complex, gave a higher molecular size (a 46 kDa

difference) when compared to hormone labelling after membrane solubilization. This difference could, most likely, represent the G protein as a genomic analysis indicates for its MW. In retrospective, had he pursued those experiments, the Nobel Prize could have been his. In our laboratory, we study the process of sperm chromatin decondensation (in humans and mice) as the first event after sperm penetration and fundamental for syngamy. The study of this process led us to identify heparan sulfate as the molecule responsible for this process, alongside with oocyte glutathione. Investigating this event, as well as other parameters such as DNA breaks, motility, concentration and success of pregnancy after assisted reproduction, we concluded that "slow decondensers" could benefit from ovodonation, even more if high DNA fragmentation was also observed. This pilot study could help to solve or, at least, include those problems related to chromatin decondensation as today's "unknown causes".

SAFE HONOR LECTURE TO DR. EDDA ADLER

Chair: María Clara Gravielle

EVOLUTION AND FUNCTION OF HAIR CELL ACETYLCHOLINE NICOTINIC RECEPTORS

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The expansion and pruning of ion channel families has played a crucial role in the evolution of nervous systems. Remarkably, with a highly conserved vertebrate complement, nicotinic acetylcholine receptors (nAChRs) are unique among ligand-gated ion channels in that distinct members of the family have non-overlapping roles in synaptic transmission, either in the nervous system, the inner ear hair cells or the neuromuscular junction. By performing a comprehensive analysis of vertebrate nAChRs sequences, single cell expression patterns and comparative functional properties of receptors from different tetrapod species, in the present talk, I will explore the evolutionary history of nAChRs. In particular, I will concentrate in a peculiar member of the family, the alfa 9- alfa 10, which mediates synaptic transmission between efferent fibers and hair cells of the cochlea. When compared to neuronal receptors, alfa 9- alfa 10 exhibits greater sequence divergence, narrow co-expression pattern and great variability of functional properties across species. Our results suggest that evolution-driven modifications of the alfa 9- alfa 10 nAChR probably allowed the efferent system to serve a differential function in the mammalian cochlea. These findings will be discussed in relation to the roles this receptor plays in fine tuning of sound amplification.

KEYNOTE LECTURES

SAIC LECTURE I

Chair: Ana María Eijan

RANK SIGNALING PATHWAY AS A NOVEL THERAPEUTIC TARGET IN BREAST CANCER

EVA GONZÁLEZ SUÁREZ

Bellvitge Institute for Biomedical Research. Spain

Using loss and gain of function genetically modified mouse models (GEMM) we demonstrated the key role of RANK signaling in mammary gland biology, as both RANK loss and overexpression leads to impaired mammary gland development. Increased RANK signaling interferes with mammary epithelial cells differentiation and expands progenitor/stem populations. RANK over-expression in the mammary epithelia impairs lactation. RANK plays a dual role on mammary gland development, a positive role acting as a mediator of progesterone, and a negative one, blocking the role of prolactin. Moreover, our work supports the use of RANKL inhibitors for breast cancer prevention and treatment: we demonstrated that RANK pathway is the main mediator of the pro-tumorigenic role of progesterone in the mouse mammary gland. Loss of RANK signaling in late stage mammary adenocarcinomas reduces the pool of cancer stem cells and induces tumor cell differentiation.

SAIC LECTURE II
Chair: Rodolfo Goya

AGEING AND THE TICKING OF EPIGENETIC CLOCKS

KENNETH RAJ
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In 2013, a mathematician penned a manuscript with the benign title; "DNA methylation age of human tissues and cell types". In it was described; the development of a mathematical tool that can predict age based on the methylation states of just 353 cytosines on the human genome. The lack of obvious cellular mechanisms that could explain how methylation profiles of just a few hundred CpGs could possibly reflect human age, meant that this remarkable progress in ageing research captured the attention of very few biologists. Instead epidemiologists and statisticians were the first to appreciate its virtue and used it to generate a plethora of publications that uncovered the link between the rate of epigenetic ageing with health, pathology and even death. Several different types of epigenetic clocks have since been developed and the biological perspective into epigenetic ageing is beginning to form, and these will be presented and discussed.

SAIC LECTURE III
Chair: Marianela Candolfi

REPROGRAMMING THE BRAIN IMMUNE SYSTEM FOR THE TREATMENT OF BRAIN TUMORS. FIRST-INHUMAN PHASE I TRIAL OF THE COMBINATION OF TWO ADENOVIRAL VECTORS EXPRESSING FLT3-L AND HSV1-TK FOR THE TREATMENT OF NEWLY DIAGNOSED RESECTABLE MALIGNANT GLIOMA

PEDRO LOWENSTEIN
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The brain immune system is unable to initiate a systemic anti-glioma immune response. We devised a strategy to reprogram the brain immune system to detect and mount an immune response against glioma tumors. We have now completed a first in human Phase I dose escalation trial of the combination of two adenoviral vectors expressing HSV1-TK or Flt3L for the treatment of newly diagnosed, resectable malignant gliomas. The absence of functional dendritic cells from the brain precludes anti-brain tumor immune responses. We combined tumor cytotoxicity (Ad-HSV1TK) with recruitment of dendritic cells to the brain (Ad-Flt3L) to induce an effective anti-tumor immune response. This strategy induced an efficacious, cytotoxic CD8 and CD4 T-dependent immune response in many animal models of glioma. This immune response also generated anti-tumor memory, and the capacity for neoantigen recognition.

The trial was approved by USA FDA and all institutional committees at The University of Michigan. Treatment was administered intraoperatively following complete glioma resection in newly diagnosed tumors. The trial consisted of vector dose escalation, starting at 1 x 10^9 i.u., and increasing to 1 x 10^{11} i.u. of each vector. Dose escalation proceeded by increasing the vector dose through a total of 6 combinations administered to 6 cohorts of 3 patients each. Two cycles of 14 days each of valacyclovir were administered to activate HSV1-TK cytotoxicity. Cycle 1 starts on day 1-3 post-surgery for 14 days, and cycle 2 on week 8-12. Standard radiation, i.e., 60 Gy in 2 Gy fractions over 6 weeks, with concurrent temozolomide, was followed by cyclic temozolomide.

Examination of tumor samples at primary resection and first recurrence show an increase in the infiltration of inflammatory cells. The experimental treatment was well tolerated. The maximum tolerated dose was not reached indicating that the gene therapy treatments is not more toxic than the standard of care. There were approx. 248 AEs, and 26 SAEs; these have not been linked to treatment. A secondary outcome is overall survival. Preliminary analysis of partial data may suggest that the combined viral vector therapy provides an approximately 5-6 months increased survival to patients.

Our results show for the first time that reprogramming of the host's brain immune system to recognize gliomas reveals a new approach for the treatment of highly malignant brain tumors.

Clinical trial information can be found in clinicaltrials.gov: NCT01811992.

SAIC LECTURE IV
Chair: Ruth Rosenstein

EPIGENETICS IN SMN2 ALTERNATIVE SPLICING: TOWARDS A COMBINED TREATMENT OF SPINAL MUSCULAR ATROPHY

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Spinal muscular atrophy (SMA) is caused by mutations on the *SMN1* gene causing the loss of function of the protein it encodes. Humans have a paralog gene named *SMN2*, that cannot compensate for the deficiency in the SMN protein because exon 7 (E7) is poorly included in its mature mRNA.

A successful approved therapy for SMA restores normal levels of SMN expression by the use of antisense oligonucleotides (ASOs, Spinraza) designed to increase E7 inclusion in the *SMN2* transcript. Our studies aim at understanding how modulation of the chromatin structure as well as transcriptional elongation affect *SMN2* E7 inclusion and to design therapies complementary to Spinraza based on drugs affecting chromatin-regulated alternative splicing.

We have found that fast transcriptional elongation, caused by chromatin relaxation due to histone acetylation, promotes *SMN2* E7 inclusion and that the combined use of the histone deacetylase (HDAC) inhibitors such as trichostatin A (TSA) or valproic acid (VPA) and Spinraza-like antisense oligonucleotides (ASO) may yield a more effective treatment for SMA. These effects were observed not only cell lines but also in fibroblasts from SMA patients. Furthermore, combined administration of the Spinraza-like ASO and HDAC inhibitors has strong synergistic effects on growth and survival of SMA mice.

Our results indicate that the ASO is acting at two levels with apparently opposite effects. As shown by the Krainer lab, at the post-transcriptional splicing regulation level, it promotes *SMN2* E7 inclusion by displacing the negative splicing factors hnRNPA1 and A2. In parallel, at the co-transcriptional level, by creating a more compact chromatin structure characteristic of higher H3K9me2 levels, the ASO inhibits RNAPII elongation around *SMN2* E7, which in turn would cause lower *SMN2* E7 inclusion, counteracting the positive post-transcriptional effect. These findings would explain why by opening the *SMN2* chromatin structure, HDAC inhibitors are able to potentiate the upregulation of E7 inclusion by the Spinraza-like ASO.

SAFE LECTURE
Chair: María José de Rosa

DEVELOPING DRUGS FOR PARKINSON'S DISEASE

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Drugs currently available for the treatment of Parkinson's Disease (PD) have several limitations and their long-term use is marred by the development of problematic side effects. Levodopa is the gold standard of treatment for the disease and the natural replacement for the loss of dopamine. Despite being the one that provides the best symptomatic effect, its prolonged use leads to the development of motor fluctuations and abnormal involuntary movements (dyskinesia), due to its short half-life and pulsatile mode of administration. A small number of patients may find relief with surgical options like deep brain stimulation (DBS), however those who benefit from DBS are young patients, cognitively intact and free of psychiatric disorders, a fact that limits the indications for the large majority of patients. Other options like the dopamine agonists, which partially mimic the effects of dopamine, are significantly less potent, its efficacy tends to diminish with time requiring in the end the addition of levodopa, and are not devoid of side effects (hallucinations, orthostatic hypotension, impulse control disorders and postural abnormalities). Moreover, none of these drugs modify the course of the disease which follows a relentless course with the development of non-drug-related motor and non-motor complications (postural instability, gait disturbances, freezing of gait, and cognitive deterioration), for which we have no effective treatments. In regards Levodopa, great efforts are being invested in improving the pharmacokinetics of the drug, to facilitate its absorption, to provide a more continuous delivery and a more sustained effect. To that effect different formulations are being developed. One of the most important developments in PD research has been the discovery that the underlying mechanism leading to neurodegeneration is the formation of aggregates of abnormally conformed alpha-synuclein (ASN) which is deposited in the cells and neurites and propagates itself in prion-like fashion to progressively involve different areas of the brain. Therefore, strategies to reduce the production of abnormally conformed ASN, prevent its deposit and propagation or to scavenge the already deposited protein are being explored. The approach that has generated more enthusiasm is the possibility of

clearing out the abnormal protein through the use of immune therapies both active (vaccines) and passive (monoclonal antibodies). Another approach is to take advantage of existing drugs already marketed for different disorders that may interfere with pathogenetic mechanisms of PD and thus the field of "drug repurposing" has exploded in recent years. This approach, together with "big data" analysis has allowed researchers to identify potentially beneficial molecules that can be applied to the treatment of PD. Finally, another rapidly expanding field is that of genetic manipulations targeting different molecular cascades that are affected by the disease. This can be achieved via different delivery systems and molecular genetics tools (antisense oligodeoxynucleotides, RNA interference, induced expression of faulty or deficient enzymatic intermediaries, CRISPR-cas9, etc., administered intrathecally or systemically, by themselves or by means of viral vectors. At present there are more than 200 molecules that are being proposed or explored for the treatment of different aspects of the disease.

SAB LECTURE I

Chair: Griselda Irusta

REGULATION OF CELL PROLIFERATION AND INFLAMMATORY PROCESSES BY CERAMIDE 1-PHOSPHATE

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Sphingolipids have long been considered fundamental structural components of cell membranes. However, some of them are bioactive and can regulate a variety of pathophysiological cell functions. In particular, the simple sphingolipids ceramide and ceramide 1-phosphate (C1P) play important roles in cell and tissue homeostasis and have been involved in inflammatory responses. Whilst ceramides promote inflammation and induce cell cycle arrest and apoptosis, C1P has anti-inflammatory properties, inhibits apoptosis and promotes cell proliferation. The mechanisms by which C1P stimulates cell growth include activation of various signaling pathways, such as mitogen activated protein kinases (MAPK) MEK/ERK1/2, phosphatidylinositol 3-kinase (PI3K)/Akt (also known as protein kinase B, PKB), c-Jun N-terminal kinase (JNK), the mammalian target of rapamycin (mTOR), or protein kinase C-alpha (PKC-α), but other mechanism may also be implicated. In fact, recent studies suggest that stimulation of vascular endothelial growth factor (VEGF) secretion or increases in lysophosphatidic acid levels and secretion can also mediate C1P-stimulated cell proliferation. Concerning inhibition of apoptosis, C1P has been demonstrated to block the activity of key regulatory enzymes of ceramide synthesis including serine palmitoyl transferase (SPT) and acid and neutral sphingomyelinases, stimulation of the PI3K/Akt pathway, upregulation of the inducible form of nitric oxide synthase (iNOS), or production of relatively low concentrations of reactive oxygen species. Recent studies have also implicated C1P and the enzyme responsible for its biosynthesis (Ceramide kinase, CerK) in the regulation of pre-adipocyte differentiation. The latter action is of critical importance given the implication of adipogenesis in the establishment and progression of obesity. In particular, obesity is a low-grade inflammatory disease associated to insulin resistance and type II diabetes, and is also involved in cardiovascular diseases, namely atherosclerosis, and development of various types of cancer. In this concern, the inhibition of adipogenesis by C1P suggests that this phosphosphingolipid may be relevant for controlling obesity and obesity-associated diseases.

SAB LECTURE II

Chair: Alejandro De Nicola

PLASTICITY AND REMODELING OF HIPPOCAMPAL CIRCUITS IN THE ADULT AND AGING BRAIN

ALEJANDRO SCHINDER

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The adult mammalian brain, including the human brain, makes thousands of new neurons each day. This process takes place in the hippocampus, a region involved in learning and memory. It is currently thought that adult neurogenesis contributes to the plasticity of preexisting networks by adding new neurons that develop slowly and display a unique potential for making new connections in a manner that depends on physiological and pathological states and, most importantly, it is tailored by experience. Thousands of neurons born each day will have to decide where to connect each one of the thousands of input and output synapses that will ultimately define their role in the circuit. My lab is interested in the functional aspects of adult neurogenesis, addressing questions such as how new neurons develop in a working circuit without affecting

normal function, what properties of new neurons that are most relevant, what molecular mechanisms are critical for their integration, how they contribute to information processing. We also use adult-born neurons as probes to understand how circuit behavior changes with age and what remaining potential for plasticity may be hidden in the senescent brain. In my talk I will discuss our views on the functional implications of adult neurogenesis, and share our most recent findings on how this process contributes to plasticity in the adult and aging brain.

SAB LECTURE III
Chair: Marta Tesone

AUTOPHAGY, ITS MOLECULAR MECHANISMS AND ITS RELEVANCE IN THE DISEASE

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The study of the molecular mechanisms that human cells set up in response to disease is a very active area of scientific research in medicine. Our work is focused on the molecular mechanisms involved in the early events that occur in pancreatic cells during the disease. In the search for new molecules that are differentially expressed during acute pancreatitis we found a new transmembrane protein that we called vacuole membrane protein 1 (VMP1). In our laboratory we discovered that VMP1 triggers autophagy in mammalian cells. Autophagy is a highly conserved process in evolution by which the cell degrades cytoplasmic components including organelles. Autophagy has been related to a variety of pathological processes such as neurodegenerative diseases and tumorigenesis, which highlights its medical importance. We demonstrated that VMP1 mediates a new type of selective autophagy that acts as a defense mechanism in secretory cells. We also show that VMP1 participates in the mechanisms of resistance to death of pancreatic tumor cells and is induced by hypoxia and chemotherapeutic agents. We recently demonstrated that the interaction of VMP1 with a specific plasma membrane protein marks a new site of autophagy initiation. In this lecture we will talk about the evaluation of autophagy and its molecular steps as a cellular response to complex pathologies such as pancreatitis and pancreatic cancer. We believe that the study of the molecular mechanisms that cells activate against the disease will allow the development of new and more rational diagnostic and therapeutic strategies.

NANOMED-ar LECTURE
Chair: Hebe Durán

IMMUNOMODULATORY NANOMEDICINES: INTRACELLULAR INFECTIONS AND BEYOND

EDER ROMERO

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Is it possible to overcome the delivery challenges imposed by intracellular infections to therapeutic agents? Complex parasites-ranging from Trypanosoma cruzi, Leishmania spp and Toxoplasma gondii to viruses-, hide within different compartments of eukaryote cells and benefit from the natural structural protection provided by their hosts. The intracellular location of such pathogens seriously limits the access of therapeutic drug doses, a drawback added to the need of providing selective drug delivery to infected targets. Such issues are partly the reason why treatments of intracellular infections are complex, extensive and a source of secondary undesired effects. In this presentation we will introduce the nanomedicines as new pre-clinical therapeutic paradigms allowing surpassing such difficulties. First some examples of nanomedicines specifically tailored for massive intracellular targeted delivery of anti-inflammatory and anti-infective agents will be presented. After that, a successful example of innovative immunomodulatory nanomedicines, designed ad-hoc to magnify the effect of TLR ligand on intracellular receptors, will be shown. Because of their ability to modify the pharmacodynamics of carried ligands, potent cellular responses with only 2-3 total subcutaneous doses could be induced in animal models without apparent toxicity. Moreover, the approach showed to be more effective than conventional benznidazole in eliminating intracellular parasites such as T cruzi in an acute murine model. It could be speculated that this new tool may contribute to reduce the length of poorly efficacious chemotherapies which are also highly toxic to adults suffering from Chaga´s disease. The therapeutic action of immunomodulatory nanomedicines has recently been assessed on veterinary patients suffering spontaneous squamous carcinoma cells. The implications of choosing the right biomaterials in the

rational design of nanomedicines, will be briefly finally addressed.

SAP LECTURE I
Chair: Oscar Campetella

DEVELOPMENT OF NEW ADJUVANTS FOR ORAL VACCINES

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In our laboratory we are working with a Brucella abortus protein called U-Omp19. We demonstrated that U-Omp19 is a broad-spectrum protease inhibitor. U-Omp19 partially inhibited gastrointestinal proteases such us pancreatic elastase, trypsin and alpha-chymotrypsin and lysosomal proteases (cathepsin L, B and S). Stability studies showed that U-Omp19 retained its full protease inhibitor activity when previously exposed to a broad pH (2-8) or temperature (25-100 °C) range. When co-delivered orally with an antigen (Ag), U-Omp19: i) can bypass the harsh environment of the gastrointestinal tract by inhibiting stomach and intestine proteases and consequently increases the half-life of the co-delivered Ag at immune inductive sites: Peyer's Patches and mesenteric lymph nodes while ii) it induces the recruitment and activation of antigen presenting cells (APCs) and increases the amount of intracellular Ag inside APCs. Besides, U-Omp19 reduces the amount of digested Ag within APCs at inductive sites increasing Ag cross presentation. Therefore, mucosal as well as systemic Ag-specific immune responses, antibodies, Th1, Th17 and CD8+ T cells are enhanced when U-Omp19 is co-delivered with the Ag orally. This bacterial protease inhibitor in oral vaccine formulations confers mucosal protection against LT or CT-induced diarrhea and reduces bacterial or parasite loads after oral challenge with virulent Salmonella, Enterotoxigenic E. coli, Enterohemorrhagic E. coli O157:H7 or Toxoplasma gondii. Thus, U-Omp19 behaves as an important component of vaccine formulations against infectious diseases. Moreover, we found that a Brucella omp19 deletion mutant is highly attenuated when infecting by the oral route. This attenuation can be explained by bacterial increased susceptibility to host proteases met by the bacteria during establishment of infection. Moreover, Omp19 deletion mutant is more susceptible to killing by macrophage derived microsomes than wt strain. Preincubation with gastrointestinal proteases led to an increased susceptibility of Omp19 deletion mutant to macrophage intracellular killing. Thus, we describe for the first time a physiological function of B. abortus Omp19. This activity enables Brucella to better thrive in the harsh gastrointestinal tract, where protection from proteolytic degradation can be a matter of life or death, and afterwards invade the host and bypass intracellular proteases to establish the chronic infection.

SAP LECTURE II
Chair: María Teresa Tellez

DECIPHERING THE QUORUM-SENSING SIGNAL IN TRYPANOSOMA BRUCEI

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Many microbial eukaryotes exhibit cell-cell communication to co-ordinate group behaviors as a strategy to exploit a changed environment, adapt to adverse conditions or regulate developmental responses. An excellent model for these processes are African trypanosomes, the causative agents of sleeping sickness in humans and 'nagana' in livestock, protozoan parasites that have a developmental cycle involving a mammalian host and an insect vector, the tsetse fly. In the mammalian host, parasites exist as replicative "slender" forms, which establish the infection, and once a critical parasitaemia level is reached, differentiate into the non-dividing short "stumpy" form, which are arrested at the G_0/G_1 phase of the cell cycle. This density-dependent differentiation, a form of quorum sensing, prolongs the survival of the host and also prepares the parasites for uptake by the tsetse fly. Recently, we elucidated the signal and signal transduction pathway underlying this activity, revealing that the parasite exploits oligopeptide signals generated by parasite-released peptidases to monitor cell density and so generate transmission stages. We identified a transmembrane protein with oligopeptide uptake-capacity necessary for cell proliferation and differentiation. The involvement of oligopeptides is consistent with the reported properties of SIF (< 500 Da, heat stable) but was unexpected: SIF had been assumed to be a directly released metabolite or other small molecule, rather than a signal generated outside the cell by the activity of parasite produced peptidases. Although we were able to identify the chemical identity of SIF, how that signal is decoded by the parasite to activate its developmental pathway remains to be elucidated. In this talk, I will discuss the approaches we are using to tackle this question.

SAP LECTURE III
Chair: Fernán Agüero

THE APPLICATION OF NOVEL GENETIC AND IMAGING TECHNOLOGY REVEALS THAT BENZNIDAZOLE UPTAKE IN TRYPANOSOMA CRUZI IS MEDIATED BY ENDOCYTOSIS

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For almost 50 years, the nitroheterocyclic agent benznidazole has been the front-line treatment for T. cruzi infections. Benznidazole is a pro-drug that is activated within the parasite by the bacterial-like mitochondrial-localised type I nitroreductase TcNTR-1. Laboratory-induced resistance to benznidazole is readily achievable and has been linked with acquired mutations within the TcNTR-1 gene, or to a reduction in copy-number. Investigations into the mechanisms of benznidazole-resistance have been restricted by the limited flexibility of T. cruzi genetic tools and the absence of the genetic machinery for RNA interference (RNAi). Because trypanosomatids share many metabolic processes, when we used a RIT-seq screen to identify T. brucei genes linked with benznidazole-resistance acquired through loss-of-function, we detected several genes encoding subunits of the vacuolar-type proton ATPase (V-type ATPase), a membrane-localised complex that mediates acidification of intracellular vacuoles, including lysosomes and acidocalcisomes. This enrichment of RNAi target fragments corresponding to V-type ATPase subunits suggested a role for the endocytic pathway in drug uptake. To validate this in T. cruzi, we used a streamlined CRISPR/Cas9 system to generate a range of V-type ATPase subunit single KO and null mutants. These each displayed benznidazole-resistance, implying a common uptake mechanism. To investigate this further, we chemically linked benznidazole to BODIPY (boron-dipyrromethene) and incubated the fluorescently tagged drug with parasites. Uptake via the flagellar pocket was readily detectable in real-time, followed by transit through the endosomal pathway. Benznidazole sensitivity of T. cruzi in the presence of the specific V-type ATPase inhibitor bafilomycin was also evaluated. This revealed inhibitor antagonism, demonstrating an association between inhibition of complex activity and reduced sensitivity to benznidazole. Therefore, both genetic and chemical validation experiments confirm a role for the V-type ATPase in benznidazole mode of action in T. cruzi. Progress in dissecting the mechanisms of benznidazole action, both in vivo and in vitro, has been facilitated by advances in transfection technology and imaging procedures. These will be described, and their further applications discussed.

SAP LECTURE IV
Chair: Adelina Riarte

EPIDEMIOLOGICAL SITUATION OF SELECTED PARASITIC DISEASES BASED ON SURVEILLANCE DATA

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There are many protozoans capable of causing many diseases in people, some of them produce serious acute clinical forms such as malaria or visceral leishmaniasis and others can make people ill in the medium or long term such as intestinal parasitosis or Chagas disease. Because the main health determinants are socioeconomic and environmental ones, the risk distribution of protozoan diseases is inequitable. Public health interventions aim at preventing and controlling these diseases require an epidemiological information for each one of them. For this reason, these are events to be notified (ENOs) in a mandatory way into the National Health Surveillance System (SNVS). This was established by law number 15,645 in 1960. The SNVS collects data needed from medical attention and laboratorial samples provided by practitioners and biochemists in more than 5,000 health-care establishments in the whole country. There are two types of public health surveillance according to the particularities of each disease as well as their possibilities to be prevented and controlled: Event-based surveillance is used to identify and track infectious diseases and involves immediate and nominal reports of cases in order to follow-up patients to implement control and prevention measures to reduce the disease transmission. It includes vector-borne diseases caused by protozoa such as leishmaniasis, malaria and acute vector Chagas, in which the detection triggers public health actions to control and to interrupt the transmission. On the other hand, notification of Chagas in pregnant women, acute congenital Chagas or toxoplasmosis gives information to follow up both, infected women and exposed children, to guarantee adequate diagnosis and timely medical treatment. Indicators-based surveillance includes reports of specific diseases from health care providers with minimum data

(clinical cases and/or laboratory samples studied and positive ones by week) to monitoring disease trends, seasonality, burden and vulnerable poblational groups. Laboratory surveillance of enteroparasites is included in this type and allows for identification of affected age groups, prevalent areas, parasite species and its trends over time to put into effect public health interventions like access to clean water, deworming treatments, etc. Surveillance of Chagas and Toxoplasmosis in pregnancy controls makes possible evaluate trends and identify age and/ or geographical vulnerable people groups. We analyzed the surveillance data collected by SNVS for ENOs related to protozoa and other selected parasitosis.

SAP LECTURE V

Chair: Alejandro Schijman

ADVANCES AND CHALLENGES IN THE ANTIPARASITIC TREATMENT OF CHAGAS DISEASE

SERGIO SOSA-ESTANI

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Chagas disease (Trypanosoma cruzi infection) is a global problem increasing public health impact. Diagnose and treatment of patients infected is consider a key intervention for controlling Chagas disease. The current tools for primary and secondary prevention are efficient to interrupt transmission and control Chagas disease. Specific anti-parasitic treatment for Chagas disease using benznidazole and nifurtimox is indicated in the following situations: a) All acute phase patients, including congenital transmission; b) Following reactivation of infection by immune suppression; c) Patients up to 18 years of age with chronic disease; d) Women of child bearing age with T. cruzi infection (with contraception during treatment). There is a relative consensus that drug treatment should generally be offered to adults aged 19–50 years without advanced Chagas heart disease and is optional for those older than 50 years. Progress were bolstered by the generation of new clinical evidence on the safety and efficacy of new antiparasitic treatment regimens after several completed clinical trials assessing also new chemical entities. BENDITA study (DNDi-Bolivia) assessed new regimens of benznidazole, as a monotherapy and in combination with fosravuconazole. A new trial to assessing short regimens of Fexinidazoleis also underway (DNDi-Spain).The MULTIBENZ trial (Berenice project-FP7-EU-Spain-Argentina-Brazil-Colombia); BENZNIDAZOLE INTERMITTENT trial (HGAEV-INP-FMS-Argentina), CHICAMOCHA Trial (COLCIENCIAS-FCI-Colombia), BETTY Trial (NIH-Tulane-IECS-Argentina) and TESEO Trial (NIH-UTEP-CEADES-ISGlobal-Bolivia) are evaluating efficacy and safety of different regimens of the current trypanocides. All of them in the chronic phase of infection in adult patients. CHICO trial (Multicenter trial-Bayer), have evaluated the safety and efficacy of a new pediatric formulation of nifurtimox. Ongoing clinical studies from DNDi, NHEPACHA Network and other groups are identifying and validating potential biological markers of therapeutic response in Chagas disease patients to support clinical development. Continued progress on these various fronts will help ensure that diagnosis and treatment finally reach the over 99 % of people with Chagas disease who have thus far been neglected. To incorporate diagnose and treatment in people infected with T. cruzi as a public health strategy, which is useful at the primary, secondary, and tertiary prevention; is essential to reduce burden of the disease and to eliminate Chagas disease as a public health issue.

SYMPOSIA

SAIC SYMPOSIUM I

AGING AND CELLULAR SENESCENCE. MOLECULAR AND PHYSIOLOGICAL BASES OF THEIR NATURAL AND PATOLOGICAL EVOLUTION

Chairs: Martín Monte / Mariana Callero

THE EMERGING VIEW OF AGING AS A REVERSIBLE EPIGENETIC PROCESS

RODOLFO GOYA

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The discovery of animal cloning and subsequent development of cell reprogramming technology were quantum leaps as they led to the achievement of rejuvenation by cell reprogramming and the emerging view that aging is a reversible epigenetic process. This short presentation will highlight the more relevant experimental achievments in the brief story of cell and animal rejuvenation. The basic facts underlying the epigenetic model of aging, including Horvath's epigenetic clock, will be outlined. The first study demonstrating that skin fibroblasts from healthy centenarians can be rejuvenated by cell reprogramming was published in 2011 and will be discussed in some detail. Other cell rejuvenation studies in old humans and rodents published afterwards will be very briefly mentioned. The only in vivo study reporting that a number of organs of old progeric mice can be rejuvenated by cyclic partial reprogramming will be described in some detail. The presentation will close with the application of the epigenetic model to two natural processes namely, the resetting of the aging clock in the mammalian zygote and the continuous resetting of the epigenetic clock along successive generations in higher vertebrates so that animal species never age. In the concluding remarks we will share with the audience a look at the wonders that rejuvenation technology promises for the not-too-distant future.

EVIDENCE OF CELLULAR SENESCENCE PROGRAM AS A PITUITARY TUMORAL SUPPRESSIVE MECHANISM

ANA LUCÍA DE PAUL

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Pituitary tumours are usually benign neoplasms, represent 25 % of intracranial lesions and rarely undergo malignant transformations, with the latter occurring in around 0.2 % of all clinical cases. Recent findings have associated premature cell senescence with the features of pituitary adenomas, a process conceived as a tumoral suppressive mechanism. In this study, we investigated the emergence of cellular senescence and the mitochondrial adaptive shift during in vivo development of experimental pituitary tumours. The quantification of Ki67immunopositive cells in the pituitaries derived from estrogenized male rats (10, 20, 40, and 60 days) revealed that the mitogenic potential rate was not sustained for the whole period analyzed and successively decreased after 10 days of estrogen exposure. In addition, the progressive rise in the SA-b-gal activity, IL6, IL1b, and TGFβ expression, was observed throughout the pituitary tumour development. Furthermore, tumoral cells also displayed nuclear pATM protein expression, indicating activated DNA damage signalling, with a significant increase in p21 expression also being detected. We also showed clear evidence of oxidative stress in tumoral cells, associated with augmented mitochondrial biogenesis and an increased fusion process. Nrf2 stress response pathway activation together with the attenuation of the oxidative damage occurring during tumoral development were also detected. Moreover, the progressive increase in lactate production suggested a metabolic shift towards glycolysis metabolism. Our data indicate that cellular senescence should be conceived as a contributing component to the benign nature of pituitary adenomas, thereby influencing the capability of the pituitary gland to avoid unregulated cell proliferation. Additionally, the results helped us to reach an overview of the progressive mitochondrial switches needed for the survival of pituitary cells in order to cope with the damage in the context of tumoral development.

ORSAI, AN ESSENTIAL REGULATOR OF LIPID METABOLISM

MARÍA FERNANDA CERIANI

Laboratory of Behavioral Genetic. Institute of Biochemical research of Buenos Aires. Institute Leloir Foundation. Buenos Aires, Argentina

Over the last decade *Drosophila* has become a well-established model to study the cellular and molecular basis of human disease. A number of years ago our laboratory undertook a misexpression screen to identify genes whose deregulation could render organisms more prone to develop age-related disorders. The screen relied on the automated evaluation of activity patterns in young and aged flies, and looked for signs of premature and progressive loss of rhythmicity as a proxy for genes contributing to sustain cellular homeostasis.
We have identified a mutation affecting a previously uncharacterized gene (*CG6115*) that causes developmental arrest during larval stages, and rename it *orsai* (*osi*). Homozygous mutants are characterized by a conspicuous feeding phenotype; while control larvae feed until reaching critical weight, *osi* mutants stop early on, and eventually die as first instars. RNAi-mediated *osi* downregulation phenocopies this behavior. Sequence analysis suggests that OSI could be part of the LYR-containing protein family, which is associated to mitochondrial respiratory complex I. Along those lines, reduced *osi* levels correlates with altered mitochondrial morphology and deficient metabolism, despite all mitochondrial complexes are correctly assembled. However, OSI localizes primarily within the nucleus. Cell autonomous knock-down dramatically impacts on cell size and morphology. Complementation analysis uncovered that osi-related phenotypes are rescued through human ETFRF1/LYRm5 expression. We propose that OSI coordinates lipid catabolism and the dynamic regulation of mitochondrial electron transport flux, providing a venue to study metabolic diseases in *Drosophila*.

AGEING AND EPIGENETICS: UNEXPECTED EPIGENETIC TWIST IN THE TALE OF THE CHROMOSOME

KENNETH RAJ
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The continuous shortening of chromosomes ends (telomeres) with each cellular division will eventually reach a point, where telomeres will be too short to be bound by proteins that would otherwise mask and prevent them from triggering a DNA damage signal. When this occurs, the cell is ushered into the senescent state where they will remain permanently unresponsive to any proliferation signals. As we age, the cells in our body would naturally accrue greater number of cumulative proliferation frequency and possess ever shorter telomeres. Hence, attempts have been made to use telomere length as a "clock" that tells the age of the cell, tissue, organ or individual. Success however, has been modest. This is owed to numerous challenges that appear intractable. In an unexpected development, the application of artificial intelligence on DNA methylation profiles of CpGs of the human genome appeared to have provided a surprisingly elegant solution to this challenge. This is particularly intriguing and unexpected because CpG dinucleotides which bear the epigenetic marks are not even present in telomeres. The successful development of a DNA methylation-based algorithm that estimates telomere length, reveals surprising properties that were beyond our prediction and expectations. This finding puts an interesting twist in tale of the chromosome.

SAIC SYMPOSIUM II

MOLECULAR AND PHYSIOLOGICAL BASES OF NEURODEGENERATION AND LEARNING

Chairs: Analía Reinés / Gustavo Murer

NEW ANIMAL MODELS OF TDP-43-RELATED NEURODEGENERATIVE DISEASES

LIONEL MULLER IGAZ
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Neurodegenerative diseases are characterized by progressive dysfunction and loss of neurons associated with depositions of pathologically altered proteins. The discovery that aggregated transactive response DNA-binding protein 43 kDa (TDP-43) is a major component of pathological ubiquitinated inclusions in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) caused seminal progress in understanding the etiology of these now so-called "TDP-43 proteinopathies". The role of TDP-43 as a neurotoxicity trigger has been well documented in different *in vitro* and *in vivo* experimental models. As such, the investigation of TDP-43 pathomechanisms in various major neurodegenerative diseases is on the rise. I will present our efforts to understand the pathophysiological roles of TDP-43, using inducible transgenic mice that recapitulate key features of the ALS/FTD spectrum.

TRANSCRIPTIONAL MECHANISMS IN THE STORAGE OF PERSISTENT MEMORIES. ROLE OF CAMKIIDELTA GENE EXPRESSION

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Although important information is available on the molecular mechanisms of long-term memory formation, little is known about the processes underlying memory persistence in the brain. In our laboratory, we recently found that persistent gene expression of CaMKIIdelta isoform participates in object recognition long-lasting memory storage in mice hippocampus. We found that CaMKIIdelta mRNA expression was sustained up to one week after training and paralleled memory retention. Antisense DNA infusion in the hippocampus during consolidation or even after consolidation impairs 7-day- but not 1-day-long memory, supporting a role of CaMKIIdelta in memory persistence. CaMKIIdelta protein is mainly present in nucleus and presynaptic terminals, suggesting a role in these subcellular compartments for memory persistence. All these results point to a key function of the sustained gene expression of this overlooked CaMKII isoform in the maintenance of long- lasting memories.

MODULATION OF MICROTUBULE ASSOCIATED PROTEIN TAU ISOFORMS: FUNCTIONAL CONSEQUENCES AND THERAPEUTIC PERSPECTIVES

MARÍA ELENA AVALE

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Tauopathies are neurodegenerative diseases characterized by the presence of intraneuronal aggregates of the protein tau in insoluble neurofibrillary tangles (NFTs). Tau is a microtubule-associated protein predominantly expressed in neurons, which participates in microtubule polymerization and axonal transport. Alternative splicing of exón 10 (E10) in the tau transcript produces protein isoforms with either three (3R) or four (4R) microtubule binding repeats, which are expressed in equal amounts in the normal adult human brain. Several tauopathies are associated with mutations affecting E10 alternative splicing, leading to an imbalance between 3R and 4R isoforms in certain brain nuclei. Correction of such imbalance represents a potential therapeutical approach for those diseases.

Here I will present our achievements using an RNA reprogramming strategy which modulates the 3R:4R tau ratio, either in cultured post-mitotic human neurons differentiated *in vitro* or into the mouse brain. Lentiviral vectors were used to express molecules that drive E10 inclusion or exclusion *via* RNA *trans*-splicing reaction with the endogenous tau transcript. Morphological analyses and live imaging axonal transport indicate that perturbations in the tau 3R:4R ratio in human neurons impaired axonal transport dynamics without altering neuronal morphology. In a mouse model of tauopathy (htau mice) local modulation of E10 inclusion in the prefrontal cortex improved cognitive deficit, restored neuronal firing patterns and reduced insoluble and hyperphosphorylated tau contents. Moreover, local shifting of 3R to 4R tau in the striatum improved motor coordination déficits in htau mice.

Together, our results evidence some of the (dys)functional consequences of tau 3R:4R imbalance and rise the potential use of RNA reprogramming to correct tau *mis*-splicing in human tauopathies.

NANOCLUSTER ORGANIZATION AND DYNAMICS OF SYNAPTIC PROTEINS IN SYNAPTOPATHIES

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Synaptic transmission relies on an adequate balance of receptor synthesis, delivery to and removal from the cell membrane and anchorage by scaffolding and cytoskeletal components. Alteration of this homeostatic balance is at the root of various neurodegenerative diseases affecting the peripheral and central nervous synapses. In order to understand the interplay between the intervening molecules, it is necessary to define their supramolecular organization, dynamics and trafficking. Two independent superresolution microscopy techniques -STED and STORM- provide complementary information on the static supramolecular organization of neurotransmitter receptors and scaffolding proteins - often occurring in nanometer-sized aggregates ("nanoclusters") in central and peripheral synapses. These can be imaged with nanometer precision and the density, number of molecules per cluster and other structural parameters defined. Furthermore, the mobility of the synaptic proteins can be followed in living cells using single-particle tracking and nanoscopy. The alterations occurring in neurodegenerative or autoimmune synaptopathies will be exemplified.

NUTRITION, METABOLISM, GENETIC, SOCIAL AND CULTURAL HABITS AS DETERMINANTS FOR ILLNESS VULNERABILITY

Chairs: Mariana Tellechea / Adriana Fraga

LIPIDS AT THE CROSSROAD OF α-SYNUCLEIN FUNCTION AND DYSFUNCTION: NEW INSIGHTS INTO NEURODEGENERATION

GABRIELA SALVADOR

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Since its discovery, the study of the biological role of α-synuclein has been the subject of increasing interest. Its aggregation and accumulation in intracellular structures called Lewy bodies are a hallmark of a class of neurodegenerative disorders grouped as synucleinopathies, of which Parkinson´s disease is the most prevalent. The different ways in which α-synuclein interacts with lipids are not only an intriguing characteristic but also an open question related with its biological function and pathogenesis. In our lab, we are mainly focused on the study of lipid signaling and metabolism in different models of neuronal injury.

Phosphatidic acid, a bioactive lipid produced by the activation of Phospholipase D (PLD), governs multiple signaling pathways. We have previously demonstrated that PLD pathways are involved in neuronal degeneration and are, in particular, associated with synaptic injury induced by oxidative stress and inflammatory response. Based on these findings and taking into account the intersections between α-synuclein and lipid biology, we have recently investigated the role of PLD signaling in a synucleinopathy cellular model. The overexpression of wild type (WT) α-synuclein was found to trigger an inhibition of phosphatidic acid production through PLD1 downregulation as well as a decrease in ERK1/2 phosphorylation. Moreover, ERK1/2 subcellular localization and nuclear sequestration were shown to be modulated by the overexpression of α-synuclein in a PLD1-dependent manner. In addition to the changes observed in PLD signaling, neuroblastoma cells expressing WT α-synuclein were found to exhibit a degenerative-like phenotype characterized by a marked reduction in the neurofilament light subunit (NFL). This NFL loss has also been reported in studies performed in post-mortem brains from Lewy body dementia. The gain of function of PLD1 through the overexpression of its active form had the effect of restoring NFL expression in WT α-synuclein neurons.

Lipid metabolism was also altered in neurons overexpressing several forms of α-synuclein (WT or the mutant A53T). The most conspicuous evidence supporting a metabolic switch induced by the different forms of α-synuclein was the presence of lipid droplets. The accumulation of lipid droplets is a rare and unusual entity for the neuronal phenotype. In this respect, WT α-synuclein overexpression was observed to trigger the nuclear localization of the lipogenic transcription factor SREBP-2 and enhancers of protein aggregation (manganese and bortezomib) were found to increase lipid droplets content. WT α-synuclein overexpression also induced Acyl-CoA synthetase activation, which explained, at least in part, the increase in triacylglycerol (usually stored in lipid droplets). The pharmacological inhibition of triacylglycerol synthesis turned neurons more vulnerable to the presence of WT α-synuclein.

Taken together, our findings reveal unforeseen roles for α-synuclein in lipid biology, namely i) PLD1 downregulation associated with NF loss and ii) a metabolic switch with increased triacylglycerol content. Both the decrease in phosphatidic acid levels by PLD1 inhibition and the increase in lipid droplets could be considered as early markers of the neurodegenerative process triggered in synucleopathies.

GLOBAL AND LOCAL NUTRITIONAL SITUATION: EFFECTS ON THE BURDEN OF CHRONIC DISEASES

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Noncommunicable diseases (NCDs) are the result of genetic, physiological, environmental and behaviours factors. NCDs are the biggest cause of death worldwide; 36 million die annually (63 % of global deaths), including 14 million before 70 y (WHO). Ninety % occur in low and middle-income countries and could have been prevented as they are linked to common causes as unhealthy diet, physical inactivity, tobacco and alcohol use. Thirty nine and 13% people over 18 years were overweight and obese in 2016, respectively; meanwhile, the prevalence of overweight and obesity among children/adolescents aged 5-19 y has risen dramatically from 4 % in 1975 to over 18 %. UN Agenda for Sustainable Development recognizes NCDs as a major challenge for sustainable development and committed to reduce one-third premature mortality from NCDs. In Argentina, ENNYS1 Survey (MOH,2004-5) demostrated that 40 % of children 6-72 months and 44 % of women 19-49 y showed excess weight, 34.1 % (6-23 mo), 9% (24-72 mo) and 18 % (19-49 y) anaemia and 14.3 % (6-23 mo) vitamin A deficiency. ENNYS2 Survey (2018-9) recently showed 41.1 % excess weight in boys and girls 5-17 y and 67.9 % in adults over 18 y and pointed out that it was 21 % higher in the lower income quintile. Exclusive breastfeeding, which assures food security, was low (43.7 %, ENNYS2).

As OMS defined obesity as excessive fat accumulation that may impair health, we found an increase in fat mass associated with childhood obesity by deuterium dilution technique in community studies; furthermore, it was also found in normal-weight children. Considering that excessive fatness may increase dyslipidemia and insulin resistance, its evaluation is useful to identify children at metabolic risk. Besides, the measurement of breast milk intake suggests that non-exclusive breastfed children (4 mo) may receive higher energy and protein intake and contribute to obesity. Additionally, the urinary sodium addressed for the excessive sodium intake in adults. The assessment of these less frequently evaluated risk factors would contribute as a tool for better diagnosis of NCDs.

DIABETES: ITS DEVELOPMENT AND SOCIOECONOMIC IMPACT AND HOW CAN BE EFFECTIVELY TACKLE THE PROBLEM

JUAN JOSÉ GAGLIARDINO

CENEXA. Centro de Endocrinología Experimental y Aplicada (UNLP-CONICET-CEAS CICPBA). Facultad de Ciencias Médicas UNLP. La Plata, Buenos Aires, Argentina

In the period 2005-2018 diabetes prevalence in Argentina increased 51 % attaining a value of 12.7 % in adult population. While traditionally its development was geared by a strong genetic component, the role of epigenetic factors has recently showed its important role. Among the latter, central obesity, different metabolic dysfunctions, sedentarism, poverty and low education level are just some of the factors that triggers the epigenetic components.

Late diagnosis of the disease, inappropriate treatment prescriptions and low patient´ adherence to their treatment allows the development of preventable diabetes chronic micro and macroangiopathic complications that increase the cost of treatment and decrease quality of life of people with diabetes. Otherwise, while microangiopatic complications which affect the retina, kidney and nerves are responsible of serious disabilities, the macrongiopatic ones (CVA, acute myocardial infarcts and lower limb amputations) are the main responsible of the patients' death. In this regard, despite new drugs and devices contributed to facilitate the control of diabetes and its associated cardiovascular risk factors (CVRF), less than half of the people with the disease attain treatment goals capable to prevent the development and progression of such complications, thus a large percentage of the diabetes population develop the above mentioned complications.

The picture described demonstrates that probably the health care team strategies currently implemented are not appropriate to tackle efficiently the diabetes problem. Thus, perhaps is the time to change the current diabetes management. From our point of view one alternative consists in improving the quality of care and appropriate disease management at the primary care level. The experience gained and reported by implementing these combined strategies and education of the health care team members as a main tool, demonstrated a significant increase in patients compliance associated with a significant improvement of clinical, metabolic and treatment indicators with the consequent decrease in costs of care. This efficient strategy is currently implemented in the health secretaries of 18 municipalities of the province of Buenos Aires.

FOOD EMERGENCY IN ARGENTINA: ECONOMY AND ITS IMPACT ON MALNUTRITION

NICOLÁS KREPLAK

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The socioeconomic crisis produced by the policies implemented since December 2015 has had a negative impact on the nutritional situation of the population. This warns of the need to review the different data available on food insecurity and put the situation of the Food Emergency that is going through our country on the agenda.

Analyze the information available to put on the political agenda the taking of urgent measures to respond to the health emergency.

The latest population surveys of the former Ministry of Health of the Nation, economic data reported by national universities, market data on access to food, FAO reports and other sources that were identified through consultation with experts were reviewed.

According to FAO, in Argentina there are 5 million people with severe food insecurity, an increase of 100 % compared to the years 2014-2016 vs. 2016-2018. In addition, there are 2.1 million people with malnutrition. Since 2005, the (former) Ministry of Health of the Nation has been carrying out different population surveys that allow characterizing the food and nutritional situation of the population, and that show that the main public health problem is excess weight (overweight and obesity) and chronic noncommunicable diseases, with a growing trend. The National Survey of Risk Factors (ENFR) in 2018 registered obesity in a quarter of the population over 18, an increase of 22 % compared to the 2013 edition and 74 % to the first edition (2005). In addition, excess weight affects 6 out of 10 adults.

However, malnutrition and excess weight are only two sides of the same coin: malnutrition. According to surveys carried out by social movements on more than 25,000 children and adolescents (NNyA) who attend

canteens and snacks, 43 % suffer from some type of malnutrition. For UNICEF, 37 % of NNyA are overweight or obese and 8.1 % are underweight or short for their age.

From the perspective of access to food, the UNDAV Public Policy Observatory notes that in the last year, food increased to 100 %. Meat consumption was reduced to the lowest levels of the last 50 years; only 6 % of the population consumes the 5 servings of fruits and vegetables (400 grams) recommended. Milk consumption fell 6.4 %, reaching lower values since 2003 (56.4 liters per person per year).

UNICEF recommends improving school environments to ensure healthy habits in NNyA. However, school canteens have deteriorated the quantity and quality of food as a result of state disinvestment. As an example, in the Buenos Aires City the budget fall between 2018 and 2019 was 19 % generating a deficit of 110 million food rations.

The increase in budget items is a necessary but not sufficient condition to transform the food-nutritional situation: it is essential that the strategies that are implemented do so taking into account the recommended nutritional parameters.

SAIC SYMPOSIUM IV

CLINICAL AND TRANSLATIONAL MEDICINE

Chairs: Carlos Davio / Geraldine Guerón

CLICK CHEMISTRY REPRESENTS A BREAKTHROUGH IN RADIOPHARMACEUTICALS

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Peptide receptor radionuclide therapy (PRRT) consists in the targeted delivery of radiopharmaceuticals to tumor cells that overexpress a specific membrane receptor, using modified peptide ligands linked to a chelator loaded with a radionuclide. This allows the localized delivery of radiation to the tumor cells. In addition, PRRTs can be used in imaging studies, such as positron emission tomography (PET). PRRTs that target the somatostatin receptors have shown therapeutic efficacy in neuroendocrine tumors, mainly in gastroenteropancreatic and bronchial NETs, but also in phaeochromocytoma, paraganglioma, neuroblastoma and medullary thyroid carcinoma. Recently, ^{177}Lu-DOTATATE was approved by the FDA, for the treatment of gastroenteropancreatic NETs. However, the use of PRRTs could be limited by the unspecific uptake of radioactive compounds in some normal tissues that do not express the specific receptors, such as the kidneys. Nephrotoxicity can be reduced considerably in patients by administering a mixture of positively charged aminoacids but reducing kidney uptake remains a desirable goal. We have used a novel click chemistry approach to reduce the kidney uptake of peptides that target the GIP receptor. To test this, we used nude mice carrying tumors formed with HEK293 cells overexpressing the GIP receptor and injected them with the peptides labelled with ^{68}Ga or ^{111}In for SPECT/CT imaging. The use of click chemistry reduced significantly the compound levels in the kidneys. To study efficacy, we used ^{177}Lu labeled peptides and measured tumor size over several weeks. The treatment showed a significant reduction in tumor volume as well as a prolongation of the survival time. This is the first example of click chemistry applied to PRRT peptides and the first proof that it significantly reduces kidney uptake while maintaining efficacy.

THE ROLE OF FGF AXIS AND FGFR1 ISOFORMS IN THE PATHOGENESIS OF METASTATIC PROSTATE CANCER

NORA NAVONE

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Bone metastases typically develop in patients with advanced prostate cancer (PCa). We have previously reported that the fibroblast growth factor (FGF) axis is implicated in the pathogenesis of PCa bone growth, and that FGFR blockade has clinical activity in advanced PCa and bone metastases (PMID:25186177). In an RNA sequencing study of 183 human PCas we found that different samples express different FGFR1 transcripts. We then mined the TCGA PCa database to determine the expression profile associated with two well characterized FGFR1 splice variants, alpha and beta, which represent the most abundant protein coding transcripts found in PCa. We discovered that each isoform is associated with expression of different genes. Also, in gene set enrichment analysis, we found that FGFR1 beta (but not alpha) is associated with many pathways. In particular, FGFR1 beta is significantly associated with MAPK signaling cascade, signaling by FGFR in disease, and pathways in cancer, among others. *In vitro* studies of FGF signaling activation in PCa cells expressing FGFR1 isoforms alpha, beta or empty vector (EV), confirmed these results suggesting that FGFR1 alpha and beta induce different genes. Importantly, when compared to PCa cells expressing EV, PCa cells expressing FGFR1 isoforms produce significantly more metastasis and reduced survival of mice injected intracardially with the cells. Furthermore, we found a significant increase of bone metastases in the group of

mice injected with PC3 FGFR1 alpha and beta compared to PC3 EV. These results suggest that FGFR1 accelerates the metastatic phenotype of PCa cells. Our studies indicate that FGFR1 isoforms activate different genes and pathways in PCa cells thus conferring different phenotypes. We further propose that FGFR1 expression in PCa cells favors its metastatic dissemination to bone and this may be mediated at least partially by activating a PCa cells-bone cells interaction.

VASOPRESSIN ANALOGS WITH ANTITUMOR ACTIVITY: FROM BASIC RESEARCH TO CLINICAL TRIALS

DANIEL ALONSO

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Desmopressin (1-deamino-8-D-arginine vasopressin, also known as dDAVP) is a synthetic peptide analog of the human antidiuretic hormone. It is a selective agonist for the vasopressin V_2 receptor (AVPR2) present in kidney tubules and blood microvessels. Activation of endothelial AVPR2 by dDAVP triggers the release of von Willebrand factor (vWF), coagulation factor VIII and tissue-type plasminogen activator. Beyond its role in hemostasis, vWF has emerged as a modulator of metastasis, playing a protective role by affecting survival of metastatic cells early after their arrest in target organs. During about two decades, our team has been studying the antimetastatic and antiangiogenic properties of dDAVP in preclinical mammary, colorectal and neuroendocrine tumor models. A pilot veterinary trial in canines subjected to surgery for locally advanced mammary cancer and administered with dDAVP infusions at high doses showed a prolonged disease-free and overall survival. Considering the well-known hemostatic effect and tolerability of dDAVP as well as its potential antitumor activity, we conducted a dose escalation Phase II clinical trial in patients with breast cancer, administering a lyophilized formulation of dDAVP (Surprex™, Elea-Phoenix) by intravenous infusion before and after surgical resection of primary tumor (ANMAT Disp. Nº7025/11; NCT01606072). A second Phase II trial was conducted in patients with rectal cancer with bleeding receiving dDAVP infusions before specific oncologic treatment with surgery and/or chemoradiation (ANMAT Disp. Nº1506/13; NCT01623206). dDAVP appeared as a promising repurposing drug in oncology for patients with bleeding cancers or tumors with high vascular perfusion, particularly in the neoadjuvant setting. In recent years, AVPR2 expression has been documented in a variety of tumors and we moved to develop a panel of novel selective vasopressin analogs. The most potent analog identified (designated [V4Q5]dDAVP) displayed enhanced cytostatic effect in vitro and remarkable antitumor activity in preclinical animal models.

SAIC-SAP SYMPOSIUM

INFECTIOUS DISEASES WITH LOCAL IMPACT

Chairs: Carlos Laino / Guillermo Alonso

TRYPANOSOMA CRUZI MEMBRANE TRANSPORTERS FOR DRUG DELIVERY AND AS DRUG TARGETS

CLAUDIO PEREIRA

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Polyamines are aliphatic polycations that participate in cell growth and differentiation. In *T. cruzi* the transport of polyamines is an essential process since it is unable to synthesize these compounds *de novo*. In a similar way, the amino acid proline is also involved in differentiation processes, cellular invasion and stress responses. *T. cruzi* polyamine and proline permeases were functionally characterized and studied as drug targets by our laboratory. Inhibitors of these permeases were identified by computational simulations combined with *in vitro* assays. In the case of the proline permease, for the similarity-based virtual screening, the compound crystal violet was selected as a starting point. Crystal violet was used in blood banks as a trypanocidal agent (discontinued due to its high toxicity) whose mechanism of action involves the inhibition of proline transport. To search for polyamine transport inhibitors, the reference molecule was a conjugate of a polyamine with anthracene, an experimental oncological drug. Using these compounds, a similarity screening was performed on structures databases of approved drugs using algorithms that compare molecule shapes and electrostatic potentials. Three drugs (loratadine, cyproheptadine and clofazimine) were found to be *in vitro* inhibitors of the proline transporter and also had trypanocidal activity with IC_{50} between 1 and 13 µM in trypomastigote and amastigote forms. Other three drugs (promazine, chlorpromazine and clomipramine) had similar effects over the polyamine transporter and the parasites with IC_{50} between 1 and 4 µM. The strategy herein applied, based on the screening of approved compounds used to treat other pathologies, is known as drug repositioning. One of the main advantages of this experimental approach is that reduces the time and the economic cost of implementation of new therapeutic alternatives, which is especially important in neglected diseases, like Chagas.

DNA REPAIR PATHWAY AS NOVEL THERAPEUTIC TARGET FOR TOXOPLASMOSIS

SERGIO ANGEL

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Toxoplasma gondii is an apicomplexan parasite of medical importance which causes toxoplasmosis in humans. Great effort is currently being devoted towards the identification of novel drugs capable of targeting such infection. The homologous recombination repair (HRR) pathway may be of particular interest in this regard. *T. gondii* presents a complex life cycle composed of two stages in human (tachyzoite and bradyzoite). Tachyzoites rapidly replicate within host cells to produce acute infection during which the parasite disseminates to tissues and organs. Highly replicative cells are subject to Double Strand Breaks (DSBs) by replication fork collapse which leads to HRR. We could observe that the parasite HRR pathway is mostly conserved in *T. gondii*, but some key molecules to modulate the HRR would be missing. Among the conserved proteins we identified a putative ATM kinase, a member of the PI3K family, which is a central factor that initiates DSB repair and activates cell cycle checkpoints. The treatment of intracellular tachyzoites with ATM kinase-inhibitor KU-55933 affects parasite replication and intracellular growth rates in a dose-dependent manner. This treatment also induces G1-phase arrest. Addition of KU-55933 to extracellular tachyzoites also leads to a significant reduction of tachyzoite replication upon infection of host cells. We observed that ATM kinase phosphorylates H2A.X (γH2AX), a marker of DSB. The level of γH2AX increases in tachyzoites treated with camptothecin (CPT), a drug that generates fork collapse. The combination of KU-55933 and other DNA damaging agents such as methyl methane sulfonate (MMS) and CPT produce a synergic effect, suggesting that TgATM kinase inhibition sensitizes the parasite to damaged DNA. By contrast, hydroxyurea (HU) did not further inhibit tachyzoite replication when combined with KU-55933. In conclusion, we have observed that the HRR pathway of *T. gondii* emerges as a new therapeutic target for toxoplasmosis.

HUMAN IMMUNE MECHANISMS THAT OPERATE DURING TUBERCULOSIS INFECTION

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Mycobacterium tuberculosis (Mtb) is the major cause of death by a microbiological agent, causing nearly 1.6 million of deaths per year. In Argentina, last reports estimated 10,733 cases of tuberculosis and almost 1,000 deaths annually. Therefore, it is crucial to elucidate the host immune mechanisms that operate during Mtb infection. The collaboration between antigen presenting cells and lymphocytes culminating in their mutual activation is mediated by the release of cytokines and other effector molecules. Accordingly, reduced IFNG production is a marker of severe disease. In line with this, we demonstrated that the activation of signaling proteins like SLAM and ICOS increased Th1 lymphocytes against Mtb, whereas PD-1, CD31 and SAP induction inhibited this population. Moreover, we found a direct association between Th1+Th17+ lymphocytes expanded by Mtb and active tuberculosis severity. Additionally, among defense mechanisms against Mtb, autophagy is an essential process that modulates the secretion of key cytokines against the pathogen and constitutes a direct mechanism of bacterial elimination. Besides, autophagy can be modulated by cytokines and other immunological signals. Thus, efforts are needed to further elucidate the basic mechanisms of autophagy in immunity against mycobacteria. Then, we used primary cells from blood from tuberculosis patients to understand the pathways that regulate autophagy during active disease. We demonstrated that: i) autophagy collaborates with human immune responses against Mtb in close association with specific IFNG secreted against the pathogen; ii) IL-17A augments autophagy in Mtb-infected monocytes from tuberculosis patients in association with the severity of the disease; iii) different mediators regulate autophagy in neutrophil from tuberculosis patients. Together, our findings suggest that modulation of specific host immune pathways might contribute to the development of new immunotherapies to control active tuberculosis.

RNA RECOMBINATION AT CHIKUNGUNYA VIRUS 3'UTR AS AN EVOLUTIONARY MECHANISM THAT PROVIDES ADAPTABILITY

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.RNA viruses contain dynamic genomes, which allow their rapid adaptation to new environments that has resulted in the expansion of re-emergent viruses. Chikungunya virus (CHIKV) is an important human pathogen transmitted by mosquitoes that has caused recent epidemics in the Indian Ocean and the Americas. Re-emerging lineages have fixed mutations in the coding sequence and large variations in the 3'UTR. As a result,

these lineages feature 3'UTRs carrying different copy numbers of conserved sequence blocks referred to as direct repeats. To investigate how CHIKV overcomes the species barriers that are imposed during transmission, we have examined the evolution of viral populations adapted to host passaging. Combination of experimental adaptation together with fitness measurements revealed that CHIKV 3'UTR is under opposite selective pressures in mosquito and mammalian cells. We observed that during RNA synthesis, viral polymerase switches templates by a copy-choice recombination mechanism and generates variants carrying different numbers of sequence repeats at the 3' end of the viral genome. Deletion variants, which accumulate mainly in mammals, are disadvantageous for replication in mosquitoes and thus, pose a constraint to host switch. Indeed, blocks of duplicated sequences act as substrates for recombination giving rise to a viral population that displays a collection of 3'UTRs. Those viral variants with more fitness in mosquitoes are positively selected to rescue replication. Our work reported for the first time that RNA recombination through template switching acts concertedly with viral selection to assure transmission of infection between hosts. Importantly, we studied the relevance of recombination in laboratory mosquito colonies and demonstrated that this mechanism also occurs in vivo. We propose a model for CHIKV transmission where RNA recombination accounts for the ability of the virus to rapidly adapt after population bottlenecks imposed during host switch.

SAIC SYMPOSIUM V

NEW MATERIALS AND TECHNOLOGIES IN REGENERATIVE THERAPIES

Chairs: Santiago Miriuka / Alberto Crottogini

NEURAL CREST-DERIVED GLAST+ PERICYTES, A SOURCE OF ENDOTHELIAL CELLS AND HEPATOCYTE-LIKE CELLS IN THE FIBROTIC LIVER

JORGE AQUINO

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Liver fibrosis results from cycles of liver damage and scar formation. Little is known regarding NCCs contribution to the liver. We have herein used different double-transgenic lines and two liver fibrosis models. Increased numbers of liver glia, with more ramified and extended processes, were found in fibrotic livers. Moreover, Schwann cell precursors (SCPs) were found to contribute with hepatocyte-like cells (HLCs) and bone marrow stromal cells during embryonic stages, through peripheral blood circulation. In the fibrotic liver, a significantly higher incidence of Wnt1- and GLAST-traced endothelial cells and HLCs were observed. Consistently, during early fibrogenesis Wnt1-traced cells get mobilized to peripheral blood. All mobilized Wnt1-traced cells co-expressed GLAST and CD44, a subpopulation showing CFU-F properties. Furthermore, a similar feature was observed in an 80 % hepatectomy model. Finally, GLAST-traced HLCs were found to co-express CD44, allowing identification of neuroectodermal-derived HLCs. This might be an evolutionary broad repair mechanism in vertebrates.

NOVEL BIOACTIVE GLASSES AND GLASS-CERAMICS WITH ANGIOGENIC PROPERTIES

LUIS HARO DURAND

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Boron (B) is a trace element, which plays an important role in different physiological processes. This presentation will focus on the angiogenic effects of B. Angiogenesis is essential for tissue regeneration and repair. B has been incorporated into bioactive glasses (BGs) in order to improve their angiogenic potential. Using both *in vitro* and *in vivo* model systems we have discovered that the ionic dissolution products (IDPs) from a B-modified BG induce a rise in angiogenesis. Human umbilical vein endothelial cells (HUVECs) seeded on 96-well tissue culture plates were treated with conditioned media (CM) containing the IDPs of BG. Cell proliferation was quantitatively assessed using the [^{3}H] thymidine incorporation assay. The migratory capacity of cells was evaluated by the wound healing assay. Cytokine expression was measured by an ELISA assay. Compared to controls, cell proliferation, migration, IL-6 and bFGF release of HUVECs were statistically significantly increased by B-containing CM. The *in vivo* angiogenic potential was evaluated using the quail chorioallantoic membrane (CAM) bioassay. The IDPs from B-doped BG affected angiogenesis increasing the number of blood vessels branch points. Additionally, studies directed towards B-delivering bioactive glass-ceramic scaffolds for regenerative medicine will be discussed.

NATURE INSPIRED TISSUE ENGINEERING SCAFFOLD DEVELOPMENT: A MECHANICAL APPROACH

FLORENCIA MONTINI BALLARIN

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Nature has developed numerous biological systems with different and characteristic functionalities, which are a continuum source of inspiration for the scientists in search for designs and strategies, for innovative and advanced engineering material systems. What is more, in the field of tissue engineering and regenerative medicine it is necessary to mimic natural tissue unique properties in order for scaffolds to succeed over time.

Tissue engineered scaffolds are being developed as treatment options for malfunctioning tissues where transplantation is no longer an alternative. In the classical paradigm, a material, in the form of a porous scaffold, is used to provide a shape to the tissue under construction and facilitate the release of molecular and mechanical signals. In addition, where it is required to replace tissues that are naturally subjected to mechanical stress, synthetic scaffolds must mimic their mechanical response. It has been studied that a mismatch between the natural mechanical properties and those of the synthetic scaffold is a cause of long-term failure.

Elastin and collagen are the two main components of elastic tissues. The hierarchical collagenous constructions of varying shape, size, and form, which elicit different toughening mechanisms, together with the high elasticity provided by elastin fibers results in a unique mechanical response. Thus, understanding the functional mechanical properties of native tissues and how to mimic these properties in an engineered construct is essential.

Electrospinning allows the production of scaffolds, consisting of nanofibers with a microstructure that mimics the natural extracellular matrix. Moreover, the use of multiscale mechanical constitutive models enables to select the scaffold characteristics based on an objective response.

This work highlights the potential of electrospinning for applications in tissue engineering, presenting examples and the challenges to consider in future work.

TISSUE UNDER CONSTRUCTION: ON 3D SCAFFOLDING FOR REGENERATIVE MEDICINE

ÉLIDA HERMIDA

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Disadvantages of transplants- a strategy to replace a damaged organ or tissue- constituted the main driving force to develop of a new paradigm in the biomedical area: tissue engineering and regenerative medicine. In this framework, Materials Science has many challenges both in the design and development of products and in the dialogue with other disciplines. This talk aims to present the world of tissue engineering and regenerative medicine through recent achievements and work done at Lab3Bio-UNSAM.

SAIC SYMPOSIUM VI

ADVANCES IN ONCOLOGY

Chairs: Elba Vázquez/ Alejandro Urtreger

NOVEL THERAPEUTIC TARGETS AND RESISTANCE MECHANISMS IN BREAST CANCER

EVA GONZÁLEZ SUÁREZ

Bellvitge Institute for Biomedical Research. Spain

Taxanes are the mainstay of treatment in triple-negative breast cancer (TNBC), with *de novo* and acquired resistance limiting patient´s survival. To investigate the genetic basis of docetaxel resistance we generated TNBC, we generated matched TNBC patient-derived xenografts (PDXs) sensitive to docetaxel, and their counterparts that developed resistance *in vivo* upon continuous drug exposure. Aiming to identify mechanisms of resistance to chemotherapy in breast cancer we characterized the cancer stem cell populations and undertook multi-OMIC approaches: genomics, epigenomics and transcriptomics. Findings have direct implications for management of breast cancer disease in the clinic.

THE DYNAMICS OF FITNESS PHENOTYPES IN CANCER CELLS

GUIDO LENZ

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Cancer cell heterogeneity has historically been credited to genetic variations produced by a long and intricate evolution process. Notwithstanding, cells change their phenotypic state constantly, altering the level of proteins, activation state of signaling pathways and complex processes. Intratumoral heterogeneity can be observed through genomic and epigenetic variability leading to a wide range of variation in mRNA and protein expression among cancer cells. This leads to a high degree of heterogeneity in several phenotypes, such as basal proliferation or resistance to therapy. However, so far most measures of genotypic or phenotypic

intratumoral heterogeneity were performed on discrete time points, with little information about the dynamics of this heterogeneity. A central characteristic of a cancer cell is its fitness, measured by the number of descendants it produces after a given time, both under unhindered growth and in the presence of challenges, such as a cytotoxic or cytostatic therapy. Although studying fitness is done by simply counting the number of descendants of a given cell, discovering if it changes over time is more complex. We developed a method to determine the rate of variation in fitness in cells in culture and applied this tool to describe the dynamics of fitness in normal and cancer cells, the fitness in the presence of chemotherapeutic agents and the stabilization of the fitness dynamic by the use of epigenetic modulators. Additionally, we used indicators of signaling mechanisms, autophagy, DNA damage, migration and cell cycle to measure the differences in these processes among sister cells and in cells of colonies of different sizes. Taken together these data indicate that phenotypes important for cancer growth and response to therapy are constantly changing and this dynamic is part of the challenge in treating cancer.

CELL DENSITY SENSING ALTERS TGF-β SIGNALING IN A CELL TYPE-SPECIFIC MANNER, INDEPENDENT FROM HIPPO PATHWAY ACTIVATION

ALAIN MAUVIEL

Institute Curie. INSERM. Paris, France.

Hippo pathway activation upon establishment of cell-cell contacts results in the phosphorylation and nuclear exclusion of its effectors YAP and TAZ, allowing cell contact-dependent growth inhibition. Cytoplasmic sequestration of activated SMAD complexes by phospho-YAP/TAZ upon activation of Hippo signaling by cell density was reported to block TGF-β responses. However, ample evidence in the literature suggests that TGF-β signaling occurs in confluent cells, suggesting that cell-cell contacts do not necessarily prevent TGF-β responses. Using a panel of cell lines of various origins, we were able to demonstrate that inhibition of TGF-β signaling by cell-cell contacts is restricted to polarized epithelial cells and independent from cytoplasmic YAP and TAZ. Rather, we demonstrate that loss of TGF-β responsiveness in polarized cells is strictly apical, due to a unique basolateral TGF-β receptor I and II distribution upon establishment of cell-cell contacts. This, in turn, prevents SMAD phosphorylation upon apical ligand delivery. Basolateral stimulation of confluent cultures or partial dissolution of cell-cell contacts by calcium depletion restored TGF-β -induced SMAD3 phosphorylation and gene responses. Thus, cell type-specific inhibition of TGF-β signaling by cell density reflect polarity domain-specific TGF-β receptor localization, irrespective of Hippo pathway activation. TAZ nuclear exclusion induced by cell-cell contacts is ubiquitous and that mechanisms driving nucleo-cytoplasmic localization of TAZ and P-SMAD are independent, eventually leading to situations whereby both proteins may be fortuitously localized in the same cellular compartment, without obvious functional consequences for TGF-β responses.

NOVEL APPROACHES IN ONCOLYTIC VIROTHERAPY AND IMMUNOVIROTHERAPY FOR GLIOMA TREATMENT

EVANTHIA GALANIS

Mayo Clinic Hospital. Rochester, USA

Oncolytic viruses represent a novel treatment modality that is unencumbered by the standard resistance mechanisms limiting the therapeutic efficacy of conventional antineoplastic agents. Attenuated engineered measles virus strains derived from the Edmonston vaccine lineage have undergone extensive preclinical evaluation with significant antitumor activity observed in a broad range of patient derived glioma models. These have laid the foundation for a phase I clinical trial in recurrent GBM, which has demonstrated safety, biologic activity and viral replication in treated tumors. Reverse engineering has allowed the generation of oncolytic measles virus strains retargeted to increase viral tumor specificity, or armed with therapeutic and immunomodulatory genes in order to enhance anti-tumor efficacy: measles virus induced glioma cell death has been shown to have immunostimulatory properties and in combination with immune checkpoint inhibitors results in synergistic activity. Continuous efforts focusing on determining optimal combinatorial strategies in conjunction with the development of biomarkers predictive of response to viral replication are expected to facilitate treatment personalization and the launching of effective clinical immunovirotherapy strategies.

SAIC SYMPOSIUM VII

CLINICAL NEUMONOLOGY

Chair: Eduardo De Vito

MICROCIRCULATION IN SHOCK STATES

ARNALDO DUBIN

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Microvascular alterations play a key role in the pathogenesis of shock. Even when systemic hemodynamics has been normalized by resuscitation, ongoing microcirculatory abnormalities might hamper tissue perfusion and oxygenation. This form of cardiovascular compromiso- the so-called microcirculatory shock– requires another kind of assessment beyond the monitoring of systemic hemodynamic and oxygen transport variables.

In this presentation, we will discuss clinical and experimental contributions of our team to the current knowledge of this issue. Briefly, we were the first to quantitatively characterize the sublingual microcirculation in patients with septic shock. Our main findings were the decrease in perfused vascular density and proportion of perfused vessels along with increased heterogeneity in septic patients, compared to healthy volunteers. In addition, red blood cell velocity was reduced and hyperdynamic flow was absent in the septic microcirculation, even in patients with high cardiac output. Thus, the state of microcirculation was unrelated to that of systemic hemodynamics. Moreover, changes in perfused vascular density and heterogeneity were more severe in nonsurvivors. In this way, microcirculation might offer valuable prognostic information.

We also studied the response of sublingual microcirculation to fluids, vasopressors, and inotropes. A key result was that the effects were strongly related to the basal microvascular condition. For example, increasing blood pressure with norepinephrine improved perfused vascular density in patients with a compromised microcirculation. On the other hand, it was worsened if microcirculation was preserved at baseline.

Furthermore, our research was focused on the heterogeneous behavior of different microvascular beds to shock and resuscitation. Therefore, the sublingual window might fail to reflect alterations in villi and peritubular microcirculation.

Unfortunately, technical difficulties associated with the acquisition and assessment of the videos are still the limiting step for the widespread clinical monitoring of the sublingual microcirculation. Consequently, it still remains as a research tool.

HEALTH INEQUITIES IN THE DIAGNOSIS AND OUTCOME OF SEPSIS IN ARGENTINA: A PROSPECTIVE COHORT STUDY

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Socioeconomic variables impact health outcomes but have rarely been evaluated in critical illness. Low- and middle-income countries bear the highest burden of sepsis and also have significant health inequities. In Argentina, public hospitals serve the poorest segment of the population, while private institutions serve patients with health coverage. Our objective was to analyze differences in mortality between public and private hospitals, using Sepsis-3 definitions.

We conducted a multicenter, prospective cohort study including patients with sepsis admitted to 49 Argentine ICUs lasting 3 months, beginning on 7/1/2016. Epidemiological, clinical, socieconomic status variables and hospital characteristics were compared between patients admitted to both types of institutions.

Of the 809 patients included, 367 (45 %) and 442 (55 %) were admitted to public and private hospitals, respectively. Those in public institutions were younger (56 ± 18 vs. 64 ± 18; p<0.01), had more comorbidities (Charlson 2 ± 1vs. 1± 0; p<0.01), fewer education years (7 ± 3 vs. 12 ± 2; p<0.01), more frequently unemployed/informally employed (30 vs. 7 %; p< 0.01), had similar previous self-rated health status (Euroqol-VAS; 70 ± 20 vs. 70 ± 25 points; p= 0.30), longer pre-admission symptoms (48 ± 24 vs. 24 ± 12 h; p<0.01), had been previously evaluated more frequently in any healthcare venue (28 vs. 20 %; p<0.01); and had higher APACHE II, SOFA, lactate levels, and mechanical ventilation utilization. ICU admission as septic shock was more frequent in patients admitted to public hospitals (47 vs. 35 %; p<0.01), as were infections caused by multiresistant microorganisms. Sepsis management in the ICU showed no differences. Hospital mortality was higher in public hospitals (47 vs. 30 %; p<0.01).

We concluded that admission to a public hospital was an independent predictor of mortality together with comorbidities, lactate, SOFA and mechanical ventilation; in an alternative prediction model, it acted as a correlate of pre-hospital symptom duration and infections caused by multiresistant microorganisms.

A MULTICENTRE DESCRIPTIVE STUDY OF EARLY AND CONTINUOUS PALLIATIVE APPROACH IN ARGENTINA: LUNG CANCER COHORT

VILMA TRIPODORO

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In Argentina, lung cancer is the most deadly neoplasm. Early identification of palliative care (PC) needs has proven benefits in terms of quality of life, survival, and decision making in Lung cancer patients. The NECPAL CCOMS-ICO© tool is face and content-validated instrument to identify patients with PC needs.
In order to implement and evaluate a demonstration multicentre program for early and continuous PC using the NECPAL-CCOMS-ICO© tool. We reported the results of lung cancer cohort (2016-2018). We categorized patients as surprise question positive (SQ+), (Would you be surprised if this patient were to die in the next 12 month?). If the healthcare professional answered 'NO', the patient was considered SQ+ and they were also considered NECPAL+ when they presented at least one additional parameter from the tool. All patients classified as NECPAL+ were considered to be in need of PC. Then using a Cox regression model, we analysed predictors for overall survival (OS).
Eighty two patients out of 206 were SQ+ and NECPAL+. Median age 64 (35-82). Forty six % had stage IVB, 18 % IVA, 19.5 % locally advanced, 7 pts. at early stage; 6 pts. presented SCLC, 59 % male, 78 pts. were analysed for OS; 4 pts. were excluded due loss of follow up; 56 % Median OS 11 months (7.2-14.7); 5/82 pts. did not receive any kind of oncology treatment due ECOG, comorbidities or patients' choice. Median OS was 17 months (10.5-23.4) for men, and 10 months (3.7-16.2) for females (p= 0.08). In the univariate analyses, only metastasis in vital organs was predicted of survival (17 vs. 8 months; p= 0.035) as well as the multivariate analysis. It was noted a small number of low PPS score (11/78), as well as nutritional (14/78) or severe functional deterioration (5/78), in spite of the majority of the cohort had advanced disease.The results presented support consideration of the NECPAL as a prognostic tool adding a palliative approach in a prospective direct method of measuring prevalence.

SAIC- SAB SYMPOSIUM

TOXICOLOGY. HOW ENVIRONMENTAL POLLUTION CAN HURT OUR HEALTH

Chairs: Andrea Randi / Claudia Cocca / Mónica Muñóz de Toro

PESTICIDE EXPOSURE IMPACT ON THE MOTHER-PLACENTA-FETUS TRIAD

NATALIA GUIÑAZÚ

CITAAC-CONICET. University of Comahue, Neuquén, Argentina.

Pesticides are substances designed to kill, repel, or control plants, insects and animals considered to be pests and that impact negatively on agricultural productivity. Once released to the environment a small amount impact on the amended pest, while the rest gain access to the soil, water and air. In rural populations, the proximity to these areas of intensive pesticide application is a risk factor favoring xenobiotic exposure. Potential health effects associated with pesticide exposure during pregnancy have become a major public health concern due to maternal and fetal high sensitivities. The High Valley of Río Negro and Neuquén provinces is the Argentine region with the largest agricultural fruit production. The insecticide families mostly used are organophosphates –OP- (chlorpyrifos), carbamates (carbofuran and pirimicarb), and neonicotinoids –NEO- (thiacloprid and acetamiprid).
Pregnant women residing in rural locations (Plottier, Centenario, General Roca, Cipolletti, Cinco Saltos) and in Neuquén city, were included in different study groups from 2008 to the present. Matrices analyzed were maternal blood, placenta and umbilical cord blood. Changes in the classical pesticide exposure biomarkers as acetylcholinesterase, butyrylcholinesterase and carboxylesterases activities, in the non-classical biomarkers of oxidative stress (glutathione content, antioxidant enzyme activity, genotoxic damage), and in hormone levels were observed between groups. We demonstrated that the three matrices studied are impacted in the population residing in these rural locations. The placenta systems such as the cholinergic, mitochondria bioenergetics and steroidogenic function were recognized to be important targets of pesticide toxicity in environmental exposure scenarios. Toxic effects of different pesticides families OP and NEO were also confirmed in human trophoblast cell lines, at concentration levels representative of human environmental exposure.

MALNUTRITION AS A RISK FACTOR IN RELATION TO ENVIRONMENTAL AIR POLLUTION: MECHANISMS OF SUSCEPTIBILITY

MELISA KURTZ

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The global impact of malnutrition is astounding. Likewise, air pollution (gases and particulate matter-PM) represents a major health problem worldwide causing 6.5 million premature deaths every year. The synergism between the nutritional deficiencies and air pollution might contribute to increase the outcome on subpopulations at risk. Both factors have been associated to changes in inflammation and oxidative metabolism. Therefore, the aim of this study was to evaluate the effect of environmental air pollution on malnourished growing rats. Our laboratory has developed a nutritional growth retardation (NGR) model

in weanling male rats placed on a 20 % restricted balanced diet during 4 weeks. Then, NGR and control (C) rats were intranasally instilled either with 1 mg/kg BW of residual oil fly ash (ROFA, a PM surrogate) or vehicle (PBS). Twenty-four hours post-exposure *1)* bronchoalveolar lavage (BAL), *2)* serum and *3)* lung, heart and liver were obtained. Additionally, ROFA (1, 10, 100 µg/ml) effect was studied *in vitro* on cultured alveolar macrophages (AM) from C and NGR rats. In C and NGR animals, ROFA exposure induced lung and liver inflammation confirmed by PMN recruitment, lung alveolar space reduction, augmentation of lymphocyte and binucleated hepatocytes. Furthermore, we found antioxidant enzymes mobilization with no changes in lipoperoxidation. On the contrary, ROFA exposure altered heart oxidative metabolism leading to lipid oxidative damage only in NGR animals. Even though we found histological and biochemical tissue alteration, systemic biomarkers of liver and heart injury were not observed. *In vitro*, C-AM exposed to ROFA induced superoxide anion and TNF-α augmentation while, in NGR-AM cultures this response was attenuated. In summary, nutritional status plays a key role in responsiveness to ambient air pollution, as it was suggested by *in vivo* and *in vitro* assays.

EPIGENETIC CHANGES INDUCED BY EXPOSURE TO LIGANDS OF ARYL HYDROCARBON RECEPTOR AND BREAST CANCER

NOELIA MIRET

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Breast cancer is the main cause of cancer death among women and epigenetic changes contribute to the disease. These alterations include the demethylation and activation of the long interspersed nuclear element 1 (LINE-1) retrotransposon, which has been linked to tumor progression. Strong ligands of aryl hydrocarbon receptor (AhR) activate LINE-1, through transforming growth factor-β1 (TGF-β1)/Smad pathway. This study analyzed the effect of two weak ligands of the AhR on LINE-1 expression and the role of AhR and TGF-β1 in their mechanism of action. The human breast cancer cell line MDA-MB-231 was exposed to the organochlorine pesticide hexachlorobenzene (HCB) or the organophosphate chlorpyrifos (CPF). Both activated the AhR/c-Src/Smad axis and enhanced LINE-1 mRNA levels (0.05-50 µM CPF, 0.005 µM HCB, $p<0.01$). This action on LINE-1 expression was prevented when cells were pretreated with the TGF-β1 receptor I inhibitor (2 µM SB431542, $p<0.05$). Considering that LINE-1 transcription is regulated by methylation, 3 sites in the 5´-UTR LINE-1 sequence (+167, +234, +373) were studied by digestion with methylation-sensitive restriction enzymes and qPCR. Only HCB reduced the methylation at the +167 site ($p<0.05$). LINE-1 encodes two proteins, ORF1p and ORF2p, which associate with their own mRNA and allow retrotransposition. ORF1p expression and localization were analyzed by subcellular fractionation and Western blot, showing that the pesticides modulated only its localization. HCB promoted ORF1p translocation to the nucleus at 0.005 µM ($p<0.05$) and its cytosolic retention at 0.5-5 µM ($p<0.05$). In contrast, 0.5-5 µM CPF increased ORF1p cytosolic localization ($p<0.01$), but induced translocation to the nucleus at 50 µM ($p<0.05$). In conclusion, HCB and CPF reactivate LINE-1, enhancing its expression through the AhR/TGF-β1 axis and regulating its localization in MDA-MB-231. Furthermore, HCB promotes LINE-1 demethylation, which could contribute to increase LINE-1 transcription.

STUDYING ENVIRONMENTAL ENDOCRINE DISRUPTORS (EDCS) IN A DEVELOPING COUNTRY (SOUTH AFRICA): JACK OF ALL TRADES?

JOHANNES H VAN WYK

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Several keystone papers echoed scientific concerns regarding the potential that exposure to many pollutants may modulate the normal functioning of the endocrine system of wildlife. South Africa produces and use most of the chemicals listed as potential endocrine disruptors (EDCs). The Water Research Commission (WRC) of South Africa published a first report on the potential of estrogenic activity in water sources in 2000. It is against this backdrop that our research, using *Xenopus laevis* as focus bioindicator to study relevant biomarkers related to endocrine disruptors, evolved. The aim of this talk is to review the progression and achievements of our research programme since 1998. Our initial focus was on estrogenicity screening, using the estrogen induced hepatic produced yolk precursor, vitellogenin (Vtg) as biomarker. To study seasonality and develop bioassays we validated available hormone assays as well as produced anti-Vtg antibodies to quantify plasma vitellogenin. We explored the potential of males having the capability of Vtg production when exposed to estrogen. We confirmed this phenomenon and exposed males in cages held in rivers and dams in different agricultural areas in the Western Cape, to assess estrogenic activity. We also developed a novel *ex vivo* liver culture Vtg bioassay. It soon became evident that EDC activity may be more than just estrogenicity and that the disruption of the androgen system needs attention. This led us to the male breeding glands that

develop under androgen control during mating season. Although, most of the international EDC focus was on the disruption of male and female reproduction the disruption of the thyroid endocrine system became a real concern. *Xenopus laevis* was internationally selected using the thyroid control of metamorphosis as biomarker complex. Although, we did not participate in the interlaboratory validation studies, the WRC helped us to validate the *Xenopus* metamorphosis assay (XEMA). More recently, the use of the Frog (*Xenopus*) embryo teratogenesis assay (FETAX) along with XEMA allowed us to gain a wider perspective on developmental concerns. In conclusion, after using *Xenopus laevis* as a model system, it is clear that this robust aquatic species remains a valuable asset with great potential as a biological indicator to explore organismal-environmental interaction in a human-disrupted environment. During the course of our research progression we relied on a wide range of biomarker systems, aiming to build a toolbox of biomarkers including molecular endpoints. In order to include multi-species variability/sensitivities we extended our research to include fish species, like Zebrafish and the local Mozambique Tilapia, as well as reptiles, for example, a local fresh water turtle species and the Nile crocodile. With this talk I show that in a developing (third world) country, with limited research resources, research groups are often forced to take a generalist approach, not only in the choice of bioindicator species, but also in the selection of focus areas and biomarkers.

SAIC SYMPOSIUM VIII

NEUTRONIC TECHNOLOGY FOR BIOLOGICAL AND MEDICAL RESEARCH

Chairs: Pablo Azurmendi / Sandra Vlachovsky

THE ARGENTINE NEUTRON BEAM LABORATORY "LAHN": APPLICATIONS OF NEUTRON SCATTERING TECHNIQUES TO BIO-SCIENCES

FLORENCIA CANTARGI; JAVIER SANTISTEBAN

Argentine Laboratory of Beams of Neutrons. National Atomic Energy Commission. Bariloche, Río Negro, Argentina.

The RA-10 is a 30 MW multi-purpose reactor under construction in Buenos Aires, designed to satisfy national and regional demand for radioisotopes, nuclear materials testing and neutron beams research. It is foreseen to start operations by the end of 2022. Within the National Atomic Energy Commission, we are working on the development of the "Argentine neutron beam laboratory for the RA-10 reactor" (LAHN); aimed at implementing state-of-the-art instruments, developing a user community and the laboratory staff. The LAHN will be a world class facility available to users from all around the world, and will become the first large-scale neutron laboratory in South America.

Taking into consideration the demands of the local and regional scientific community, a suite of instruments has been planned for the facility. Two instruments are being designed in-house: (i) a neutron imaging instrument placed on a cold beam; and (ii) a multi-purpose diffractometer placed on a thermal beam, optimized for non-destructive studies on large objects. Other instruments will be installed at LAHN coming from laboratories in Europe which are closing their reactors. Besides this, an ambitious program is running to popularize neutron techniques in Argentina and create new users. In this talk, the current state of the project will be described, providing details of the instruments to be installed, training opportunities and strategies to develop the Argentine user community. The use of neutrons in the area of bio-sciences will be specially addressed.

HCS SYMPOSIUM

VASCULAR IMAGING - FORM AND FUNCTION IN DEVELOPMENT AND DISEASE

Chair: Alejandro Adam

STRUCTURAL STUDIES OF MOUSE LYMPHATIC VESSELS IN HEALTH AND DISEASE

PETER BALUK

Cardiovascular Research Institute. University of California. San Francisco. USA.

Lymphatic vessels form a system of tubes parallel to blood vessels but have generally received much less attention than their vascular cousins. Their main functions are to collect and transport excess extracellular fluid and immune cells back to the vascular system. When lymphatics do not function properly tissue swelling or edema can occur and other pathologies also exist. Lymphatic endothelial cells share many features and characteristic molecules with vascular endothelial cells such as Pecam1, and junctional adhesion molecules, but also have their own distinct markers, including the transcription factor Prox1, and cell surface markers LYVE-1 and VEGFR-3 and podoplanin. Prox1-EGFP transgenic mice have been useful reporter mice for visualizing lymphatics. Lymphatic vessels are unusual in that they have a unique double system of valves that permits the uptake of fluid and immune cells and facilitate unidirectional flow. Small diameter initial lymphatics (or lymphatic capillaries), where fluid uptake is believed to occur, have

intricate overlapping oak-leaf shaped endothelial cells with discontinuous flap valves at their borders and little or no smooth muscle coverage. Larger diameter conducting lymphatics have continuous endothelial junctions and internal flap valves. Together, the system of specialized endothelial junctions has become known as 'buttons and zippers'. Growth of lymphatics by sprouting is known as lymphangiogenesis and is analogous to the process of angiogenesis in blood vessels, but different growth factors are involved, e.g. VEGF-C, and receptors, e.g. VEGFR-3. CCSP-rtTA/TetO-VEGF-C double transgenic mice are useful for experimental overexpression of the lymphatic growth factor VEGF-C in a doxycycline-regulated fashion to induce lymphatic growth in the respiratory tract. In pathological situations, lymphangiogenesis commonly occurs in inflamed tissues. The mTOR signaling pathway is a major intracellular mechanism involved. Antibodies to phosphorylated downstream metabolites in the mTOR pathway identify activated and dividing lymphatic endothelial cells. Lymphatic growth is prevented by the mTOR inhibitor rapamycin, but reversal of already grown lymphatics is slow compared to the rapid reversal of new blood vessels deprived of growth factors. In other words, established lymphatics are remarkably resistant to regression. The molecular basis for this difference is unknown. Finally, lymphatics have an intimate relationship with the immune system in lymph nodes and temporary tertiary lymphoid organs, for example, in bronchus-associated lymphoid tissue in the inflamed lung.

REAL TIME IMAGING OF ENDOTHELIAL CALCIUM SIGNALING DURING TRANSENDOTHELIAL MIGRATION

WILLIAM MULLER

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Extravasation of leukocytes across endothelium of postcapillary venules into the inflamed tissues is a critical stage in the inflammatory response. Extravasation encompasses leukocyte capture, rolling, activation, tight adhesion, intraluminal crawling, diapedesis, and migration across the endothelial basal lamina. Each of these steps employs different molecules and signaling pathways of the leukocyte and endothelial cells. Previous studies from our lab have determined that the process of diapedesis, or transendothelial migration, in which the leukocyte squeezes between tightly apposed endothelial cells, is itself a multistep process controlled by sequential engagement of particular leukocyte and endothelial cell adhesion molecules and their respective signaling pathways. The order and mechanisms by which these molecules regulate transmigration has been worked out largely in vitro. Now, using spinning disc confocal intravital microscopy, we have imaged this process in real time to validate the temporal order in which these molecules mediate transmigration. Furthermore, using mice with endothelial cell restricted genetically encoded fluorescent calcium sensors (GCaMP3), we can visualize the spatially and temporally localized increase in cytosolic free calcium ion concentration required for transmigration downstream of PECAM (CD31) engagement on the endothelial cell. These studies offer novel insights into the regulation of inflammation and identify new targets for anti-inflammatory therapy.

DYNAMIC IMAGING OF THE VASCULAR BARRIER

LENA CLAESSON-WELSH

Dept. Immunology, genetics and pathology. Uppsala University. Uppsala, Sweden.

The vasculature responds to stimuli with transient, dynamic changes such as vessel dilation, blood flow changes and leakage. In a chronic setting such as in cancer and retinopathies, these changes may aggravate the condition and promote disease progression. In order to image these processes live, we have established a multi-photon imaging set-up and software for quantification of the changes observed. By immobilizing the mouse ear and injecting fluorescent tracers in the tail vein and substances in the ear dermis, we can follow vascular dynamics noninvasively (Honkura et al., 2018). The effects of stimuli such as vascular endothelial growth factor (VEGF) and the inflammatory cytokines histamine can moreover be mapped to different vessel types, identified by virtue of their anatomical location, their diameter, and blood flow rate. Thereby, we have shown that VEGF/histamine induces leakage in prevenular capillaries and postcapillary venules in the skin. The leakage is transient; and is over in about 10 min. Leakage is accompanied by a 3-fold dilation of capillaries and a dramatic reduction in blood flow. This methodology is being applied on different mouse genetic models to identify positive and negative regulators of blood vessel dynamics, which ultimately can serve as drug targets.

AACYTAL SYMPOSIUM

RUSSEL AND BURCH'S 3RS PRINCIPLES, SIXTY YEARS LATER: ADVANCES AND CURRENT CHALLENGES

Chair: Marcelo Asprea

RUSSELL AND BURCH'S 3RS PRINCIPLES, 60 YEARS LATER: EXPERIENCES AND INITIATIVES IN EUROPE

JUDITH VAN LUIJK

Evidence-Based Laboratory Animal Science and SYstematic Review Centre for Laboratory animal. Experimentation (SYRCLE), Department for Health Evidence, Radbouduniversity medical center. Nijmegen, the Netherlands.

.The 3R principles were introduced by Russell and Burch in 1959 and became well known in the laboratory animal sciences in the 70's. The 3Rs stand for Replacement, Reduction and Refinement. Over the years, the 3Rs have been incorporated in the legislation on animal experimentation of many countries In the EU, the Directive 86/609 on animal experimentation was first accepted in 1986 and updated in 2010 (2010/63/EU). In Directive 2010/63/EU the 3Rs are an important basic principle to promote scientifically and ethically justifiable animal experiments and implementation of non-animal methods. In order to further promote 3R implementation, various local, national and international initiatives have emerged. For example various countries have investigated how 3R implementation on a national level can be improved. Various stakeholders, including researchers, journals, funders and legislators have been working on the development and implementation of guidelines such as the ARRIVE and PREPARE. To further promote the implementation of Directive 2010/63/EU, the European Commission issued calls for a number of related projects last year, including development of education. During this presentation various initiatives and their output will be presented and discussed.

CYS-LOOP RECEPTORS IN CAENORHABDITIS ELEGANS AS PHARMACOLOGICAL DRUG TARGETS

CECILIA BOUZAT

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The free-living nematode Caenorhabditis elegans has emerged as a powerful model for the study of the nervous system and human diseases and as a model for antiparasitic drug discovery. This nematode has also shown promise in the pharmaceutical industry search for new therapeutic compounds by high-throughput screening. Pentameric ligand-gated ion channels, which include Cys-loop receptors, mediate rapid synaptic transmission by converting the chemical signal given by the neurotransmitter into an electrical one. These receptors play key roles in physiological processes, such as neuromuscular transmission, cognition, memory, and are targets of pharmacological compounds of clinical relevance. C. elegans has one of the most extended families of Cys-loop receptors, which have multiple functions including neuromuscular transmission. In particular, nicotinic (nAChR) and GABA receptors are essential for worm locomotion and are of clinical importance as targets of antiparasitic drugs. We combined paralysis assays, locomotion measurements and electrophysiological recordings from C. elegans cultured cells to identify the subunit composition, molecular function and antiparasitic drug modulation of muscle nAChR and GABA receptors. We also identified plant terpenoids and novel synthetic compounds that emerge as potential antiparasitic compounds by inducing rapid paralysis of C. elegans and deciphered the main drug targets and mechanisms underlying their anthelmintic actions. In order to use C. elegans as a model of human neuromuscular diseases, we generated transgenic strains containing mutant nAChRs that mimic those found in congenital myasthenic syndromes. We found that it is possible to recapitulate the molecular functional changes observed in patients, thus validating C. elegans as a model for these disorders.

MASS SPECTROMETRY TECHNOLOGY AND DRIED BLOOD SPOT METHODOLOGY IN ACCORDANCE WITH THE RUSSELL AND BURCH´S 3RS PRINCIPLES

MATÍAS BALDO

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In the area of Health Sciences, the main purpose of analytical chemist is to achieve reliable measurements without compromising the health of the subjects involved in the study. In this sense, the pharmacokinetic (PK) analysis of a given product requires a considerable number of experimental units to achieve a reliable statistical result. In mammals, PK analysis is performed in plasma obtained from whole blood at different time intervals1. The first issue arises from the large volume of blood required by conventional sample preparation techniques, finally compromising the survival of the animals. For small animals (rats, mice, rabbits), this volume is large enough to demand the sacrifice of the animal per time point. This is unacceptable due to the intrinsic variation added to the assay and the practical and ethical considerations[1, 2]. Therefore, the block designs are usually an option to address this problem. In recent years, the use of dried blood spot (DBS) for the miniaturization of analytical procedures has become more important due to the large number of advantages, which leads to significant benefits in accordance with the 3Rs principles for animal research. In addition, liquid chromatography coupled to triple quadrupole tandem mass spectrometry (LC-Ms/Ms) represents the "Elite"

instrument for analytical quantifications due to its high sensitivity and specificity, and the reduction of sample preparations. In this context, the aim of this presentation is to show the development of an alternative methodology for the determination of phenytoin in rabbits and its application to PK studies. The comparison of two types of experimental designs (classical and block) was addressed by coupling a methodology such as DBS with LC-Ms/Ms technology.

References: 1. Beaudette, P., & Bateman, K. P. Discovery stage pharmacokinetics using dried blood spots. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci*. 2004; 809: 153-8.

2. Diehl, K. H. et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. *J. Appl. Toxicol*. 2001; 21: 15–23.

AACyTAL-SAP SYMPOSIUM

PRECLINICAL RESEARCH IN PARASITOLOGY: IMPROVING THE REPRODUCIBILITY THROUGH APPLYING THE 3RS PRINCIPLES

Chair: Eduardo Caturini

USING *IN SILICO* TOOLS TO OPTIMIZE OUTCOMES OF *IN VIVO* DRUG SCREENING

ALAN TALEVI

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In agreement with the three Rs Principle, International guidelines related to the use of animal models advise the use of mathematical models, computer simulations and *in vitro* systems prior to biomedical research involving laboratory animals. However, lack of correlation between *in vitro* and *in vivo* drug screening outcomes is frequently observed in drug discovery (leading to the occurrence of both in vitro false negatives and false positives). Lack of activity *in vivo* is usually related to inappropriate choice of drug targets, pharmacokinetic issues (mostly, insufficient free drug bioavailability at the site of action) or resilient physiological or non-physiological states that are not likely to be disturbed through single target perturbations and are inadequately modeled *in vitro*. Machine learning tools, molecular modeling and network analysis may provide a rational framework to explain and, most importantly, predict lack of concordance between *in vitro* and *in vivo* results, avoiding or reducing unsatisfactory results at preclinical level, and also, in some cases, rescuing drug candidates that would otherwise be disregarded. We will provide a brief overview of such *in silico* tools, from *in silico* druggability and essentiality assessment, to rules of thumb and supervised machine learning approximations directed to the prediction of biopharmaceutically relevant properties (e.g. oral and central nervous system bioavailability, interaction with anti-targets, plasma protein binding, biotransformation, etc).

LOW DOSES OF BENZNIDAZOLE-NANOFORMULATIONS ON THE EXPERIMENTAL TRYPANOSOMA CRUZI INFECTION

LAURA FICHERA

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Chagas disease caused by Trypanosoma cruzi, is progressive and endemic in Latin America. Specifically, a chronic disease develops with cardiac involvement among others. Benznidazole and nifurtimox are effective drugs used to treat Chagas disease although their administration in patients in the chronic phase of the disease is still limited, mainly due to the adverse effects related to these drugs. In our laboratory of trypanocidal preclinical studies, we studied the infection with T. cruzi, isolated from endemic area and with affinity for the heart muscle. Our aim was to reduce the amount of benznidazole in both phases of the experimental infection (Grosso et al., 2013), to study different treatment schemes evaluating the effects of daily doses vs those with BNZ administered intermittently evaluated in different chronic models (Rial et al., 2018) and finally, to study the effects of new nanoformulations of benznidazole (BNZ-NP) with both treatment schemes (Scalice et al., 2016; Rial et al., 2017).To evaluate the response of the treatments we carried out differ results. The electrocardiography, a non-invasive method, was performed to assess conduction disorders caused by the parasite. All treatment schemes showed parasite load and antibody levels reduction, as well as reversion of alteration of heart conduction. Treatment with BNZ-NP on chronic mice showed, besides, a reduction of T. cruzi-specific IFN-γ producing cells and a reduction in the inflammation and fibrosis in the heart compared with untreated T. cruzi-infected animals. The BNZ-NP decreased even more the amount of administered dose and improved the absorption of BNZ to control the T. cruzi infection.

COULD ULTRASONOGRAPHY BE A RELIABLE NONINVASIVE METHOD TO FOLLOW EXPERIMENTAL CYSTIC ECHINOCOCCOSIS IN MICE?

MARÍA CELINA ELISSONDO

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The metacestode Echinococcus granulosus causes hydatidosis or cystic echinococcosis (CE) in humans and livestock. Hydatidosis is characterized by the presence of cysts in different organs and tissues, but liver and lungs being the main locations. The search for therapeutic alternatives to optimize the treatment of CE is performed at two levels: *in vitro* on the larval stage and *in vivo* in mice infected intraperitoneally with E. granulosus protoscoleces. In the current murine model of CE, the cysts are located in the peritoneal cavity. In order to establish a new murine model presenting similar characteristics to the disease in humans, we infected CF-1 mice via the portal vein. In this new model, the cysts developed in the orthotopic and primary infection organ. In an attempt to apply to the 3Rs principle, we used ultrasonography to monitor the development, the growth and the localization of the cysts in the liver of infected mice. In this presentation, we discuss the advantages and disadvantages of ultrasonography to follow the experimental hepatic CE in mice.

REFINEMENT STRATEGIES TO BALANCE SCIENTIFIC PURPOSES AND ANIMAL WELFARE

ERNESTO GULIN

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Animal models play a key role in biomedical research. Regarding parasitology research, the use of several animal species had contributed to unveiling physiopathology process, explore new diagnosis and chemotherapeutic options and develop vaccine candidates to improve animal and human health. However, animal use for research purposes carries legal and ethical responsibilities to preserve animal welfare to the greatest extent possible during the assays. Although many animal species and models have been developed for parasitology research, there is still a lack of uniformity and harmonization in pre-clinical *in vivo* studies. This situation could contribute to the lack of reproducibility crisis in science, increasing the gap between basic and clinical research. In this lecture, we will introduce some new concepts such as therioepistemology and different criteria for animal model validation. Also, we will discuss some practical examples to address the 3Rs principles in animal experimentation (replacement, reducing and refinement) during planning, conducting and reporting process as well as some considerations to choose the most robust and reliable animal model focusing in parasitology research.

SAP SYMPOSIUM

DRUGS FOR NEGLECTED DISEASES

Chairs: Alan Talevi/ Claudio Pereira

MONITORING REDOX CHANGES IN LIVING TRYPANOSOMATID PATHOGENS USING A GENETICALLY-ENCODED BIOSENSOR

MARCELO COMINI

Pasteur Institute of Montevideo. Montevideo, Uruguay

Thiol-redox homeostasis is key to sustain biosynthetic processes, to cope against oxidative stress and xenobiotics. Overwhelming experimental and clinical evidences support the important role of the thiol-redox system of trypanosomatids in parasite virulence and survival. Given their uniqueness and indispensability, several components of the parasite redox system are attractive drug-target candidates. Here we aimed to develop redox-reporter cell lines of pathogenic trypanosomatids suitable to study fundamental questions of the parasite´s redox biology, to study drug mode of action and for high-content screening applications. Different redox-sensitive fluorescent proteins were developed and proved specific to detect changes in the trypanothione (major thiol-redox substrate of trypanosomatids) and glutathione pool, via thiol-disulfide exchange reactions accelerated by oxidoreductases. Stable redox-reporter cell lines of Trypanosoma brucei, T. cruzi and Leishmania infantum were generated. The biosensor was capable to detect intracellular redox unbalance triggered by different exogenous redox stimuli within a minute time-scale and using flow cytometry or confocal microscopy as read-out techniques. Screening of a small compound library allowed the identification of anti-trypanosomal compounds that affect (or not) the redox homeostasis. The on-target effect as well as the mode of action of several drugs and drug candidates was successfully

addressed with the redox reporter cell lines. Redox changes occurring during the infection process were monitored on real-time in a mouse model of African trypanosomiasis. The redox-reporter cell lines allow monitoring intracellular redox changes in trypanosomatids in a non-invasive, dynamic and in situ fashion. The transgenic cell lines proved useful for high-throughput/high-content flow cytometry-based screening of compounds, for addressing the mechanism of action of drugs and drug candidates, and to study host-pathogen interaction.

ANTIPARASITIC DRUG DISCOVERY FROM PLANT SOURCES

VALERIA SÜLSEN

Institute of Chemistry and Metabolism of the Drug (IQUIMEFA). Buenos Aires, Argentina.

Despite the advances and development of synthetic chemistry, drugs derived from natural sources continue to be outstanding. The chemical diversity of natural compounds determines a greater possibility of finding new molecules with unique structures and with potential biological activities. Among them, plant sources have been of great interest and compounds isolated from them have been used to treat a large number of diseases. The cardiotonic agent digoxin, morphine and codeine, the antihypertensive drug reserpine, ephedrine, vinblastine and vincristine are examples of therapeutic agents obtained from plant sources. Among antiparasitic drugs, quinine and artemisinin, can be mentioned. These drugs, currently in use for malaria treatment, have been used as scaffolds for the synthesis of more effective and selective compounds. Plant kingdom comprises about 250,000 species, of which only about 6 % have been studied for their biological activities, and approximately only 15 % have been studied phytochemically [1]. Neglected diseases are a group of parasitic, viral or bacterial diseases that mainly affect populations of tropical and subtropical areas of the world that live in a situation of poverty and/or marginality. The World Health Organization estimates that more than 1 billion people suffer from one or more of these diseases and live in areas at high risk of contracting them[2]. Our research group is dedicated to the investigation of Asteraceae species in the search of compounds that can be used as a basis for the development of new antiparasitic drugs mainly for the treatment of Chagas disease and Leishmaniasis, both of them considered neglected diseases. Most of the promising bioactive molecules found belong to terpenoids, being specifically sesquiterpene lactones isolated from genus Ambrosia, Mikania and Stevia. The presentation will provide the most important results of our research involving antiparasitic sesquiterpene lactones.

References: 1- Katiyar C, Gupta A, Kanjilal S, Katiyar S. Drug discovery from plant sources: An integrated approach. *Ayu.* 2012; 33(1): 10–9. doi:10.4103/0974-8520.100295. 2- World Health Organization, 2019. Neglected tropical diseases. https://www.who.int/neglected_diseases/diseases/en/

NEW ANTIKINETOPLASTIDS 123-TRIAZOLYL AZASTEROLS TARGETING 24-SMT

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Unlike humans, T. cruzi, T. brucei and Leishmania spp. have ergosterol as the main sterol instead of cholesterol. Ergosterol biosynthesis have different demethylation steps, while 14alpha-demethylase (SDM) is shared between human, fungi and protozoa; Delta24-sterol methyltransferase enzyme (24-SMT) is only present on the lasts organisms. 24-SMT catalyze the conversion of zymosterol to fecosterol, transferring a methyl group from the SAM cofactor to generate a high-energy intermediate as the first step. As part of our ongoing neglected tropical diseases drug development program and looking to mimic 24-SMT carbocation intermediate a series of azasterol derivatives were designed as potential inhibitors of the 24-SMT. A collection of azasterols was prepared with a 1,5-substituted amino-123-triazole on lateral chain as diversity entry. The prepared compounds were assayed against the T. cruzi epimastigotes, T. brucei blood stream form and L. donovani promastigotes. The most active analogs have IC50s on the low micromolar or at submicromolar concentration. Looking to validate the target in vivo, the compounds were also assayed on a polyene resistant L. mexicana strain. Additionally, L. mexicana 24-SMT was cloned and expressed in E. coli. The enzyme was subjected to a substrate specificity assays with a pool of steroids and the enzyme kinetics parameters were also determined using zymosterol as substrate. Compounds were screened as 24-SMT inhibitors and the IC50 of the most potent was measured. Out of the 20 compounds assayed, 14 analogs have IC50s below 200 µM, where the most active were below 8 µM, validating the target. Finally, a mass spectrometry-based metabolomics study was conducted to identify metabolic differences on treated parasites to identify the affected biochemical pathways and their variation. In summary, the newly introduced 123-triazolyl sterols that targets 24-SMT are promising structures to develop wide spectrum antiparasitic agents.

Short talks from SAP selected abstract:

0490 - COMPUTATIONAL REPOSITIONING OF BIOACTIVE COMPOUNDS FROM LARGE CHEMOGENOMIC SCREENS: IDENTIFICATION OF CONSERVED DRUGGABLE MODULES BETWEEN YEASTS AND TRYPANOSOMES

Lionel URAN LANDABURU | Mercedes DIDIER GARNHAM | Fernán AGÜERO

INSTITUTO DE INVESTIGACIONES BIOTECNOLÓGICAS (IIB-UNSAM-CONICET)

Detailed characterization of the cellular response to chemicals is fundamental to understand the mechanism of action of drugs. One strategy to do this is to analyze the growth capacity (fitness) of gene mutants exposed to different drugs. Recently, a number of genome-wide fitness profiling assays were performed on Saccharomyces cerevisiae. These chemical-genomics screens were based on whole-genome collections of heterozygous and homozygous deletions and quantified the growth fitness of each strain in the presence of different chemicals. Now, several such chemogenomic datasets are available, providing a rich source of pharmacogenomic associations between drugs and genes ("druggable modules"). In contrast, in trypanosomes pharmacogenomic associations are scarce, hence these yeast chemogenomic screens may serve as good starting points to guide repurposing opportunities. The aim of this project is the curation and standardization of yeast-based chemogenomic assays from published studies, and the development of an orthology mapping pipeline. Using this pipeline to find conserved druggable modules between yeasts and T. cruzi, we obtained 93,758 gene-drug interactions, with a set of 3,005 unique genes and 2,430 unique drugs. Further filters were applied to each set. For drugs, filters were applied to retain compounds that are drug-like, novel, commercially available, and with low potential promiscuity; with a final iteration to maximize chemical diversity within the set. For genes, we selected those that have T. brucei orthologs with significant fitness phenotypes when knocked down (through an orthology mapping between T. cruzi genes and T. brucei whole-genome RNAi essentiality assays described in Alsford et al, 2011). After standardization and filtering we obtained a library of 50 compounds, associated with 78 candidate protein targets in T. cruzi. References: Alsford S (2011) Genome Research 21: 915-24. DOI:10.1101/gr.115089.110

0604 - HIT OPTIMIZATION OF A TRYPANOSOMA CRUZI BROMODOMAIN INHIBITOR IDENTIFIED USING COMBINATORIAL CHEMISTRY

Victoria ALONSO (1) | Andrea ESCALANTE(2) | Ricardo FURLAN(2) | Esteban SERRA(1)

INSTITUTO DE BIOLOGÍA MOLECULAR Y CELULAR DE ROSARIO (IBR, CONICET/UNR) (1); UNIVERSIDAD NACIONAL DE ROSARIO (2)

Recently, our group has used Dynamic Combinatorial Chemistry targeting the Trypanosoma cruzi Bromodomain factor 3 (BDF3), as a strategy for the identification of a parasite inhibitor, this hit is an acylhydrazone with a Kd of 1.7 µM and and IC50 for epimastigotes, amastigotes and trypomastigotes between 13 and 23 µM. TcBDF3 interacts with acetylated alpha-tubulin present in the cytoskeleton and flagella of T. cruzi and is essential for the viability. TcBDF3 is an interesting target for the development of new trypanocidal drugs that disrupt the bromodomain-acetylated ligand interaction during the parasite differentiation. There are six other proteins with bromodomain in T. cruzi, among which TcBDF2 has also been shown to be essential for the parasite. Today it is a challenge to find selective inhibitors that can distinguish between the different bromodomains, and are more effective and less toxic than the trypanocidal drugs currently use. We prepare a small library of acylhydrazones synthesized from an acylhydrazide nucleus and various aldehydes selected according to the hit previously described by our group for TcBDF3, with the goal of finding more potent and selective inhibitors against TcBDF2 and TcBDF3. The interaction of each hydrazone with TcBDF2 and TcBDF3 from T. cruzi was determined by microplate protein fluorescence quenching assays and Thermal Shift. The results obtained so far allow us to conclude that i) all synthesized hydrazones interact with the hydrophobic pocket of TcBDF3, ii) none of them interact with TcBDF2 up to the highest concentration tested (20 µM), iii) two of the synthesized hydrazones are attractive due to its affinity to TcBDF3 and iv) only one of these hydrazones inhibits the development of epimastigotes of T. cruzi (IC50 <10 µM).

0927 - HIGH-THROUGHPUT SEROLOGICAL FOLLOW-UP OF CHAGAS DISEASE PATIENTS TREATED WITH BENZNIDAZOLE AND E1224 IN A RANDOMISED, PLACEBO-CONTROLLED TRIAL: SEARCHING FOR EARLY INDICATORS OF TREATMENT SUCCESS AND FAILURE

Alejandro Daniel RICCI (1) | Leonel BRACCO(1) | Faustino TORRICO(2) | Isabela RIBEIRO(3) | Bethania BLUM(3) | Sergio SOSA ESTANI(3) | Joaquim GASCON(4) | Fernán AGÜERO(1)

INSTITUTO DE INVESTIGACIONES BIOTECNOLÓGICAS (IIB-UNSAM-CONICET) (1); FUNDACIÓN CEADES (2); DRUGS FOR NEGLECTED DISEASES (DNDI) (3); BARCELONA INSTITUTE FOR GLOBAL HEALTH (ISGLOBAL) (4)

During an infection, the immune system produces antibodies against pathogens. With time, the immune repertoires of infected individuals become specific to their clinical history and thus represent a rich source of diagnostic markers. Chagas Disease (CD) is caused by the protozoan parasite Trypanosoma cruzi. When developing new drugs, it would be advantageous to reduce the followup time in clinical trials. The current criteria for cure in CD is negativization of at least one of 3 independent samples by PCR or at least 2 serological tests. However PCR assays have low sensitivity and seroconversion is slow using standard assays that measure antibodies to several antigens at once. Recently a new drug was tested for CD. In this study, fosravuconazole (E1224) was tested alongside benznidazole (BZ) and a placebo (Torrico et al 2018). E1224 displayed a transient, suppressive effect on parasite clearance, whereas BZ showed early and sustained efficacy until 12 months of follow-up. Using high-density peptide arrays displaying ~400,000 peptides derived from a large collection of recently identified T. cruzi antigens, we assessed changes in individual antibody repertoires along time for 36 patients from the 3 main study arms (BZ, E1224, placebo). The antibody repertoire of each patient was analyzed at recruitment (0), end-of-treatment (day 65) and at the end of follow-up (12 months). A total of 108 serum samples were analyzed in duplicate to assess the dynamics of the antibody immune in response to drug treatments (or placebo). A preliminary analysis of the data allowed us to identify antigens that increased or decreased their signal in treatment groups and also a number of subjects that showed more or earlier changes in their serological profile in response to treatment. We will discuss advances in our aim to find candidate prognostic markers to follow-up CD patients in clinical trials.

SAP SYMPOSIUM II

BIOCHEMISTRY AND MOLECULAR BIOLOGY

Chair: Sergio Angel

REGULATION OF TRANSLATIONAL EFFICIENCY IN T. CRUZI

PABLO SMIRCICH

"Clemente Stable" Biological Research Institute. Department of Genomics. Montevideo, Uruguay.

Trypanosomatid parasites depend almost exclusively on post-transcriptional mechanisms to regulate gene expression. In eukaryotes, mRNAs translation is controlled by several mechanisms that mostly affect the initiation rate. So, to further characterize this process in T. cruzi, our group has studied the influence of translation in the determination of the gene expression profiles in different parasite stages by using the ribosome profiling method. This technique allows to quantify both the translation rates as well as the translation efficiency (TE) of mRNAs in a genome wide fashion. The results show that the translation rate is an excellent proxy of protein levels as measured by proteomic approaches. Besides, the observed changes in the TE indicate that translation is indeed a key regulatory step which affects gene families of physiological relevance such as surface antigens and ribosomal proteins. To describe specific mechanisms acting on translation efficiency, several approaches were carried out. The subcellular localization of mRNAs was shown to affect TE by specifically partitioning mRNAs. Also, the analysis of the presence of small upstream ORFs showed that these elements on the 5'UTR affect the TE of hundreds of T. cruzi transcripts. Finally, the presence of primary and secondary structure cis acting signals in the UTRs of highly regulated gene families was observed. Overall, the results shed new light into the mechanisms that may be acting to fine tune translation levels in these parasites.

DECIPHERING THE MECHANISM OF LACTOFERRIN AS A NATURAL ANTIGIARDICIDAL

CAROLINA TOUZ

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A large amount of research has focused on the structure and function of the iron-binding protein lactoferrin (LF), achieving considerable advances in our understanding of its synthesis, distribution, and degradation. It is now recognized as a molecule with multiple biological roles, including regulation of iron absorption, and as a protein with anti-oxidant, anti-microbial, anti-carcinogenic and anti-inflammatory functions, among others recently discovered. Human and bovine milk are the most abundant source of LF but it is also present in saliva, seminal fluid, glandular epithelial cells, and neutrophils. It has been suggested that LF is effective in the treatment of giardiasis, an intestinal disease caused by the protozoan parasite Giardia lamblia. However, the molecular mechanisms by which LF exerts its effect on this parasite are unknown. Most of the microbicidal activity of human or bovine LF (hLF or bLF) has been associated with the N-terminal region of the mature LF - lactoferricin (LFcin). LFcin is produced by pepsin cleavage of the native protein *in vitro* and likely *in vivo*. Our studies display the role of the receptor-mediated endocytosis machinery in the regulated cell entry of bLF and bLFcin and the initiation of cell damage that induces cell differentiation but blocks encystation to infective-mature cysts. Our findings support the use of bLF to treat giardiasis since it may be beneficial in several aspects: at high doses, it produces trophozoite killing, while traces (low concentration) cause microstatic effects and the production of non-infective cysts. This finding, together with the fact that LF protects the intestinal cell barrier, modulates the immune response, and produces no side effects, gives to LF great therapeutic potential to eradicate giardiasis.

EXPLORING THE CONTENT OF NONCODING RNAS, PUTATIVE REGULATORY ELEMENTS, IN THE PROTOZOAN PARASITE LEISHMANIA

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Several classes of noncoding RNAs (ncRNAs) have been revealed in recent years. ncRNAs are involved in a variety of regulatory processes in a wide range of organisms. Our laboratory is focused on understanding some of the layers at which regulation of gene expression occurs in Leishmania. Serendipitously, studying a group of short unannotated and polyadenylated transcripts from Leishmania major, we identified and partially characterized one of them, ODD3, a ~150 nucleotide-long transcript arising from the 3'UTR of one of the two copies of a ribosomal protein gene (RPS16). We extended the search for similar uaRNAs to other species and an in-depth study on the modulation of gene expression across the life cycle stages of Leishmania braziliensis covering coding and noncoding RNAs (ncRNAs) was conducted. Analyses of differentially expressed (DE) genes revealed that most prominent differences were observed between the transcriptomes of insect and mammalian proliferative forms (6,576 genes). A computational

pipeline and five ncRNA predictors allowed the identification of 11,372 putative ncRNAs. Of the DE ncRNAs, 295 were DE in all three stages and displayed a wide range of lengths, chromosomal distributions, and locations; many of them had a distinct expression profile compared to that of their protein-coding neighbors. The presence of 22 of the predicted transcripts of similar length was confirmed by Northern blotting analysis. Knockout (KO) and tagging of 6 transcripts were obtained using CRISPR/Cas9 editing machinery, and the parasites' phenotypes are under analysis. Modification of macrophage infection profile was witnessed for one of the evaluated ncRNA KO, and pulldown assays were conducted to identify ncRNA binding proteins. The novel putative ncRNAs uncovered in L. braziliensis might be regulatory elements and are under investigation in our laboratory.

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Shorts talks from SAB selected abstracts:

0554 - TRYPANOSOMA CRUZI TCHTE PROTEIN EXPRESSION IS REGULATED BY INTRACELLULAR HEME LEVELS

Evelyn TEVERE | Cecilia DI CAPUA | Julia CRICCO

IBR-CONICET, UNR

Trypanosoma cruzi is a heme auxotroph, therefore it must scavenge heme (as free heme or as hemoglobin) from their hosts. The protein TcHTE (T. cruzi Heme Transport Enhancer) is involved in the uptake of this cofactor. It is localized to the flagellar pocket of the parasite and there is evidence that it forms a homotrimer. Conversely to other trypanosomatids, no hemoglobin (Hb) receptor has been found in T. cruzi yet. Given these precedents, we investigated if TcHTE has a role in Hb uptake from the extracellular medium or in Hb-derived heme transport. At mRNA and protein level, TcHTE is higher in heme starved Wild Type epimastigotes, and it gradually decreases when increasing amounts of a heme source (hemin or Hb) are added to the media. However, this response is faster when hemin is used as heme source, which may be related to the different biodisponibilities and/or uptake mechanisms of both heme sources. Surprisingly, epimastigotes that overexpress rTcHTE.His-GFP incubated in media supplemented with Hb have a significantly higher intracellular heme content compared to control epimastigotes; as previously reported using hemin as heme source. Altogether, these results mean that TcHTE is also involved in Hb uptake. Conversely to Trypanosoma brucei ortholog rTbHRG, rTcHTE.His-GFP does not change its localization when Hb is used as heme source, which discards that TcHTE has a role in the salvage of Hb-derived heme in internal compartments. We concluded that T. cruzi is able to sense intracellular heme level and regulates TcHTE expression according to it. Based on these and our previous results we propose two models of heme uptake in T. cruzi. In the first one, Hb is endocyted via a non-canonical Hb receptor and Hb-derived heme is transported through an unknown protein, meanwhile heme enters the cell via TcHTE. In the other model, Hb is degraded by external proteases in the parasite's surface, heme is released and enters the cell via TcHTE.

0778 - FUNCTIONAL ROLES OF AMP-ACTIVATED PROTEIN KINASE (AMPK) COMPLEXES CONTAINING TCAMPKA1 OR TCAMPKA2 IN ENERGY HOMEOSTASIS REGULATION AND CELL CULTURE PROGRESSION IN TRYPANOSOMA CRUZI

Tamara STERNLIEB (1) | Alejandra C. SCHOIJET(2) | Patricio D. GENTA(1) | Guillermo D. ALONSO(2)

INGEBI-CONICET (1); INGEBI- CONICET- UBA (2)

The AMP-activated protein kinase (AMPK) is a heterotrimeric enzyme involved in maintaining energy homeostasis in response to different stresses in many organisms. During the transition between the mammalian host and the insect vector, Trypanosoma cruzi, the causative agent of Chagas disease, faces different types of environmental fluctuations, all of which prompt the parasite to remodel its metabolism. Recently, it was shown that Trypanosoma brucei AMPK is involved in the differentiation from the bloodstream slender to stumpy stage and in surface protein expression changes in response to nutritional stress. This underscores the relevance of AMPK for parasite life cycle progression. We identified four candidate genes for the AMPK subunits of T. cruzi (alpha1 and alpha2 catalytic subunits, beta and gamma regulatory subunits). The beta and gamma subunits are largely conserved in their domain structure relative to the mammalian orthologs. However, the alpha subunits show significant sequence and structure differences from the human counterparts. The presence of these subunits in T. cruzi epimastigotes was confirmed by RT-PCR, Western blot using a phospho-AMPKa specific antibody, mass spectrometry and by kinase activity assays using the specific AMPK substrate SAMS. TcAMPKa1 over-expressing epimastigotes showed a lower growth rate in basal culture conditions compared to the control. On the other hand, alpha2 over-expression had the opposite effect. Additionally, we observed upregulation of AMPK activity under epimastigote starvation, and that dorsomorphin, a specific AMPK inhibitor, also inhibits T. cruzi AMPK. Moreover, each of these subunits could complement S. cerevisiae conditional mutants lacking the respective subunit of the AMPK ortholog SNF1. Finally, starving assays with AMPKa over-expressing parasites also showed a possible role of AMPK in autophagy. Overall, our results show for the first time, the presence of a functional AMPK orthologue in Trypanosoma cruzi.

0688 - THE GENOME OF THE SYLVATIC SPECIES ECHINOCOCCUS OLIGARTHRUS: PHYLOGENETIC HISTORY OF ECHINOCCOCUS THROUGH WHOLE GENOME VARIANTS ANALYSIS

Lucas MALDONADO (1) | Juan ARRABAL(2) | Gabriel LICHTENSTEIN(1) | Mara ROSENZVIT(1) | G OLIVEIRA(3) | Laura KAMENETZKY(1)

IMPAM (UBA-CONICET) (1); INSTITUTO DE MEDICINA TROPICAL "DR. FELIX PIFANO" (2); INSTITUTO TECNOLÓGICO VALE (3)

The first parasitic helminth genome sequence was published in 2007, since then only ~200 genomes have become available, most of them being draft assemblies. Nevertheless, despite the medical and economical global impact of helminthic infections, parasites genomes in public databases are under-represented. Recently, through an integrative approach involving morphological, genetic and ecological aspects, we have demonstrated that the complete life cycle of Echinococcus oligarthrus (Cestoda: Taeniidae) is present in South America. The neotropical E. oligarthrus parasite is capable of developing in any felid species and producing human infections. Neotropical echinococcosis is poorly understood yet and only a few cases of echinococcosis have been unequivocally identified as consequence of E. oligarthrus infections. Regarding phylogenetics, the analyses of mitogenomes and nuclear data sets have resulted in discordant topologies and there is no unequivocal taxonomic classification so far. In this work, we sequenced and

assembled the genome of E. oligarthrus that was isolated from agoutis (Dasyprocta azarae) naturally infected and performed the first comparative genomic study of a neotropical Echinococcus species. The E. oligarthrus genome assembly consisted of 86.22 Mb which showed ~90% of identity and 76.3% of coverage with Echinococcus multilocularis and contained the 85.0% of the total expected genes. Genetic variants analysis of whole genome revealed a higher rate of intraspecific genetic variability (23,301 SNPs; 0.22 SNPs/Kb) rather than for the genomes of E. multilocularis and Echinococcus canadensis G7 but lower with respect to Echinococcus granulosus G1. Comparative genomics against E. multilocularis, E. granulosus G1 and E. canadensis G7 revealed 38,762; 125,147 and 170,049 homozygous polymorphic sites respectively, indicating a higher genetic distance between E. oligarthrus and Echinococcus granulosus sensu lato species. Phylogenetic analysis using whole genome SNPs demonstrated that E. oligarthrus is one of the basal species of the genus Echinococcus and is phylogenetically closer to E. multilocularis. This work sheds light on the Echinococcus phylogeny and settles the basis to study sylvatic Echinococcus species and their developmental evolutionary features.

SAP SYMPOSIUM III

IMMUNOLOGY AND VACCINES

Chair: Karina Gómez

ROLE OF THE ARYL HYDROCARBON RECEPTOR (AHR)-INDOLEAMINE 2,3 DIOXYGENASE (IDO) AXIS IN THE REGULATION OF IMMUNITY AND IMMUNOPATHOLOGY DURING TRYPANOSOMA CRUZI INFECTION

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After acute infection with T. cruzi approximately 30 % of infected individuals develop chronic Chagas cardiomyopathy (CCC) while the rest remain asymptomatic (Asy). Although the mechanisms underlying the differential progression to CCC are still not fully understood, CCC display a more intense inflammatory response than Asy patients, who appear to have a more regulated immune response. We have demonstrated that in mice the IDO-Tryptophan (Trp)-AhR axis is associated with both the development of a strong Th1 response able to control parasite replication and its regulation by inducing, depending on the levels of AhR activation, Treg or IL-10+ producing cells. AhR can be activated by several ligands many of them being derivatives of Trp generated by IDO activity. To determine whether IDO-Trp-AhR axis is associated with CCC development, we analyzed the levels of IL-6, Trp metabolites and AhR agonists in serum samples from healthy controls, CCC and Asy patients by using ELISA, targeted LC-MS and AhR agonistic activity. CCC patients were subclassified as mild (altered ECG without congestive cardiac failure) or severe (altered ECG, congestive cardiac failure and other alterations). Decreased global AhR agonistic activity was detected in sera from infected patient as compared to healthy controls, with CCC patient's sera showing lower levels than Asy patients. Moreover, infected patients showed increased levels of circulating L-Kyn (the first Trp catabolite of IDO pathway) and IL-6 compared to healthy controls, thus associating increased IDO activity with persistent inflammation. Interestingly, sera targeted LC-MS metabolomic revealed differences in Trp metabolism pathway related with Severe CCC. Identification of a metabolic signatures for T. cruzi infected patients or the disease-stage associated metabotypes, could enable a personalized therapy and the direct follow up of drug effect in each patient-aproposition of a new field called pharmacometabolomics.

GALECTIN-8 ROLE IN TRYPANOSOMA CRUZI EXPERIMENTAL INFECTION

SUSANA LEGUIZAMÓN

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.Galectins (Gals) are animal lectins with high affinity for ß-galactosides via carbohydrates recognition domains that drives the immune response by several mechanisms. In particular, Galectin-8 (Gal-8) has been involved in the regulation of both, homeostatic and pathological processes. The role of Gal-8 on the inflammatory response remains controversial. To analyze it we infected mice with Trypanosoma cruzi, the causal agent of Chagas disease, as model of chronic inflammatory disease. Infected C57BL/6J (iWT) and Gal-8 deficient mice (iGal-8KO) were analyzed at 4 months post-infection. The absence of Gal-8 favors inflammation development in liver, skeletal muscle and heart during infection, but do not modified fibrosis levels. Fibrosis degree follows the inflammation level. An increase of neutrophils and macrophages M2-type in iGal-8KO was observed in heart, by flow cytometry analysis. Similar values of specific chemoattractans were detected in cardiac lysates and serum samples from both infected mice groups. Neutrophils increase is associated to the absence of preaparesis mechanism (stimulation of phosphatidylserine surface expression on viable leukocytes that is recognized by phagocytes and then removed) that is dependent of Gal-8 participation. Our study shows the relevance of Gal-8 as an anti-inflammatory mediator in chronic inflammatory infectious disease and the involvement of Gal-8 in preaparesis in vivo for the first time.

NEW SCHEDULES OF IMMUNOCHEMOTHERAPY FOR CUTANEOUS AND VISCERAL LEISHMANIASIS

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The present work shows data on the treatment of Leishmania (Leishmania) amazonensis-infected mice with a palladacycle complex, DPPE 1.2, associated with a recombinant cysteine proteinase from L. (L) infantumchagasi, rLdccys1, plus Propionibacterium acnes as adjuvant. Results from the treatment of L. (L.) infantumchagasi-infected hamsters with Allopurinol associated with rLdccys1 plus P. acnes are also shown. The treatment of L. (L.) amazonensis-infected BALB/c mice with DPPE 1.2 associated with rLdccys1 plus P. acnes resulted in a reduction of parasite load which was significantly higher compared to that observed in animals treated with DPPE 1.2 alone. The reduction of parasite load was followed by increase of TCD4+ and TCD8+ lymphocytes and IFN-gamma, besides the reduction of active TGF-beta in treated animals. Furthermore, there was not relapse of parasite load one month after the end of treatment in animal group treated with DPPE 1.2 associated with rLdccys1 plus P. acnescompared to that observed in mice treated with DPPE 1.2 alone. Data from the treatment of L. (L.) infantumchagasi-infected hamsters with Allopurinol and rLdccys1 alone or associated showed a significant reduction of spleen parasite load. On the other hand, the parasite reduction of group treated with Allopurinol associated with rLdccys1 plus P. acnes was not significantly different from that found in hamsters treated with Allopurinol plus rLdccys1. There was a small increase of IFN-gamma and IL-10 mRNA expression in the spleen of hamsters treated with rLdccys 1 plus Allopurinol, indicating a mixed Th1 and Th2 response in these animals. So far, our data indicated that the association with rLdccys1 potentiated the leishmanicidal action of Allopurinol and DPPE 1.2, increasing the efficacy of these compounds for the disease treatment.

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ORAL VACCINATION USING GIARDIA'S SURFACE PROTEINS

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Vaccination is one of the most successful and cost-effective health interventions known, has played a key role in reducing diseases, disabilities and deaths caused by infectious diseases since its introduction more than 200 years ago. Although parenteral immunization is generally effective in eliminating systemic infections, it often fails to establish protective responses on mucosal surfaces, where most infectious agents initiate infection. Mucosal immunization pathways, where the oral route has significant benefits, are being increasingly sought, as they provide a painless and safe administration, with induction of systemic and local protective responses. During the development of a vaccine against Giardia lamblia, containing the entire repertoire of its Variant-specific Surface Proteins (VSPs), we proposed a new oral vaccination strategy. The use of VSPs is a mechanism that can be used to generate oral vaccines due to their outstanding resistance to proteases and to changes in pH, high immunogenicity and absence of toxicity. Meanwhile, the development of vaccines composed of Virus like particles (VLPs) has demonstrated the immunogenic potential of these particulate antigens, which exhibit on their surface repetitive arrangements of epitopes requiring less doses of antigen. In a recent study we generated a novel oral vaccination platform which takes advantage of the properties of G. lamblia VSPs in combination with VLPs harboring hemagglutinin (HA) and neuraminidase (NA) from influenza virus, as model antigens. The oral vaccination of mice with only the VLPs that were pseudotyped with VSP achieved protection from live influenza challenge and from HA-expressing tumors, highlighting that this oral vaccine was able to activate the different components of the immune system, generating antibodies in mucosa that prevent the binding and invasion of pathogens, and serum antibodies that control invasive pathogens at systemic level, in addition to an effective cellular immunity.

Short talk from SAP selected abstracts:

0404 - REGULATION OF T CELL RESPONSE BY B CELLS IN TRYPANOSOMA CRUZI INFECTION

Martin SOMOZA | Juan MUCCI | Oscar CAMPETELLA

INSTITUTO DE INVESTIGACIONES BIOTECNOLÓGICAS "DR. RODOLFO UGALDE", UNIVERSIDAD NACIONAL DE SAN MARTÍ

Previous studies in mice with partial (Xid) and absolute (µMT) deficiencies have shown that B cells in the context of infection with T. cruzi are able to regulate the T response. However, little is known about the mechanism by which this regulation occurs. Consequently, we aimed to evaluate the modulation by B cells induced by trypomastigotes and which are the implications in the T response. In the present work, we demonstrate that the co-culture of naive B cells (purified by CD43 negative selection) with trypomastigotes induced the proliferation of these cells and secretion of cytokines such as IL-6 and IL-10. These effects are enhanced in the presence of a CD40 agonist. On the other hand, we observed that the co-culture with trypomastigotes (CL Brener strain) increases the expression of MHC-II and the co-stimulatory molecules CD80 and CD86, all of which are essential for the B-T cell interaction. We have observed that IL-10 secretion by B220+ cells is associated with cells previously reported as regulatory phenotypes (marginal zone and T2-MZP). Subsequently, it was

tested if the supernatant of B cells-trypomastigotes co-cultures is able to regulate the T cell response. For this, we cultured purified naive CD4+ cells in the presence of anti-CD3/CD28 and conditioned medium from the co-cultures. A decreased proliferation accompanied by a drop in IL-2 secretion was observed. In addition, a decrease in IFNg; secretion was observed together with an increase of IL-4 secretion indicating the regulation of the Th1/Th2 balance. Moreover, the co-culture of CD4+ cells together with trypomastigote-pretreated B cells induced a significant increase in the proportion of CD4+/Annexin-V+ cells, indicating an increase in their apoptosis rate. Therefore, our results support that T. cruzi trypomastigote interacts and educates B cells to modulate CD4+ responses by various mechanisms: inhibiting their proliferation, altering Th1/Th2 balance and inducing apoptosis.

SAP SYMPOSIUM IV

LINKING MOLECULAR BIOLOGY TO DISEASE TRANSMISSION

Chairs: Laura Kamenetzky / María Victoria Cardinal

NEUROBIOLOGICAL STUDIES IN TRIATOMINES. TOOLS FOR AN INTEGRATED CONTROL STRATEGY

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In despite of the efforts for triatomine control, vectorial transmission of Tripanosoma cruzi could not be prevented in Argentina to date. High insecticide resistance emerged and expanded in Triatoma infestans populations from the Gran Chaco ecoregion during the last 15 years, exposing the urgent need of an integrated vector control strategy. This approach requires the combination of both resistance management and the development of environmentally sustainable insecticidal tools, to replace or complement neurotoxicsin the near future. Our research group performs neurobiological studies in triatomine insects to address both insecticide resistance monitoring tools, and the search of neuroendocrine targets for new-generation insecticides. We have identified that the main mechanism of pyrethroid resistance in T. infestans from the Gran Chaco ecoregion is the presence of punctual mutations in the gene that encodes their target site: the voltage-gated sodium channel gene. This finding allowed us to develop sensitive and high-throughput molecular tools for resistance monitoring in field populations. Furthermore, we implemented genomic, transcriptomic and proteomic approaches combined with physiological experiments and RNAi-mediated gene silencing to study the physiological roles of understudied neuropeptide families such as Orcokinins, CCHamide and ITG-like. Interestingly, we found that these poorly studied neuropeptide families have fundamental physiological roles in the regulation of either post-embryonic development, reproduction, diuresis or stress response. The results indicate that some of the studied neuropeptides could be good targets for further studies oriented to the development of next-generation tools for the control of triatomines.

FUNCTIONAL CHARACTERIZATION OF T. CRUZI MUCINS IN THE INFECTION OF THE INSECT

MILAGROS CÁMARA

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Chagas disease, caused by the protozoan Trypanosoma cruzi, is a life-long and debilitating neglected illness of major significance to Latin America public health, for which no vaccine or adequate drugs are yet available. In this scenario, identification of novel drug targets and/or strategies aimed at controlling parasite transmission are urgently needed. In this work using a genetic and a biochemical approach we functionally characterised the role of T. cruzi surface mucins in the infection of the invertebrate host. By using *ex vivo* binding assays together with different biochemical and genetic approaches, we herein show that Gp35/50 kDa mucins, the major T. cruzi epimastigote surface glycoproteins, specifically adhere to the internal cuticle of the rectal ampoule of thetriatomine vector, a critical step leading to their differentiation into mammal-infective metacyclic forms. *Ex vivo* binding assays in the presence of chemically synthesized analogs allowed the identification of a solvent-exposed peptide and a branched, galactofuranose (Galf)-containing trisaccharide (Galfß1-4[Galpß1-6]GlcNAcAlpha) from their O-linked glycans as Gp35/50 kDa mucins adhesion determinants. Furthermore *in vivo* infection assays revealed that parasites overexpressing Gp35/50 KDa mucins presented a higher infectivity in the insect host which was also correlated with a higher number of metacyclic forms in the rectal ampoule in comparison with control lines. Overall, these results provide novel insights into the mechanisms underlying the complex T. cruzi triatomine interplay. Most importantly, and taking into account that Galf residues are not found in mammals, we propose Gp35/50 kDa mucins and/or Galf biosynthesis as appealing and novel targets for the development of T. cruzi transmission-blocking strategies.

SAP SYMPOSIUM V

DIAGNOSIS

Chairs: Silvia Longhi / Jacqueline Búa

STANDARDIZATION AND MULTI CENTER VALIDATION OF A CONSENSUS QPCR ASSAY TO MONITORE THE PARASITIC LOAD IN SKIN LESION SAMPLES OF PATIENTS WITH CUTANEOUS LEISHMANIASIS IN THE AMERICAS

OTACILIO MOREIRA

Laboratory of Molecular Biology and Endemic Diseases, Oswaldo Cruz Foundation. Brazil.

From 2001-2016, 892,846 new cases of cutaneous Leishmaniasis (CL) were reported to PAHO/WHO, distributed in 17 of the 18 endemic countries in the Americas. In the last years, the highest number of cases were registered by Brazil, Colombia, Nicaragua and Peru. The diagnosis of leishmaniasis is challenging because of the wide spectrum of clinical manifestations. For CL, skin lesions vary in severity, clinical appearance and duration. Leishmania parasites in tissue specimens are detected using microscopic examination, cultivation and/or molecular techniques. However, the lack of standardization and validation of a consensus protocol for molecular diagnosis and parasite load quantification represents a need to conduct studies which look at the development of new drugs, epidemiological surveillance and routine clinical diagnosis. Therefore, in this study, we aimed to perform the standardization and multicenter validation of a real-time PCR-based methodology for the parasite load quantification in skin lesion samples of patients with CL in the Americas. After the previous development of TaqMan assays targeting the Leishmania 18S ssrDNA, kDNA and HSP70 (FAM/NFQ-MGB), in multiplex with the human RNAse P gene (VIC/TAMRA), the analytical validation using DNA from reference strains of different Leishmania species (L. (V.) braziliensis, L. (V.) guyanensis, L. (V.) panamensis, L. (L.) amazonensis) was initiated, according to the Clinical Laboratory Improvement Amendments (CLIA) regulations for laboratory-developed tests. In addition, the TaqMan exogenous internal positive control reagents (Exo-IPC, Applied Biosytems) was used to monitoring the reproducibility in DNA extraction and absence of PCR inhibitors. Following, the multicenter validation of the qPCR assays will be performed using skin lesion samples obtained from patients with CL from Argentina, Bolivia, Brazil, Colombia, Mexico, Panama, Peru and Spain (Bolivian immigrants), totalizing 180 specimens.

INNOVATIONS IN THE DIAGNOSIS AND POST-TREATMENT MONITORING OF CHAGAS' DISEASE: FLOW CYTOMETRY IN THE SEARCH OF ANTI-AMASTIGOTE, TRYPOMASTIGOTE AND EPIMASTIGOTE- SPECIFIC ANTIBODIES IN SINGLE AND MIXED INFECTIONS

MARTA DE LANA

Federal University of Ouro Preto. Brazil

One challenge regarding treatment in the chronic phase (CP) of Chagas disease (CD) is the methodologies limitations available for detection of parasitological cure. Initially, was stablished a methodological innovation using the FC-ATE-Triplex technique for simultaneous detection of anti-Trypanosoma cruzi IgG1 (amastigote-AMA), (trypomastigote-TRIPO) and fixed (epimastigote-EPI). Treated and cured (TC) showed low reactivity and treated not treated (TNC) had high reactivity, not treated (NT) was positive and not infected (NI) was negative with all antigens. The reactivity of IgG1 anti-AMA, TRIPO and EPI in samples from TC and TNC groups in a new population, TNC group were 100 % positive with the three antigens, while 100 % samples from the TC group were negative with AMA, 71 % negative with TRIPO and 85 % negative with EPI. Following, the study evolved for to explore the correlation of T. cruzi genetic diversity with the diagnosis of this infection. Thus, Chagas-Flow-ATE technique was standardized for the universal and genotype-specific diagnosis of simple/mixed experimental T. cruzi infections, using different T. cruzi DTUs as antigen. Sera from non-infected mice and mice with single infections of T. cruzi [Colombian/TcI, CL/TcVI (hybrid) and Y/TcII] and mice with mixed infections of T. cruzi: Colombian/TcI + CL/TcVI, CL/TcVI + Y/TcII and Colombian/TcI + Y/TcII, in the acute (AP) and chronic (CP) phases were evaluated by TcI/TcVI/TcII Chagas-Flow-ATE-IgG2a. An excellent performance for the universal diagnosis of T. cruzi infection (100 % of sensibility/specificity) with a good performance for the genotype-specific diagnosis of single infections in the CP (accuracy of 69 % to discriminate TcI/TcVI/TcII infections and 94 % to differentiate TcI/TcII infections), to distinguish T. cruzi infection in AP/CP (accuracy of 81 %), to discriminate simple and mixed infections in AP/CP (accuracy of 85 and 84%, respectively) were verified.

FASCIOLA HEPATICA: AN ORGANISM THAT NEEDS AN INTEGRATIVE APPROACH

SILVANA CARNEVALE

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Fascioliasis is a foodborne, waterborne, vector-transmitted and zoonotic trematode infection. After the incubation period, three clinical phases can be distinguished in humans, corresponding to acute or invasive, latent and chronic or obstructive phases. The diagnosis is based on coproparasitological studies and

immunological methods. We have developed a molecular amplification technique based on a 117 bp-repetitive element. The use of PCR in the diagnosis of human fascioliasis has a high value in the detection of low parasite burden and early infections and allows distinguishing between current and past infections. Fascioliasis is a common disease of ruminants. Diagnosis in animals is usually carried out by parasitological analysis and molecular techniques in stool. We developed a PCR reaction based on the amplification of a fragment of the cox1 gene. Fascioliasis is transmitted by lymnaeid snails. The differentiation of species is crucial for their different ability of transmission to humans. We employ molecular techniques for snail species identification and infection detection. For the identification of species we use nuclear and mitochondrial markers in real-time PCR based on the E10-1 helix of the variable region V2 of the 18S rRNA gene; for PCR and sequencing of the ITS1 and ITS2 ribosomal nuclear markers and mitochondrial 16S and cox1 markers. For the detection of infection, we use the same amplification technique as for animals. For the study of parasite adult worms we perform molecular characterization based on nuclear markers (ITS1) for species and mitochondrial markers (cox1, nad4 and nad5) for intraspecific variation. We carry out the study of this zoonosis in areas of different altitude. We currently employ a diagnostic algorithm for humans that combines clinical, direct, immunological and molecular methodologies in order to detect infection in all clinical phases, variable epidemiological situations and new transmission patterns.

ARGENTINA CERTIFIED MALARIA FREE BY THE WHO: LABORATORY APPROACH

GERMÁN ASTUDILLO

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Malaria is caused by infection with protozoan parasites belonging to the genus Plasmodium transmitted by Anopheles female mosquitoes. Four species of Plasmodium are known to infect humans: P. falciparum, P. vivax, P. malariae and P. ovale. Among these, P. falciparum is the most dangerous species and responsible for vast majority of deaths. P. vivax accounts for more than half of all malaria cases outside sub-Saharan Africa and is the more prevalent species in countries that are optimal candidates for disease elimination. In Argentina, since 2010 there are no reports of indigenous malaria cases, and in 2019, WHO certified the elimination of this parasitic disease in the country, thus entering the group of malaria-free countries. During the certification process it was essential to ensure good diagnostic capabilities in the national territory. For this purpose, workshops were held for malaria microscopy training and certification of the competence of microscopists, accordingly to WHO standards. They began in Salta, Jujuy and Misiones. The last workshop was in Buenos Aires city, with the participation of delegates responsible for malaria diagnosis the remaining Argentine provinces. The total number of trained microscopists was 64, 47 of which were certified. There is actually at least one trained and certified agent for malaria diagnosis in each of the 24 jurisdictions that constitute the national network. The Infectious Diseases Hospital "Dr. Francisco J. Muñiz" is the reference center for treatment and diagnosis of malaria in Buenos Aires city, with a laboratory service that provides quality-assured parasitological diagnosis of the disease, based on microscopic examination of thick and thin blood smears. In order to ensure accurate results, the laboratory service participates in direct and indirect quality control programmes. This laboratory service is responsible for coordinating the servicing at the internal network, the confirmatory testing and the training of human resources in Buenos Aires city.

THE SEROLOGICAL ANTIBODY REPERTOIRE IN CHAGAS DISEASE

FERNÁN AGÜERO

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During an infection the immune system produces antibodies against pathogens. With time, the immune repertoires of infected individuals become specific to the history of infections and thus represent a rich source of diagnostic markers. A comprehensive description of the specificities of global antibody repertoires has been hindered by the lack of powerful tools. In Chagas Disease the immune response is directed to Trypanosoma cruzi, a protozoan parasite that evades immune mediated elimination and mounts long-lasting chronic infections. Although available diagnostic tests give satisfactory results in most cases, there is currently no gold standard for diagnosis of infection and discordant results remain a possible cause of undetected cases. Here, using state of the art high-density peptide arrays we examined the global human antibody repertoire developed by Chagas Disease patients. Peptide arrays displaying 2.8 million unique peptides from the complete proteomes of T. cruzistrains CL-Brener (DTU TcVI, 19,668 proteins) and Sylvio X10 (DTU TcI, 10,832 proteins)were assayed with 72 serum samples from infected subjects from Argentina, Bolivia, Brazil, Colombia, Mexico and the US, as well as negative samples from the same regions. A cascading screening strategy was designed to identify the antigenic core of these genomes using pooled samples, and in a secondary screening assay individual serumsamples to study seroprevalence of identified antigens and fine map all epitopes. Our analysis uncovered >4,500 antigens and >22,000 antibody-binding regions,

delineating both public (shared) Chagas antigens as well as private (non-shared) individual anti-T. cruzi responses. To our knowledge, this the first and largest collection of Chagas Disease antigens and epitopes described to date. This dataset will enable the study of the human antibody repertoire in Chagas Disease at an unprecedented depth and granularity, while also providing a rich dataset of serological biomarkers.

Short talks from SAB selected abstracts:

0355 - EGPE CELLS, ANALYTICAL LABORATORY SOURCE FOR ECHINOCOCCUS GRANULOSUS ANTIGENS.

Andrea MAGLIOCO (1) | Facundo A AGÜERO(1) | Alejandra JUÁREZ VALDEZ(2) | Jorge GENTILE(3) | Claudia HERNÁNDEZ(3) | Oscar JENSEN(4) | María Laura GERTISER(4) | Elizabeth SÁNCHEZ ROMANÍ(5) | Gabriela CANZIANI(6) | Alicia Graciela FUCHS(7)

UNIVERSIDAD ABIERTA INTERAMERICANA; CONICET (1); UNIVERSIDAD ABIERTA INTERAMERICANA (2); HOSPITAL MUNICIPAL RAMÓN SANTAMARINA (3); CENTRO DE ZOONOSIS (4); INSTITUTO NACIONAL DE SALUD (5); INSTITUTO DE CIENCIA Y TECNOLOGÍA "DR. CESAR MILSTEIN" ; CONICET (6); UNIVERSIDAD ABIERTA INTERAMERICANA/INP FATALA-CHABEN, ANLIS-MALBRÁN (7)

Cystic echinococcosis (CE) is a zoonoses worldwide distributed produced by Echinococcus granulosus sensu lato. In Argentina, CE is an endemic disease with active dissemination reporting more than 450 human cases per year. Disease diagnosis is performed by ultrasound. Serology tests for diagnosis, population screening and patients follow-up have poor or variable sensitivity and specificity. A cell line from E. granulosus G1 (EGPE cells) obtained in our laboratory (Echeverría et al, 2010) expresses antigen B and protein extracts from EGPE in different culture stages were recognized by sera from CE patients with high sensitivity by Western blot. The aim of this study was to identify, by proteomics, the CE antigens present in EGPE from 20-day-old culture. We tested sera from 34 CE patients (Tandil, Chubut and Perú), 21 healthy donors (Tandil), 5 cysticercosis (Perú) and 3 fascioliasis (Perú). Protocols were approved by the UAI Ethical Committee. Protein extracts were obtained with ice-cold lysis buffer, after 20 days of cell culture. Sera reactivity was detected with AP- goat anti-human IgG and BCIP/NBT, bands were analyzed with GelAnalyzer software. Protein extracts were separated through Sephacryl S-200 and further by affinity column performed with a pool of antibodies from: CE patients sera (CE column) or sera from patients with other parasitoses (OP column). Eluted proteins from affinity columns were identified by proteomics (CEQUIBIEM - FCEyN, UBA). CE patients sera recognized bands from 12 to 94 kDa, few bands were also recognized by sera from healthy donors or from patients with other parasitoses. Elution profile of the gel filtration column showed three peaks, all of them recognized by CE patients´ sera. Proteins obtained from CE column allowed the identification of 15 proteins from E. granulosus. No detectable proteins were identified from OP column. EGPE cells can be used as a laboratory tool for identification of epitopes involved in the immune response.

0915 - SYNTHESIS AND EVALUATION OF NEO-GLYCOCONJUGATES AS TOOLS FOR THE SEROLOGICAL DIAGNOSIS OF CHAGAS DISEASE.

Ivana DUCREY (1) | María de Los Milagros CÁMARA(1) | Virginia BALOUZ(1) | Rosana LOPEZ(2) | María Eugenia GIORGI(2) | Linda TORO MELGAREJO(2) | Fernán AGÜERO(1) | Rosa M. DE LEDERKREMER(2) | Carla MARINO(2) | Carlos A. BUSCAGLIA(1)

INSTITUTO DE INVESTIGACIONES BIOTECNOLÓGICAS 'DR RODOLFO UGALDE' (IIBIO, UNSAM-CONICET) (1); UBA-CONICET (CIHIDECAR). FACULTAD DE CIENCIAS EXACTAS Y NATURALES. DEPARTAMENTO DE QUÍMICA ORGÁNICA (2)

The immunodominant glycotope alfa-Galp-(1-->3)-ß-Galp-(1-->4)-GlcNAc (also known as alfa-Gal), expressed in the mucins of the infective trypomastigote stage of Trypanosoma cruzi has been proposed for multiple clinical applications, from xenotransplantation or cancer vaccinology to serodiagnosis of protozoan caused diseases, including Chagas disease. However, methodological limitations have precluded its consistent clinical. It was previously shown that the trisaccharide analogue to alfa-Gal, with Glc in the reducing end, was as efficient as the natural trisaccharide for recognition of antibodies to alfa-Gal elicited during T. cruzi infections. We describe here the synthesis of alfa-Galp-(1-->3)-ß-Galp-(1-->4)-Glcp and alfa-Galp-(1-->3)-ß-Galp both functionalized as the 6-aminohexyl glycosides, and their conjugation to BSA. For the synthesis of the trisaccharide a lactose derivative, which already has the ß-Galp-(1-->4)-ß-Glcp motif, was used as starting material. For conjugation, the squarate method was chosen. The synthesized neoglycoconjugates were structurally characterized by biochemical and mass spectrometry studies and antigenically validated by conventional ELISA immunoassays. Both compounds were specifically recognized by serum samples of T. cruzi-infected patients. Moreover, competition assays allowed us to map the disaccharide alfa-Galp-(1-->3)-ß-Gal as the glycotope recognized by anti-alfa-Gal antibodies, thereby supporting the 'antigenic mimicry' between alfa-Galp-(1-->3)-ß-Galp-(1-->4)-Glcp and the natural alfa-Gal structure. The disaccharide was next conjugated to immunodominant peptides present in selected T. cruzi antigens. Further immunoassays using unconjugated peptides and 6-aminohexyl alfa-Galp-(1-->3)-ß-Galp as controls indicated that it is possible to develop bivalent serological reagents, able to display peptidic- and carbohydrate-based epitopes. Overall, these results indicate that our neo-glycoconjugates provide suitable, cost-effective and much needed tools for the improvement of currently used Chagas disease diagnostic applications.

NANOMED-AR SYMPOSIUM I

NANOMEDICINE I

Chair: Romina Glisoni

CHANGING THE IDENTITY OF DRUG NANOCARRIERS: FROM SYNTHESIS TO LIVING SYSTEMS

CARLA GIACOMELLI

Department of Physicochemistry. Faculty of Chemical Sciences. National University of Córdoba. Institute of Research in Physicochemistry of Córdoba (INFIQC). CONICET-UNC. Córdoba, Argentina.

The surface properties of drug nanocarriers are drastically and rapidly modified when administered to a living system. This bio-transformation is strongly controlled by the different components of the biological fluids, especially plasma proteins. In fact, the self-assembly in mono or multilayers of these proteins on

the nanocarriers surface has been described as a protein corona since 2007. However, this concept is not new since it dates back to the pioneering works of the 50s and 60s, where it was described the essential role of adsorbed proteins on the interactions and biological response of different materials. Since the protein corona completely transforms the original interfacial properties of the nanocarriers (synthetic identity) into a new biological identity, it can cause unexpected or adverse responses, such as increased cytotoxicity and immunogenicity, altered endocytic pathways to target different intracellular locations. Consequently, the bio-transformation of the nanocarriers by the formation of the protein corona unpredictably modulates their pharmacological and toxicological profiles. The formation and stabilization of the protein corona mainly depends on two types of interactions: nanocarrier-protein and protein-protein. Both interactions not only control the formation of the protein corona, but also the cellular response of the nanocarriers biological identity. The purpose of this presentation is to discuss different features of this bio-transformation, particularly related to the composition, structure and interaction network of the protein corona on drug nanocarriers. The emphasis of the discussion will focus on nanocarrier-protein and protein-protein interactions and their effect on the response of model cellular systems and intact cells.

NANOPARTICLES IN MOLECULAR IMAGING

PABLO CABRAL

Center for Nuclear Research (CIN). Science Faculty. Montevideo, Uruguay.

The advent of molecular imaging has led to unprecedented progress in non-invasive visualization, characterization and quantification of biological processes at molecular and cellular levels. Multifunctional nanoparticles for multimodal images and theragnosis are being developed. Nuclear medicine imaging, particularly positron emission tomography (PET) and single photon emission computed tomography (SPECT), has played a crucial role in the development of molecular imaging. With the advantage of high sensitivity, PET and SPECT images can offer physiological, metabolic and functional information. The hybrid imaging techniques developed PET/MR, PET/CT, SPECT/CT and SPECT/Fluorescence have gained wide acceptance as powerful tools in basic research and clinical applications. However, few new imaging agents have been provided for clinical purposes during the last decade, in particular, multifunctional radiolabeled agents for these hybrid imaging techniques. Therefore, current research efforts mainly focus on the development of multifunctional image probes in this field.

NEBULIZABLE ARCHAEOLIPID NANOVESICLES FOR DRUG DELIVERY TO THE LUNGS

JULIA ALTUBE

Center for Research and Development in Nanomedicines, National University of Quilmes. Buenos Aires, Argentina.

To improve safety and efficacy in the treatment of lung diseases like asthma and lung infection, medications are routinely inhaled rather than administered systemically. Inhaled medication can achieve the same effective concentration in lungs at doses lower than by oral or intravenous routes. Moreover, the efficacy of inhaled medication can be enhanced by performing targeted delivery to selected body sites, with tailored nanoparticulate carriers. Inhaled nanoparticles preferentially accumulate in lungs, thus limiting drugs penetration into the bloodstream, consequently decreasing adverse systemic side effects. Inhaled nanoparticulate medication, however, needs to overcome some critical drawbacks. Firstly, nanoparticles must to withstand the physical stress produced by the nebulizer forces, and to surpass the barriers imposed by the lung morphology. Secondly, nanoparticles must avoid the trapping into the mucociliar escalator, being free to cross the surfactant layer covering the alveolar epithelium. Thirdly, safety issues related with lung epithelium integrity must be evaluated. This presentation will describe the performance of nebulizable nanovesicles, as delivery systems for antibiotics or anti-inflammatory drugs to the lungs, on *in vitro* models of mucus and surfactant layers and of inflamed alveolar epithelium in an air-liquid interface. The advantages of these novel nebulizable nanovesicles made of archaeolipids extracted from the cellular membrane of archaebacterias will be analyzed in comparison with conventional inhaled liposomes.

Short talks of Nanomedicine selected posters:

0757 - DENDRITIC CELL TARGETING IN CULTURED FISH IN ARGENTINA FOR VACCINE DEVELOPMENT

Federica GHERSA (1) | Ivana SORIA(2) | Valeria QUATTROCCHI(2) | Mariela GAMMELLA(2) | Cecilia Ana LANGELLOTTI(2) | Patricia ZAMORANO(2) | Carlos Alejandro RAUQUE(1) | Juan Sebastina PAPPALARDO(3)

INSTITUTO DE INVESTIGACIONES ES BIODIVERSIDAD Y MEDIOAMBIENTE (INIBIOMA-CONICET) (1); INSTITUTO DE VIROLOGÍA E INNOVACIONES TECNOLÓGICAS (IVIT, CONICET-INTA) (2); INSTITUTO FORESTAL AGROPECUARIO (IFAB- INTA CONICET) (3)

Aquaculture is a fast-developing sector in the food industry worldwide. In Argentina two main species present an important economic value, these are Pacú (Piaractus mesopotamicus, Pm) and Rainbow Trout (Oncorhynchus mykiss, Om). Due to stress and changing environmental conditions cultured fish are exposed to, the use of antibiotics has become a common solution for

treatment and avoidance of disease. This practice presents several problems such as overdose, contamination and resistance generation. The development of effective and affordable vaccines is necessary for aquaculture in order to produce safe products for consumption and the environment. This work focuses on the evaluation of a species unspecific nanovaccine platform in Pm and Om, composed of liposomes decorated with a1,2-mannobiose, a specific disaccharide that targets DC-SIGN receptor, mainly expressed on dendritic cells (DC). We cultured DC obtained from head kidney (HK), of Pm and Om in complete D-MEM (10 % FBS) for 1, 7 and 14 days at room temperature (RT) in order to obtain non-adherent cells, enriched in DC. These cells where later incubated for 30 m or 12 h at RT in D-MEM without FBS with undecorated liposomes for unspecific cell targeting (plain-L), a1,2-mannobiose decorated (Mana-L) and DOTAP (DOTAP-L) liposomes as a positive control, all marked with rhodamine. Prior liposome formulation and characterization with Z sizer was done. Incubation was stopped adding complete D-MEM. Cells were washed and fixed with PFA at 0.02 % w/v final concentration and then analyzed by flow cytometry. Results were statistically analyzed with two-way ANOVA followed by Bonferroni's Test. Results demonstrate that HK cultures at day 7 and 14 are enriched in DC-SIGN expressing cells, and Mana-L targets specifically these cells (***p<0.0001). These preliminary results indicate that the nanovaccine platform would be efficient in targeting DC, therefore could be an important tool in aquaculture vaccine development.

0636 - TOPICAL CO-DELIVERY OF VITAMIN D3 (VD3) AND BACTERIORUBERIN FOR PSORIASIS TREATMENT: FORMULATION AND IN VITRO STUDIES

Yamila SIMIONI | Priscila SCHILRREFF | Eder ROMERO | María José MORILLA

UNIVERSIDAD NACIONAL DE QUILMES

Psoriasis, a chronic immune-mediated disease, is characterized by excessive growth and abnormal differentiation of keratinocytes, angiogenesis, infiltration of neutrophils and inflammatory cells and cytokine production. Calcipotriol (vitamin D3 analogue) inhibits dendritic cells and IL-2 and IL-6 production by epidermal T cells, suppressing epidermal proliferation and differentiation. On the other hand, bacterioruberin (BR), the major C50 carotenoid found in halophilic Archaea, is one of the highest efficient natural antioxidants. However, the high hydrophobicity of VD3 and BR, and their susceptibility to chemical degradation impair its topical administration. The objective of this work was to combine the high antioxidant activity of BR with VD3 in one nanoparticle (VD3-BR-NP) to be administered by the topical route. The BR extract (350 µg BR/g dry Halorubrum tebenquichense culture) showed the typical three-fingered profile (460, 490 and 525 nm) spectrum and an inhibitory concentration providing 50 % reduction of the DPPH radical (IC50) of 21 µg/ml. NP made of a core of compritol and BR extract (50, 75 and 100 % of BR) covered by a shell of polar archaeolipids from H. tebenquichense and Tween 80 (2; 2; 1.2; 3 % w/w) were prepared by homogenization-ultrasonication. The best formulation (50 % BR) in terms of smaller size (67 nm), high colloidal stability (6 months), lower cytotoxicity (5 % at 0.02 mg/ml LN) and high uptake by macrophages and keratinocytes was loaded with VD3. Nanosized (75 ± 2 nm), monodisperse (polydispersity index 0.496 ± 0.053), negative potential (-36 ± 0.3 mV), 6.5 mg/ml VD3 and IC50 to 4,8 µg/ml (activity was potentiated by co-encapsulation) VD3-BR-NP were obtained. Effectiveness of VD3-BR-NP was assessed on an in vitro psoriatic model and compared with conventional NP (lacking BR). VD3-BR-NP, and no conventional NP, showed anti-inflammatory and antioxidant activity. Concluding, VD3-BR-NP deserves further studies as promising topical option for treatment of psoriasis.

NANOMED-AR SYMPOSIUM II

NANOMEDICINE II

Chair: Eder Romero

BIOPOLYMER NANOCARRIERS FOR ONCOLOGICAL DRUGS: SYNTHESIS, CHARACTERIZATION AND IN VITRO AND IN VIVO EVALUATION

VERA ÁLVAREZ

Research Institute in Materials Science and Technology (INTEMA)- CONICET. Mar del Plata, Buenos Aires, Argentina.

Nanoparticles (NPs) are submicron size entities which can be made from a wide variety of polymers. The existing anticancer agents usually do not show selectivity between cancerous and normal cells leading to systemic toxicity and adverse effects which limits the maximum permissible dose to be applied. This is the case of tamoxifen (TMX), fundamental drug for the treatment of breast cancer conforming to the WHO. This selective estrogen receptor modulator has been the Trojan horse for the endocrine treatment of estrogen-receptor-positive breast cancer. Depending upon the dose and the concentration has several side effects as endometrial carcinoma for postmenopausal women, liver cancer, venous thrombosis, pulmonary emboli and an ocular effect includes retinopathy and corneal opacities. Another interesting drug to treat breath cancer is desmopressin (DDAVP); an innovative peptide in cancer treatment. Considering biopolymers capability for high loading drugs and to modulate drug release, this work studies the physicochemical and biomedical properties of PLGA nanoparticulated systems that could carry TMX or DDAVP in order to improve its therapeutic effect. TMX and DVAP loaded PLGA NPs were fully characterized by scanning electron microscopy (SEM), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and Fourier transform infrared spectroscopy (FTIR). Additionally, encapsulation efficiency and in vitro cell viability assay were performed. TMX and DDAVP loaded PLGA NPs were successfully obtained showing an average size around 200 nm. Morphologically, a quasi-spherical regular shape was observed for all of the PLGA NPs, which demonstrating that the selected experimental conditions for each case allow control of the formation of the polymeric particles, their dimensions and their properties. Suitable encapsulation efficiencies for both active principles were achieved whereas in vitro an in vivo test give promising results. The capitalization of the therapeutic effect as a result of the fulfillment of the

proposed biotechnology objectives was achieved in both cases.

PROTEORAL - THE EFFECTIVE SOLUTION OF ORAL PROTEIN THERAPIES

JOAQUÍN ANTONIO GONZÁLEZ

PANARUM SAS. Argentina.

Proteoral® is the universal innate oral delivery system of the solid and liquid oral formulation of protein nanoparticles totally effective in oral delivery of therapeutic proteins to the systemic circulation. Proteoral® combines biotechnology, nanotechnology and pharmaceutical technology and generates an optimum disruptive change in therapy with proteins. Proteoral is a recombinant fusion protein of 92 kDa molecular weight produced by biotechnology manufacture. Proteoral has the intestinal absorption activity with the essential secretion signal located in the C-terminal with a number of glycine-rich tandem repeats in the consensus sequence responsible for the enhancing of transepithelial paracellular secretion efficiency in the intestine. The protein nanoparticle synthesis method starts from buffer solutions containing the Proteoral a therapeutic protein, a gastro-resistant polymer, and a stabilizing hydrophilic polymer. The protein nanoparticles, with an average size between 250 and 350 nm, are dispersed in an aqueous solution and they act as a vehicle carrying the therapeutic protein. In the oral administration, the protein nanoparticles are formulated using liquid vehicles or solid vehicles. We present the carrier Proteoral is a totally effective strategy in therapy of oral administration of Follicle-stimulating Hormone in an optimized oral formulation that transports the therapeutic protein and releases to the circulation system with biological activity by transepithelial paracellular passage.

ADVANCES ON BIO-NANOMATERIALS BASED ON SILICA AND MAGNETIC TARGETING

MARIELA AGOTEGARAY

Southern Chemistry Institute (INQUISUR) - CONICET. Bahía Blanca, Buenos Aires, Argentina.

Conventional treatments for various pathologies currently find limitations related to the low bioavailability of drugs used in the blank site and the adverse effects associated with them. Nanotechnology allows us to create platforms with biocompatible materials for anchoring drugs and biomolecules that can target the specific organ or tissue. Silica nanomaterials provide a versatile surface, added to the fact that from the implementation of specific synthesis methods, self-luminescent materials can be achieved. Thus, it is possible the conjugation in a same material of high value theranostic properties for applications not only in biomedicine but also in the field of research to elucidate the mechanisms by which nanomaterials enter the cell and are metabolized. Another tool for addressing nanomaterials to specific sites in the body is the development magnetic nanoparticles. They consist of an iron oxide core with superparamagnetic properties coated with biocompatible materials that allow the incorporation of drugs. The application of an external magnetic field allows to concentrate the nanoparticles at the desired site. The main pathologies to which we apply this nanotechnology in our laboratory are based on inflammatory, tumor and bone disease. We have developed various platforms of silica and magnetic nanoparticles evaluating the effect of biocompatible coatings and their physicochemical properties. We perform studies on biocompatibility in vitro on vascular and bone cells and on in vivo invertebrate models such as C. elegans as well as on murine mammalian models, achieving magnetic in vivo targeting to bone system in mice. In this way, the great applicability of these nanosystems in the biomedical field is demonstrated in order to improve the conventional therapies of different pathologies with great social impact.

Short talks of Nanomedicine selected posters:

0669 - STUDY OF ROUTES OF ADMINISTRATION OF ALBUMIN NANOPARTICLES FOR THE TREATMENT OF RETINAL PATHOLOGIES

Carlos Gastón VALVERDE (1) | Mariano GRASSELLI(2) | Pablo CHIARADIA(3) | Martin RADRIZZANI(4) | Alejandro BERRA(1) | Ana Vanesa TORBIDONI(1)

LAB. TRASLACIONAL DE INMUNOPATOLOGÍA Y OFTALMOLOGÍA - DEPTO. DE PATOLOGÍA - FAC. MEDICINA - UBA (1); LABORATORIO DE MATERIALES BIOTECNOLÓGICOS, UNIVERSIDAD NACIONAL DE QUILMES-IMBICE (CONICET) (2); HOSPITAL DE CLINICAS, FAC MEDICINA UBA (3); LABORATORIO DE NEURO Y CITOGENÉTICA MOLECULAR- ESC. DE CIENCIA Y TECNOLOGÍA - UNSAM (4)

Retinal pathologies are treated with therapeutic agents that are administered invasively and periodically. Nanoparticles (NPs) could be a new way of performing therapies directed to the retina. the aim of this study was to evaluate different routes of administration of NPs to reach the retina and to analyze the response of the retinal pigment epithelium (RPE) to the presence of NPs. We evaluated NPs of 20 and 100 nm in diameter of human albumin associated with a quantum dot. We inoculated the 20 nm NPs through intravitreal (IV), subconjunctival (SCj), and suprachoroidal (SC) injections and the NPs of 100 nm only through

SCj injections. The distribution was observed at 3 and 24 hours after the inoculation in wholemounts of retinas and choroids in a fluorescence microscope. In addition, the RPE cell line, ARPE-19, was incubated with NPs for 3 and 24 h, and were observed under fluorescence microscopy. In the IV inoculations, the NPs were detected mainly in the retina and vitreous humor, both at 3 and 24 h. After 24h, we observed inflammatory cells containing NPs. The SC inoculations showed NPs in the choroid and in the retina mainly in regions associated with blood vessels (after 3 h), persisting in inflammatory cells after 24 h. In SCj inoculations, NPs of both 20 and 100 nm were detected in the choroid (after 3 and 24 h) and in the RPE (after 24 h). In the ARPE-19 cells, we observed presence of NPs in the cytoplasm and also in the extracellular matrix, mainly after 24 h of incubation, for both NPs. We concluded that NPs compose of protein showed high biocompatibility, since we did not observed aggregation in none of the tissues analyzed. From the *in vitro* studies we can infer that the ARPE-19 cells could endocyte the NPs. From the *in vivo* studies the most promising route of administration would be the SCj, because of the NPs reach the target tissues being less invasive than the others studied.

0082 - NANOFILMS OF ADSORBED THYMOL FORMED ON TITANIUM SURFACES FOR BIOMEDICAL APPLICATIONS. ANTIMICROBIAL ACTIVITY AND BIOCOMPATIBILITY

Ariel GONZALEZ | Alejandro MIÑAN | Claudia Alejandra GRILLO | Patricia Laura SCHILARDI | Mónica Alicia FERNÁNDEZ LORENZO

INSTITUTO DE INVESTIGACIONES FISICOQUÍMICAS TEÓRICAS Y APLICADAS (INIFTA); CONICET-UNLP

Titanium (Ti) and its alloys are widely used in the construction of permanent orthopedic and cardiovascular implants. However, one of the most frequent causes of failures are bacterial infections by Staphylococcus aureus. This is aggravated by the abusive use of antibiotics that generate microbial resistance to conventional therapies. As a consequence, new antimicrobial nanotechnologies (AMN) emerge as promising alternatives to prevent prosthetic infections. The aim of this work was to evaluate the antimicrobial effect of an innovative AMN: thymol (TOH, phenolic phytocompound) nanofilms adsorbed on Ti (NPTOH-Ti) against S. aureus. The biocompatibility was also determined using preosteoblast cells (MC3T3-E1). To that end, 1 cm diameter grade 2 Ti discs were used and TOH was adsorbed onto their surface by 2 h immersion in 0.1 M TOH acid solution. NPTOH-Ti was detected by infrared spectroscopy (FTIR-ATR). The antibiofilm activity of NPTOH-Ti and Ti (control) was determined by immersing the metal discs in a suspension of S. aureus (10^8 bacteria/ml) for 3 h. Subsequently, the number of bacteria adhered on the discs was caunted after sonication by colony forming unit (CFU). In addition, Live/Dead (Invitrogen) staining was used to determine if the adhered bacteria were alive or dead. Finally, biocompatibility of NPTOH-Ti and Ti was assessed by staining the preosteoblast cells with acridine orange. The results showed that NPTOH-Ti has effective anti-biofilm properties. On the one hand, viable bacteria were not observed by the plating count method and Live/Dead staining exhibited only dead (red) bacteria on the surface. On the other hand, control Ti revealed $4 \pm 0.5 \times 10^5$ adhered bacteria that were mostly (95 %) alive (green). In addition, NPTOH-Ti and Ti showed similar cell adhesion and growth (107 ± 12 and 100 ± 16 % respectively; $p>0.05$). It was concluded that NPTOH-Ti are biocompatible and have anti-biofilm properties which make them promising to prevent prosthetic infections.

SAFE SYMPOSIUM I

STRATEGIES TO IMPROVE ANTIMICROBIAL DRUG BIOAVAILABILITY

Chairs: María Celina Elissondo / Hector Alejandro Serra

THE CHALLENGE OF IMPROVING ANTIMICROBIAL THERAPY WITH CURRENTLY AVAILABLE DRUGS

VIRGINIA AIASSA

Research and Development Unit in Pharmaceutical Technology (UNITEFA) - CONICET. Department of Pharmaceutical Sciences. Faculty of Chemical Sciences - National University of Córdoba. UNC. Bionanotechnology Innovation Laboratory- LINBIO. Córdoba, Argentina.

Nowadays antimicrobial resistance (ATMR) is a global health crisis. At the current rate of emergence and spread of ATMR, annual loss of life is expected to reach 10 million deaths by 2050 with a very high economic cost. Combating ATMR requires a multifaceted approach that facilitates sustainable and equitable use of antimicrobials (ATM), thwarts the spread of infectious disease, preserves existing ATM therapies and fosters innovation of new therapies and diagnostic tools. A critical component of the ATMR solution is the development of novel ATM drugs to cover the diminishing effectiveness of existing ATM that are relied on every day for essential clinical care. However, due to a variety of inherent market failures, the present business model for ATM has not adequately responded to the growing demand for innovation. First, the success rates of moving an ATM through the different clinical phases suggests that of the ~ 39 drugs in development, only 13 will translate into a market. Second, most new ATM do not have the novel mechanisms of action or novelty in chemical matter targeting well validated targets, which are necessary to significantly ensure effectiveness against resistant pathogens. Numerous of the products in the pipeline are redevelopments or combinations of existing ATM. In third place, many of these drugs do not target the highest priority ATM resistant pathogens. Therefore, scientific and clinical advancements in ATM development are inherently challenging, particularly relative to other therapeutic fields. For this reason, our research group is choosing to focus development efforts on alternatives such as the preparing and characterizing supramolecular systems and new nanomaterials for the treatment of infectious diseases.

USE OF CYCLODEXTRINS TO IMPROVE THE BIOAVAILABILITY OF ANTIMICROBIAL DRUGS

ARIANA ZOPPI

Research and Development Unit in Pharmaceutical Technology (UNITEFA) - CONICET. Department of Pharmaceutical Sciences. Faculty of Chemical Sciences- National University of Córdoba. UNC. Bionanotechnology Innovation Laboratory -LINBIO. Córdoba, Argentina.

For many years, researchers have worked with supramolecular structures involving binary inclusion complexes with cyclodextrins (CDs). CDs are cyclic oligosaccharides used for the improvement of water-solubility, stability and bioavailability of drugs. More recently, the formation of multicomponent complexes with CD (i.e. the inclusion of a third auxiliary substance) has become more frequent due to of the versatility and further synergistic optimization possibilities that can be reached. In this context, an interesting approach in which we have been working is the development of drug delivery systems containing an ATM drug in combination with CDs and auxiliary substances with recognized antibiofilm properties. The structural and energetic features driving the inclusion of guest molecules within the CD cavity is a complex phenomenon, involving a dynamic network of intermolecular interactions and solvent effects, among other. Consequently, in order to form efficient multicomponent inclusion systems, it is necessary to identify auxiliary agents able to successfully interact with the binary complex, which in turn implies a structural complementarity. In this way, a wide range of chemicals have been studies as potential third compounds. Among them, essential aminoacids have gained our particular interest due to their varied physiochemical and structural properties, potential to interact binary systems, biocompatibility and recognized capability to act as antibiofilm substances. Therefore, our interest is focused towards the understanding and rationalization at the molecular level, of the physicochemical and structural properties governing the formation of multicomponent cyclodextrin complexes. This allows us to screen auxiliary agents able to optimize the complexation behavior, and result in systems with improved bioavailability and antimicrobial properties.

DEVELOPMENT AND BIOCOMPATIBILITY OF ANTIMICROBIAL NANOMATERIALS

MARÍA JAZMÍN SILVERO COMPAGNUCCI

Multidisciplinary Institute of Plant Biology (IMBiV) - CONICET. Department of Pharmaceutical Sciences. Faculty of Chemical Sciences- National University of Córdoba. UNC. Bionanotechnology Innovation Laboratory - LINBIO. Córdoba, Argentina.

New biocompatible nanomaterials can be designed to have antimicrobial properties. Those could be used as alternative to antibiotics in the treatment of multiresistant infections. An important step before its clinical application is the evaluation of their biointeractions, biodistribution and bioavailability. In example, one of the strategies involving metal nanoparticles is to use visible light to excite their plasmon and achieve a photothermal effect, what is called “Photodynamic Antimicrobial Therapy” (PACT). Gold nanoparticles are often chosen for PACT because they are considered non-toxic to mammals; however, their biocompatibility is directly link to their selectivity. In order to make them more selective for prokaryotic cells, gold nanoparticles are synthesized and stabilized by amoxicillin. This ATM guides the nanoparticle towards the bacterial wall. An in vivo study shows that they do not produce any alteration in blood cells, and most of them (spheres below 50 nm diameter) are excreted in urine after just 5 hours. Interesting interactions inside internal organs are shown through TEM images. Minor alterations found in tissue cells are possibly due to the amoxicillin toxicity; therefore, the next generation of gold nanoparticles for PACT are synthesized employing a natural ATM peptide: casein. These nanoparticles are able to inhibit the growth of pathogenic strains after only 15 min with green LEDs. They were thought to be used as topical ATM gel, due to casein gelation property. Their skin permeability study shows that they penetrate the skin and are mostly eliminated in the urine, due to their size and shape (spheres of 10 ± 2 nm diameter). The biocompatibility of other nanomaterials, like titanium dioxide nanoparticles (employed in cosmetics), zinc oxide nanoparticles (for dental treatments) and silver nanoparticles (added to disinfectants to increase their efficiency) are currently being investigated and presented in this work.

SAFE SYMPOSIUM II

EXPERIMENTAL PHARMACOLOGY II

Chair: Ana Genaro

CHRONIC PAIN AND DRUGS, AN APPROACH TO THE MOLECULAR MECHANISM INVOLVED

ALEXIS MEJÍAS DELAMANO, HÉCTOR SERRA

First Cathedra of Pharmacology. Medical Faculty - UBA. Buenos Aires, Argentina.

For along time chronic pain was associated with neuropathic pain. However, newpathophysiological knowledge classifies pain according to its origin innociceptive (triggered by a noxious stimuli), neuropathic (due to neuraldamage) and nociplastic (caused by neurogenic inflammation). Obviously, due toneuroglial plasticity any of these forms can intermingle and become chronic.

The aim of this lectureis to explain the neuroplastic mechanisms involved in the genesis and maintenance of chronic pain of any origin and provide the basis for therational use of analgesics, drugs that mitigate pain. Such is the case of "theformer number one pain killers" opiates, which gave way to other drugs"best fitted" for chronic pain and called adjuvants analgesics sincetheir primary indication was not pain (for example, antiepileptics, antidepressants, corticosteroids, etc.). Precisely, gabapentinoids (gabapentinand pregabalin) far from their primary development as antiepileptics are nowconsidered analgesics in their own right in neuropathic forms of pain.

SAB SYMPOSIUM I

SYMPOSIUM SOCIETIES OF BIOLOGY

Chairs: Pablo Cetica / Leandro Miranda

SCAVENGING OF INTRACELLULARLY PRODUCED HOCL WITH RESVERATROL PROTECTS INSULIN SIGNALING IN ADIPOCYTES

DARÍO RAMÍREZ

Biology Society of Cuyo (SBCuyo). Laboratory of Experimental and Translational Medicine-IMIBIO-SL-CONICET. San Luis, Argentina.

Resveratrol is a cell permeable *trans*-stilbene derivative that can scavenge intracellularly produced HOCl (*J. Biol. Chem*, 285:20062/71). Our diet-induced obesity B6-mouse model showed that epididimal AT expresses myeloperoxidase (MPO) in adipose tissue macrophages (ATM) that also express M1-pro-inflammatory markers (e.g., IL-6, iNOS). MPO expression was observed in ATM located in the typical crown-like structures; and also inside adipocytes. Tissue fractionation showed MPO mRNA in the ATM, but not in the stromal-vascular fraction. Treatment of these isolated adipocytes with H_2O_2 blocked glucose uptake. Based on these findings, we hypothesized that MPO interferes with AT insulin signaling by producing HOCl and causing oxidation of intracellular components involved in insulin-triggered signaling. To test this hypothesis, we differentiated human adipocytes and loaded them with human MPO. Treatment of MPO-loaded adipocytes with H_2O_2 caused intracellular production of HOCl, reduced insulin-triggered GLUT-4 translocation to the membrane and glucose uptake. Furthermore 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO)-based immuno-spin trapping and MS-tandem showed radicalization of specific components of the insulin signaling (e.g., IRS-1/2, SOCS3, GLUT-4). These effects were prevented by resveratrol, but not by taurine or methionine because these cannot pass across the membrane. Together our findings suggest that resveratrol can protect insulin sensitivity in the obese AT by scavenging intracellularly produced HOCl. These findings are also in agreement with the role of resveratrol in protecting genomic DNA against intracellularly produced HOCl in MPO-loaded epithelial cells.

Supported by PICT-3435, PIP-916, PROICO-023418 and PUE-013 grants.

STUDY OF THE ROLE OF MODULATORS OF SMALL GTPASES IN MELANOMA

MAURICO MENACHO MÁRQUEZ

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Melanoma is the most dangerous form of skin cancer, accounting for the third highest number of lives lost across all cancers. Since the incidence of melanoma is steadily increasing in the population, finding prognostic and therapeutic targets appears a crucial task in cancer. Vav proteins are guanosine nucleotide exchange factors (GEFs) of the Rho GTPase family whose involvement in melanoma is yet to be elucidated. As GEFs, Vav proteins are capable of regulating processes associated to cytoskeleton rearrangement, mostly through their regulatory function on RhoA and Rac1 GTPases. In this way they manage to modulate cellular migratory capacities and other processes highly associated to the development of cancer and metastasis. In our work we explored the role of Vav proteins in events associated to aggressiveness in melanoma. By modulating the expression or activity levels of these GEFs we were able to observe that Vav proteins play important roles modulating proliferation, apoptosis, cell morphology and migratory behaviour. Also, members of this family are critical to maintain epithelial traits in melanoma cells. All these events were controlled both in GTPases dependent and independent manners. Interestingly, different members of this GEF family, although close related, play opposite roles controlling melanoma proliferation. Altogether our data indicate a critical role

for Vav proteins in development of melanoma and highlight that the elucidation of the role of these proteins in the generation of tumors, their growth and subsequent invasion of nearby tissues, could provide new therapeutic alternatives that contribute to an improvement in current therapies against the main tumor types.

NEUROTROPHINS IN ASTROCYTES, OTHER ROLES BEYOND SURVIVAL AND DEATH

ANDREA CRAGNOLINI

Biology Society of Cordoba (SBC). Institute of Biological and Technological Research (IIByT). Faculty of Exact, Physical and Natural Sciences, National University of Córdoba. Córdoba, Argentina.

Neurotrophins can influence multiple cell functions depending on the cell context and the specific receptors they interact with. These trophic factors have been extensively studied in neurons for their ability to support neuronal survival via Trk receptors and to induce apoptosis via p75NTR. However, neurotrophins and their receptors are expressed in other cellular populations, such as glia, in which their functions are starting to be elucidated. Astrocytes represent a large population of glial cells that produce and secrete neurotrophins, especially after a lesion in the central nervous system (CNS). Astrocytes are also the target of the neurotrophins since they express their receptors. In astrocytes, we demonstrated that the expression of the p75NTR receptor increases after inducing seizures in rodents or after inflicting a mechanical injury in vitro. The treatment of astrocytic primary cultures with the neurotrophin NGF caused a reduction in the number of cells via activation of the p75NTR receptor but, unlike neurons, it was not associated with apoptosis. Rather, the activation of p75NTR by NGF interfered with the expression of cyclins and their association with specific cyclin-dependent kinases, which are associated with the progression through the cell cycle. These results suggest that the activation of p75NTR promotes withdrawal of astrocytes from the cell cycle, which may have important consequences during development or after injury. Next, we wondered if astrocytes show specific brain region-associated responses. We used an *in vitro* model of mechanical injury (scratch injury) to evaluate the wound closure in monolayers of cells obtained from the cortex, hippocampus and striatum. We observed that migration and proliferation varied between astrocytes obtained from different regions of the brain, as well as their responses to NGF and BDNF which potentiate wound closure. These results reinforce the idea that astrocytes constitute a heterogeneous cell population and that their brain-region origin may determine their responses to a lesion and to neurotrophins.

TRACE MINERALS FROM DIFFERENT SOURCES AND THEIR IMPORTANCE IN SWINE PRODUCTION

MÓNICA LUNA

Association of Biology of Tucumán (Asoc.BT). Faculty of Vetenary Sciences, National Universit of theLitoral. Santa Fe, Argentina.

Argentina production has increased mainly at expense of intensive systems where selected genetic lines aimed at achieving a greater meat production in quantity and quality, enhance fertility, prolificity and litter size; clearly, the nutritional needs increase. Pig sector has become very competitive in recent years and a good mineral nutrition is essential to achieve profitable production. Technology for formulation rations in establishments is based in information on the composition of foods and nutritional requirements from foreign countries. For responsible management of mineral nutrition, it should adjusted according to: new genetic lines; chemical interactions; source and mineral bioavailability of diet and critical production stages of high nutritional demand, as well as the influence of environmental conditions. Organic minerals have better bioavailability than inorganic sources, minimize interactions between minerals and not pollute the environment. In pig establishment, peripartum period represents stage of higher importance from the point of view productive. While it is known that mineral needs of sow are higher during late gestation and lactation, there is very little research related to impact on production level of mineral source in those stages. It is necessary that nutrition adjusts to genetic potential to maximize the production. In order to achieve effective nutritional management of animals and obtain larger and better weight litters at time of weaning, minerals profile and productive parameters were evaluated in intensive bristle systems during stages of gestation and lactation fed with different sources of microminerals. Below at symposium we present results obtained, projections and advances on topic.

SAB SYMPOSIUM II

CELL DEATH: ITS IMPLICATION IN PHYSIOLOGY AS IN PATHOLOGY

Chairs: Silvina Pérez Martínez / Fernanda Parborell

EFFECT OF FATTY ACIDS ON HYPOTHALAMIC AUTOPHAGY

EUGENIA MORSELLI

Laboratory of Autophagy and Metabolism, Faculty of Biological Sciences, Pontificia Universidad Católica. Santiago, Chile.

Chronic consumption of high fat diets (HFDs), rich in saturated fatty acids (SatFAs) like palmitic acid (PA), is associated with the development of obesity and obesity-related metabolic diseases such as type II diabetes mellitus (T2DM). Previous studies indicate that SatFAs accumulate in the hypothalamus following consumption of HFDs; in addition, HFDs consumption inhibits autophagy and reduces insulin sensitivity. Recently, we showed that dysregulation of autophagy reduces insulin sensitivity in hypothalamic neuronal cells. Interestingly, consumption of HFDs, as well as SatFAs treatment, also affects the primary cilium in the same cell type. The primary cilium is a "cellular antenna" that functions as a signaling platform, and its depletion in hypothalamic neurons, triggers obesity and insulin resistance in mice. Furthermore, it has been demonstrated autophagy id required for the formation of the primary cilium. We demonstrated exposure of hypothalamic neurons to SatFAs, and not poly-unsaturated fatty acids, reduces both the percentage of ciliated cells and primary length in neurons. Pharmacological or genetic inhibition of autophagy decreases primary cilium expression and, conversely, depletion of primary cilium modulates autophagy. Importantly, insulin signaling following primary cilium depletion is reduced, as well as following SatFAs treatment or autophagy inhibition, suggesting SatFAs impair ciliogenesis through the inhibition of autophagic flux thereby reducing insulin sensitivity in hypothalamic neurons. In conclusion, our research demonstrates a crosstalk between autophagy and the primary cilium in hypothalamic neurons.

GROWTH AND CELL DEATH. TWO OPPOSITE FUNCTIONS FOR THE SAME PROTEIN IN MAMMALS OR PLANTS

ELINA WELCHEN

Litoral Agrobiotechnology Institute (IAL-CONICET-UNL). Cathedra of Cellular and Molecular Biology (FBCB-UNL). Santa Fe, Argentina.

Cytochrome c (CYTc) is a soluble heme protein that transfers electrons between complexes III and IV during the last step of cellular aerobic respiration, for ATP production through the mitochondrial respiratory chain. The release of CYTc from mitochondria to triggers the intrinsic apoptosis pathway is a hallmark of programmed cell death (PCD) in mammals. In plants, PCD is involved in growth and developmental processes, and their activation is part of the defense responses established by plants against environmental stresses. To date, no studies have conclusively reported that CYTc has a direct function in positively regulating PCD in plants. Conversely, the CYTc could be involved in the prevention of the autophagy process during starvation or in response to abiotic stresses, through their connection with the TOR signalling pathway. Plant CYTc is a multi-functional signalling molecule that, apart from its canonical role as an electron carrier, participates in the alternative mitochondrial protein import pathway, in the final step for the synthesis of the antioxidant molecules like ascorbate, and is connected with the detoxification of toxic compounds on the D-lactate and Methylglyoxal pathway. CYTc is essential during embryogenesis in plants and differentially influences the balance between life and death; acting in energy provision for cellular functions that regulate growth and developmental process during the whole plant life cycle and also triggering PCD in mammals.

HUMANIN, AN ANTIAPOPTIC PEPTIDE AS THERAPEUTIC TARGET IN CANCER

MARIANELA CANDOLFI

Biomedical Research Institute (INBIOMED-CONICET), UBA. Buenos Aires, Argentina.

Humanin (HN) is a mitochondrial-derived peptide that signals within the cell or is released to act as an autocrine/paracrine/endocrine antiapoptotic factor through binding to membrane receptors. HN also regulates the mitochondrial apoptotic pathway through the modulation of expression and intracellular localization of proteins of the Bcl-2 family. It exerts protective effects against cytotoxic stimuli in many cell types. These effects have led to the evaluation of HN and its analogs as therapeutic targets for different chronic diseases such as neurodegenative diseases, cardiovascular disorders, diabetes and infertility. Reports on the involvement of HN in tumor development are very scarce. HN was shown to be over-expressed in bladder tumor cells and gastric cancer being proposed that upregulation of HN could be an important molecular event in tumorigenesis and chemoresistence. Although the role of HN in the response of tumor cells to cytotoxic drugs and tumor progression is controversial, this peptide was shown to have cytoprotective effect in normal cells exposed to chemotherapeutic drugs. However, we demonstrated that the inhibition of endogenous HN by intratumoral injection of baculoviral gene therapy vectors encoding a short-hairpin RNA targeting HN (shHN), upregulated the expression of Bax, increased the tumor apoptotic rate, inhibited tumor growth and increased the survival of prolactinoma xenograft models. Meta-analysis of transcriptomic data indicated that HN and its receptors are expressed in human breast cancer specimens. Exogenous HN in vitro protected triple-negative breast cancer (TNBC) cells from cytotoxic insults and reduced the antiproliferative effect of chemotherapy. Systemic administration of HN in TNBC-bearing mice reduced

tumor apoptotic rate, accelerated tumor progression and impaired the antitumor and antimetastatic effect of chemotherapy suggesting that HN inhibits the response of TNBC cells to cytotoxic stimuli, facilitating tumor progression and chemoresistance. Inhibition of endogenous HN increased apoptosis and chemosensibility of TNBC and glioblastoma cells. In addition, chemotherapeutic drugs increased HN expression. Our results put into question the safety of systemic administration of HN or its analogs for chronic diseases and suggest that local administration of gene therapy vectors that silence endogenous HN could hold therapeutic potential in cancer.

IMPLICATION OF THE PROLIFERATION / APOPTOSIS BALANCE IN OVARIAN ALTERATIONS IN BOVINES

NATALIA SALVETTI

Veterinary Sciences of Litoral (ICIVET). National University of Litoral. Santa Fe, Argentina.

Follicular persistence is caused by the failure of ovulation and the consequent permanence of the follicular structure in the ovary, which alters the cyclicity of the female and causes infertility. This process is one of the main components of cystic ovarian disease (COD) and other diseases of ovarian origin that causes great economic losses in the dairy industry because of unsuccessful artificial inseminations, veterinary treatment and decrease in milk production related to the increase in the interval from calving to conception. Along folliculogenesis, the cells that compose the ovarian follicles normally proliferate and then differentiate. Finally, the follicle can take one of two pathways: ovulation, if the follicle is dominant in an ovulatory wave, or atresia, which occurs with most of the non-dominant follicles. Successful follicle development depends on the presence of survival factors that promote follicle growth and also protect cells from apoptosis. These include factors produced within the ovary like 17-β estradiol, progesterone, insulin-like growth factor (IGF)-1, as well as gonadotropins. In the absence of survival factors, endogenous apoptotic pathways within the follicle become activated and lead to follicular atresia. Many studies in different species have shown that the processes of proliferation and apoptosis are altered in the cysts already formed, where there is a decrease in cellular proliferation and an increase in the survival of the cells, which contribute to the persistence of these follicles, preventing their ovulation or regression. However, the study of initial events in the development of persistence indicates that, initially, the proliferation rate is maintained in the absence of ovulation, with low levels of apoptosis and an increase in cell survival due to the increase in anti-apoptotic proteins. It is probable that the hormonal changes that occur later, both at endocrine and paracrine level, are responsible for the alterations observed in the parameters of cell proliferation and differentiation of already developed cysts.

SAB SYMPOSIUM III

YOUNG RESEARCHERS

Chairs: Débora Cohen / Clara Marín Briggiler

INTEGRATING IMMUNOREGULATORY AND VASCULAR SIGNALING PROGRAMS THROUGH LECTIN-GLYCAN INTERACTIONS

DIEGO CROCI

Institute of Histology and Embryology of Mendoza Dr. Mario Burgos (CCT-CONICET). Mendoza. Mendoza, Argentina.

Recent efforts toward decoding the glycosylation signature of immune cells have revealed dramatic changes in N- and O-glycan structures during immune cell maturation, activation and differentiation. The responsibility of deciphering these glycosylation changes is assigned to endogenous lectins which expression is dynamically regulated during chronic inflammatory responses. We will discuss recent findings from our laboratory demonstrating the contribution of glycosylation-dependent mechanisms and lectin-glycan interactions to the regulation of a broad range of immunological programs including T cell survival, dendritic cell fate, microglia activation and endothelial cell signaling. These mechanisms, which could be usurped by tumors to evade and thwart immune responses, have been proposed to shift the balance of immune responses and control immune cell tolerance, inflammation and angiogenesis.

THE YIN AND YANG OF HISTAMINE IN THE REGULATION OF TESTICULAR LEYDIG CELL PROLIFERATION

CAROLINA MONDILLO

Laboratory of Molecular Endocrinology and Signal Transduction. Experimental Medicine and Biology Institute (IByME CONICET). Buenos Aires, Argentina.

Histamine (HA) is a biogenic amine with indisputable significance for medicine and biology. It is synthesized exclusively by histidine decarboxylase (HDC) in all mammalian tissues, albeit tissue-specific mechanisms operate to keep its concentration within strict limits: both a deficit of HA or a slight excess can lead to health

loss. With regard to the male gonad, previous studies have linked increased mast cell numbers and mast-cell related HA with the pathogenesis of infertility. In contrast, HA levels are normally higher in the neonatal testis, and in a former report we described that HDC gene knockout mice show reduced testis weight already at 7 days of age, implying that important HA-dependent events may occur during testicular development. To assess this apparent conflict and considering the ubiquitous role of HA as modulator of cell proliferation, we speculated that testicular HA might contribute to the regulation of LC number. Firstly, we studied the effect of intratesticular HA treatment in adult male rats injected with the specific LC cytotoxic agent ethane dimethanesulphonate (EDS), a convenient model to study normal LC proliferation and development from LC precursors in vivo. As expected, LC re-population was faster in HA-treated rats. Thus, to better identify the stage/s of testicular development in which HA would play a role, we conducted in vitro experiments to evaluate its effect on the proliferation of progenitor and immature LC, isolated from 21- and 35-day old male rats, respectively. However, none exhibited a proliferative response upon stimulation with HA. We then studied HDC immunoexpression in testes of rats aged 7 to 240 days, and found that it was highest in 7-day old rats but then decreased abruptly, with remnants of the fetal LC population being the most intensively stained. In line with previous observations in HDC KO mice, these results suggest that local HA synthesis may be important to influence LC numbers in the early stages of normal testicular development, while it would be negatively regulated with age. Interestingly, we have recently detected HDC overexpression in 2 murine Leydig tumor cell lines, as well as in human LC hyperplasia and prepubertal LC tumors. Moreover, HA promoted the proliferation of Leydig tumor cells in vitro, while specific HDC inhibitors had the opposite effect. Importantly, these findings indicate that autocrine overproduction of HA might be related to abnormally increased proliferation in LC.

UNDERSTANDING THE CRITICAL ROLE OF MONOCYTE-MACROPHAGE LINEAGE IN INFLAMMATORY DISEASES AND HOW THE COOPERATION WITH OTHER CELL TYPES DEFINE THE EFFECTOR RESPONSE

EUGENIO CARRERA SILVA

Experimental Thrombosis Laboratory, Institute of Experimental Medicine (IMEX), National Academy of Medicine - CONICET. Buenos Aires, Argentina.

Monocyte-macrophage lineage cells are multifunctional and found in nearly all tissues throughout the body. They orchestrate the initiation and resolution phases of both innate and adaptive immunity, significantly impacting protective immunity and immune-mediated pathological damage through their ability to adopt distinct functional capacities in different microenvironment. A major goal of the macrophage field is to link specific functions with specific cellular and molecular pathways associated with different macrophage activation profiles. Negative regulatory feedback is a critical aspect of the homeostatic immune response and disruption on this point could lead to inflammatory-based disease. The tyrosine kinase receptors TYRO3, AXL and MERTK (TAM) and their ligands Protein S (PROS1) and growth arrest-specific 6 (GAS6) are critical players in maintaining immune homeostasis by dampening inflammatory response, mediate efferocytosis and to contribute to tissue repair process. Our research is focus in understanding the participation of monocyte-macrophage compartment and TAM axis in the protection or development of some chronic inflammatory human diseased such as Inflammatory Bowel disease, Langerhans Cell Histiocytosis or Multiple Sclerosis as well as in acute infection and sepsis. We have found that monocyte-macrophage linage play central role not only in immune-mediated diseases but also because regulatory T cell or platelets can orchestrate macrophage effector responses improving clinical outcome.

DECONSTRUCTING THE GLUCOCORTICOID RECEPTOR: PHARMACOLOGICAL IMPLICATIONS

DIEGO PRESMAN

Institute of Physiology, Molecular Biology and Neurosciences (IFIBYNE-UBA-CONICET). Buenos Aires, Argentina.

Synthetic glucocorticoids (GCs) are one of the most prescribed pharmaceuticals world-wide due to their powerful anti-inflammatory and immunosuppressive activities. The action of GCs is mediated by the glucocorticoid receptor (GR), a member of the nuclear receptor superfamily of transcription factors. The GR is involved in many physiological processes, including the regulation of cell death and proliferation. Historically, GR transcriptional activity and clinical outcomes have been linked to its dimeric/monomeric state. A widely discussed model suggests that dimeric GR regulates unfavorable metabolic pathways, while monomeric GR is responsible for anti-inflammatory activities. Hence, GR ligands that preferentially induce the monomeric rather than the dimeric pathway should retain the desired pharmacological effects but will lack the undesired adverse reactions. However, the search for improved ligands under this paradigm has produced no significant results for the last 25 years. During this talk, I will present evidence against this predominant "dissociated model". By combining advance quantitative fluorescence microscopy techniques with genomic analysis on oligomeric mutant receptors, I will propose a new relationship between GR's quaternary

structure, chromatin binding and transcriptional output. This has important implications for the therapeutic uses of steroid hormones and the goal of finding selective anti-inflammatory drugs that do not create unwanted side-effects.

AWARDS

SAIC AWARD I

'LUCIO CHERNY FOUNDATION'- MULTIDISCIPLINARY

Juries - Alicia Belgorosky | Graciela Cremaschi | Alejandro De Nicola | Mirta Schattner | Elba Vázquez

0296 - INTEGRIN-MECHANOSIGNALING ROLE IN SMALL GTPASES ACTIVATION AND CANCER

Georgina COLÓ (1) | Lucía FERNÁNDEZ CHÁVEZ(1) | Karen SCHWEITZER(1) | Nazarena BARRERA- LAMAS(2) | Norberto GANDINI(1) | Exequiel ALONSO(1) | Marilina MASCARÓ(2) | Pamela PICHEL(1) | Sergio RECIO(3) | Reinhard FÄSSLER(4) | María Marta FACCHINETTI(1) | Alejandro CURINO(1)

INIBIBB-CONICET, DEPTO. BIOLOGÍA, BIOQUÍMICA Y FARMACIA-UNS (1); INSTITUTO DE INVESTIGACIONES BIOQUÍMICAS BAHÍA BLANCA INIBIBB -CONICET (2); UNIDAD DE CIRUGÍA DE CABEZA Y CUELLO, HOSPITAL MUNICIPAL DE AGUDOS "DR. L. LUCERO" (3); DEPARTMENT OF MOLECULAR MEDICINE MAX PLANCK INSTITUTE OF BIOCHEMESTRY (MPI) (4)

The ability of cells to adhere and simultaneously probe their mechanical environment is central to many physiological and pathological processes. Extracellular matrix sensing and mechanotransduction are mediated by the integrin family of cell adhesion receptors. Using genetically engineered cells, we studied the specific fibronectin integrin binding signaling and its role in tumor development. We observed that a5ß1-integrins promoted the formation of small adhesions, low RhoA activation and high force, while aß3-expressing cells showed large adhesions, thick stress fibers, high RhoA activation and low force. To further analyse these cellular phenotypes, we looked for specific RhoA activators (GEFs). For this purpose, we performed Mass Spectrometry (MS) analysis follow by biochemical assays and observed that GEF-H1 activation is aVß3-integrin dependent. Furthermore, using integrin-tail pull-down and MS assay, we observed that GEF-H1 binds to ß3-tail, suggesting that specific integrins may activate different Rho-GEFs during tumor progression. In order to study the role of GEF-H1 in cancer, we analysed by immunohistochemistry GEF-H1 expression in human biopsies. We observed overexpression of GEF-H1 in breast (p= 0.0053, n= 61) and thyroid (p= 0.0006, n= 32) tumor biopsies compared with normal tissue. Similar results were obtained in cancer cell lines (CCL). To further study the role of GEF-H1 in tumor development using CRISPR/Cas9 technology, we generated GEF-H1-knock out (KO) clones in a murine invasive breast CCL. We observed a decrease in the proliferation, migration and invasion rates (p<0.001) in GEF-H1-KO cells. These results showed that GEF-H1-RhoA activation is aVß3-integrin dependent and that may mediate the signaling involved in controlling cell structure, force generation, proliferation, migration and invasion of breast cancer cells. In addition, the studies in human tumor samples suggest that GEF-H1 might be a molecular biomarker in cancer.

0331 - REGULATORY MECHANISMS UNDERLYING FUNCTIONAL MATURATION OF SERTOLI CELLS IN RESPONSE TO ANDROGENS DURING POSTNATAL DEVELOPMENT

Nadia Yasmín EDELSZTEIN | Helena Fedora SCHTEINGART | Rodolfo Alberto REY

CENTRO DE INVESTIGACIONES ENDOCRINOLÓGICAS "DR. CÉSAR BERGADÁ" (CEDIE) - CONICET - FEI

Androgen-dependent maturation of Sertoli cells during postnatal testicular development is key for the establishment of spermatogenesis. Meiosis in the male begins at puberty and relies on androgens and retinoic acid. Immature Sertoli cells produce high levels of AMH, which is inhibited by androgens at puberty. The molecular mechanisms underlying androgen-mediated AMH decline are unknown. CYP26B1 degrades retinoic acid in the prenatal testis preventing meiosis initiation. The concurrence of meiotic entry and Sertoli cell maturation in response to androgens led us to propose that CYP26B1 —like AMH— is downregulated by androgens in the Sertoli cell during puberty, thus enabling meiosis initiation. By immunohistochemistry, we saw that CYP26B1 expression declines in the postnatal mouse Sertoli cell as androgen receptor (AR) expression increases, closely before meiotic spermatocytes appear. Luciferase reporter assays in the SMAT1 Sertoli cell line showed a direct negative effect on Amh promoter activity in the presence of dihydrotestosterone (DHT, p<0.001). Site-directed mutagenesis and ChIP-qPCR assays showed that androgen-mediated inhibition requires the SF1 sites in the Amh promoter. Regarding Cyp26b1, we saw no changes in promoter activity in response to androgens (p= 0.34). This lack of response was further supported by invariant levels of endogenous Cyp26b1 expression in SMAT1 cells transfected with the AR (p= 1.0) and in primary Sertoli cells of 10-day-old mice in culture (p= 0.7), after DHT treatment. ChIP-qPCR showed no enrichment in AR sequences analyzed, indicating a lack of functional binding of the AR. In sum, we confirmed a negative correlation between the immature Sertoli cell markers AMH and CYP26B1 and AR expression and meiotic initiation in postnatal development. We identified the molecular mechanism underlying AMH inhibition by androgens but found that the decline in CYP26B1 expression is not caused by a direct inhibitory androgen effect on Sertoli cells.

0359 - CDC42 ACTIVITY IS NECESSARY FOR THE INTERPLAY BETWEEN CAMP/PKA PATHWAY AND CATSPER FUNCTION

Guillermina LUQUE (1) | Ana ROMAROWSKI(1) | Cintia STIVAL(2) | Nicolas GILIO(1) | Tomas DALOTTO-MORENO(1) | Paula BALESTRINI(1) | Martina JABLOÑSKI(1) | Jamaica SCHIAVI-EHRENHAUS(1) | Diego KRAPF(3) | Dario KRAPF(2) | Mariano BUFFONE(1)

INSTITUTO DE BIOLOGÍA Y MEDICINA EXPERIMENTAL (IBYME-CONICET) (1); IBR-CONICET, UNR (2); COLORADO STATE UNIVERSITY (3)

Sperm acquire the ability to fertilize in the female genital tract in a process called capacitation. During capacitation, sperm undergo changes in the motility pattern called hyperactivation, which depends on Ca^{2+} transport by the sperm-specific Ca^{2+} channel CatSper. CatSper is essential for fertilization and therefore, it is subjected to a complex regulation that is not fully understood. Recent reports found that mouse CatSper is upregulated by cAMP-dependent activation of protein kinase A (PKA). From a molecular point of view, bicarbonate stimulation of the soluble adenylyl cyclase (sAC) leads to an increase in cAMP, PKA activity and tyrosine phosphorylation of sperm proteins. It remains incompletely understood if PKA itself phosphorylates CatSper or if its activation relays on other intermediary events. By using super-resolution microscopy, we report that similar to CatSper, the small

GTPase Cdc42 distribution in the principal piece is confined to four linear domains and this localization is disrupted in CatSper-null sperm. Cdc42 inhibition impaired CatSper opening, assessed by different approaches including analysis of downstream signaling events and membrane potential recordings. Consequently, Cdc42 inhibition abrogated the increase in intracellular Ca^{2+} and other Ca^{2+}-associated events, resulting in a severe compromise of the sperm fertilizing potential: decreased percentage of sperm undergoing hyperactivation and in vitro fertilization. We also demonstrate that Cdc42 is essential for CatSper function by modulating cAMP production by sAC, providing a new regulatory mechanism for the upregulation of CatSper by the cAMP/PKA-dependent pathway. These results reveal a broad mechanistic insight into the regulation of Ca^{2+} in mammalian sperm, a matter of critical importance in male infertility as well as in contraception.

0531 - TRISTETRAPROLIN (TTP) EXPRESSION IS REQUIRED FOR MAINTENANCE OF THE MAMMARY PROGENITOR CELL POPULATION

Micaela STEDILE | Inés BECKERMAN | María Victoria GODDIO | Lourdes PÉREZ CUERVO | Martín GARCÍA SOLÁ | María Victoria MEDINA | Ana RAIMONDI | Omar COSO | Edith Claudia KORDON

IFIBYNE, CONICET-UBA

Messenger RNA (mRNA) stability is regulated mainly by proteins that bind sequences enriched in adenine and uracil in their 3´untranslated regions, called AU-binding proteins (AUBPs). Tristetraprolin (TTP) is an AUBP that promotes mRNA degradation of proteins involved in inflammation, proliferation and tumor invasiveness. In particular, RNA-seq data indicates that TTP is up-regulated in mammary progenitor cells ($p<0.01$). Considering this fact, our goal was to determine whether expression of this AUBP is relevant for the maintenance of the mammary stem cell compartment. Our results showed that upon successive pregnancies TTP knockout mice exhibited underdeveloped lactating glands that also presented decreased pre-neoplastic lesions when crossbred with mice expressing RasG12D oncogene (H&E analysis). Hence, our hypothesis is that diminished expression of TTP in the mammary progenitor compartment caused impairment of mammary gland development and differentiation. To test it, we generated TTP knockdown cells (TTP-KD) by stable transfection of stem-like HC11 mammary epithelial cells with specific TTP-shRNAs. These cells exhibited impaired survival rates (MTS assay, $p<0.01$) and increased apoptosis (TUNEL, $p<0.01$). Then, we analyzed several pathways related to survival and cell fate. TTP-KD cells displayed high expression of IL-6, an inflammatory cytokine whose mRNA is a recognized TTP target (RT-qPCR, $p<0.01$), increased levels of STAT3 and P-STAT3 (WB, $p<0.05$) as well as p65/RelA (WB, $p<0.05$) and phospho-p38 (WB, $p<0.05$). Conversely, we observed inhibition of phospho - ERK 1/2 (WB, $p<0.05$). TTP-KD cells also revealed decreased capacity to form mammospheres in 3D culture ($p<0.05$) and to repopulate cleared fat pads of virgin BALB/c female mice. Taking together, our results indicate that TTP expression is required for mammary progenitor cell survival by preventing spontaneous pro-inflammatory and stress-associated events which are able to induce mammary stem cell death.

0693 - PROGESTERONE RECEPTOR ISOFORM RATIO DICTATES ANTIPROGESTINS/PROGESTINS EFFECTS ON METASTATIC BREAST CANCER MODELS.

María Florencia ABASCAL (1) | Michelle ALVAREZ(1) | Gonzalo SEQUEIRA(2) | Silvia VANZULLI(3) | Andres ELIA(1) | Gabriela PATACCINI(1) | Luz GIBBONS(4) | Hugo GASS(5) | Paula MARTINEZ VAZQUEZ(5) | Javier BURRUCHAGA(5) | Sebastian GIULIANELLI(6) | Claudia LANARI(1)

IBYME-CONICET (1); HOSPITAL DE AUTOGESTIÓN DR. ARTURO OÑATIVIA (2); CEDIE (3); EFECTIVIDAD CLÍNICA Y SANITARIA (IECS) (4); HOSPITAL MAGDALENA V. MARTINEZ (5); INSTITUTO DE BIOLOGÍA DE ORGANISMOS MARINOS, IBIOMAR-CCT CENPAT-CONICET (6)

The role of the progesterone receptor isoforms A (PRA) and B (PRB) in breast cancer (BC) is still controversial as well as the use of PR ligands for BC treatment. Our aim was to evaluate a) the role of PRA and PRB in metastatic growth, b) the effect of different progestins (P) and antiprogestins (AP) in metastatic models c) the involvement of two metastasis suppressor genes, NDRG1 and NME1. Mifepristone and 3 other AP inhibited the onset and growth of lymph node and lung metastases of the murine C7-2-HI tumor with PRA>PRB (PRA-H). When tumors were removed, and the long-term metastasis were evaluated after interruption of AP neoadyuvant/adjuvant treatment, an increase in disease free survival rate was observed in all AP-treated mice as compared to controls ($p<0.001$). Contrariwise, the P medroxyprogesterone acetate (MPA), increased the metastatic growth ($p<0.001$). In the murine C7-HI tumor (PRB>PRA; PRB-H), AP increased metastatatic growth ($p<0.001$) whereas MPA showed an opposite trend. The previous findings were confirmed in the human MDA-MB-231 model transfected with, or induced to express PRB or PRA growing in NSG mice. NDRG1 and/ or NME1 expression was evaluated in these models and in BC samples by immunohistochemistry. These genes are up-regulated in luminal B vs luminal A tumors (TCGA) and in the PRB-H tumor models. A similar trend was found in PRB-H BC samples as compared to those PRA-H obtained from Magdalena V. Martinez Hospital (n= 93). In the PRA-H models, P down-regulate and AP up-regulate NDRG1 expression, and the opposite occurs in PRB-H models. Our data highlights the relevance of determining the PR isoform ratio previous the administration of a P/AP therapy and suggests that NDRG1 and/or NME1 may participate mediating the anti- or pro-metastatic ability of P and AP in these models.

SAIC AWARD II:

'BIGAND FOUNDATION' YOUNG INVESTIGATORS- ONCOLOGY

Juries - Carolina Pérez Castro | María Fernanda Rubio | Enrique Sánchez Pozzi | Laura Todaro

0807 - LOW DOSES OF DOCETAXEL PRIOR TO IMMUNOTHERAPY LEAD TO PROSTATE TUMOR-FREE OUTCOME THROUGH GALECTIN-3 NEGATIVE REGULATION.

Carolina Daniela TIRABOSCHI (1) | Lucas GENTILINI(1) | Enrique CORAPI(1) | Felipe JAWORSKI(1) | Carla VELÁZQUEZ(1) | Anne CHAUCHEREAU(2) | Diego LADERACH(1) | Daniel COMPAGNO(1)

INSTITUTO DE QUÍMICA BIOLÓGICA DE LA FACULTAD DE CIENCIAS EXACTAS Y NATURALES (IQUIBICEN) (1); INSTITUT GUSTAVE ROUSSY-INSERM U981 (2)

Based on animal model results and the analysis of clinical data from metastatic and castration resistant prostate cancer (mCRPC) patients, we were able to demonstrate the advantages and the mechanisms by which Docetaxel (DTX)-based chemotherapy induces the correct pre-conditioning of the tumour microenvironment to allow high cytotoxic T cell activity to avoid prostate tumour growth and recurrence. First, analysis of the gene expression of human PCa cells showed that treatment with DTX decreases the expression of Galectin 3 (Gal-3) in either chemo-sensitive or -resistant prostate cancer (PCa) cell lines (98 ± 3 %) but also in clinical samples of mCRPC patients treated by taxane-based chemotherapy (79 ± 17 %). To evaluate whether this DTX-dependent Gal-3 downregulation in PCa tumors could be one of the principal molecular factors responsible for chemotherapy enhancement of immunotherapy, we decide to inoculate mice with an autologous BM-DC vaccine loaded with lysates from TRAMP-C1 (TC1) cells pretreated by DTX in vitro or expressing an anti-Gal-3 shRNA. This immunotherapy protocol completely protects the animals to the growth of Gal-3 deficient-PCa tumors, and suggests that Gal-3 expressed by the tumor could be one of the major negative checkpoints of the immune response against PCa. We finally clearly demonstrate that a low doses DTX treatment decreasing gal-3 expression in tumor prior vaccination protects 6/7 treated mice for prostate cancer recurrence and metastasis development in mice after primary tumor-resection. In conclusion, our results strongly suggest that Gal-3 is one of the principal immunosuppressor in prostate cancer that limits the success of immunotherapy. Finally, we show that low doses of DTX-based chemotherapy would promote a decrease of Gal-3 expression by the tumors to create a permissive tumor microenvironment for successful immunotherapy, which in turn could control tumor recurrence for all PCa patients.

SAIC AWARD III

'BIGAND FOUNDATION' YOUNG INVESTIGATORS- INTERDISCIPLINARY

Juries - Hernán Farina | Mariana Farina | Silvia García | María Laura Ribeiro

0138 - C-C MOTIF CHEMOKINE RECEPTOR 2 (CCR2) AS A NOVEL INTERMEDIATE IN THE OVULATORY CASCADE

Marina Cinthia PELUFFO

CENTRO DE INVESTIGACIONES ENDOCRINOLÓGICAS "DR. CÉSAR BERGADÁ" (CEDIE)-CONICET

Shortly before ovulation the LH surge induces processes critical for fertility, including cumulus-oocyte expansion (C-OE) and resumption of meiosis. Since the molecular mechanisms responsible for initiating such complex processes are not fully understood, we hypothesized that interactions between chemokines and the chemokine receptor CCR2 have a direct effect promoting C-OE and/or oocyte maturation. To test this hypothesis, two separate novel feline culture systems were established in our laboratory. Studies were designed to a) evaluate the mRNA expression of CCR2 and its chemokine ligands, within the cumulus oocyte complex (COC) and follicle wall after a LH stimulus in cultured feline antral follicles; b) examine the direct effects of exogenous recombinant MCP1 (monocyte chemoattractant protein-1) on mRNA expression of key periovulatory genes in the COC, using a feline COC culture system; and c) determine whether inhibition of CCR2 signaling in the COC interferes with the expression of periovulatory genes and/or oocyte maturation. Results demonstrated mRNA expression of CCR2 receptor and its ligands within the feline COC and follicle wall, and a significant increase in CCR2 mRNA by LH within the COC. MCP1 treatment promoted a significant increase in mRNA levels of key periovulatory genes within the COC. Interestingly, a highly selective CCR2 antagonist significantly affected the stimulation of periovulatory genes induced by either gonadotropin, amphiregulin or prostaglandin E2. In summary, this is the first report in any species, describing a direct effect of MCP1 within the COC. More importantly, the stimulation of periovulatory genes occurs, at least in part, through the CCR2/MCP1 pathway, suggesting CCR2 receptor as a novel mediator of the ovulatory cascade. A better understanding of this novel role of CCR2 could eventually aid in the diagnosis or treatment of infertility; otherwise, this may help identifying novel targets for non-hormonal contraception.

0159 - OSTEOPOROSIS AND NEUROLOGICAL IMPAIRMENT ARE ASSOCIATED WITH SYSTEMIC OXIDATIVE STRESS IN OLD RATS

Maria Luz TORRES(1) | Nahuel WANIONOK(1) | Antonio MCCARTHY(1) | Gustavo Ramon MOREL(2) | **Juan Manuel FERNÁNDEZ** (1)

LABORATORIO DE OSTEOPATIAS Y METABOLISMOS MINERAL (LIOMM), FACULTAD DE CIENCIAS EXACTAS, CICPBA-UNLP (1); INSTITUTO DE INVESTIGACIONES BIOQUÍMICAS DE LA PLATA, CÁTEDRA DE HISTOLOGÍA B, FC DE CSMEDICAS, UNLP. (2)

Alzheimer´s disease (AD) is a neurodegenerative disorder that accounts for 50-75 % cases of dementia in the elderly. It is characterized by high morbidity (mainly memory loss) and a scarcity of treatments to revert disease progression. As reported by Alzheimer's Disease International 2015 report, 46 million people worldwide suffer from this disease, with an estimated total cost of U$S 818 billion. Osteoporosis (OS) is defined as a decrease in bone density with compromised bone resistance and increased fracture risk. OS is a degenerative disorder that mainly affects the elderly, showing high morbidity and mortality due to osteoporotic fractures. Recent studies have found that there is an increase in the incidence of OS and hip fractures in patients with AD, compared to control (non-AD) individuals. On the other hand, it has been demonstrated that there is an increased prevalence of AD in women with OS. Thus, in ageing individuals there appears to be a bidirectional association between AD and OS. In this work, we have evaluated bone properties in two groups of 30-month-old Sprague Dawley rats with unimpaired (control) or impaired memory, assessed by NOR (Novel Object Recognition test). Histological and pQCT evaluation showed a decrease in bone quality of rats with impaired memory vs. control. Bone marrow progenitor cells (BMPC) were obtained from both groups, and their ex vivo ability to differentiate into osteoblasts was evaluated by PCR and phenotypic progression. We found that BMPC from rats with impaired memory have a lower capacity to differentiate into osteoblasts and a higher osteoclastogenic profile vs. control. Because both diseases have been related to oxidative stress, we also evaluated serum TBARS and conjugated dienes, and found these parameters to be elevated in rats with impaired memory vs. control. Our results suggest that oxidative stress could be a causal link between the decrease in bone quality and impaired memory observed in old individuals.

0911 - EXPERIMENTAL NON-EXUDATIVE AGE-RELATED MACULAR DEGENERATION: THE MACULA IS TO BLAME

Damián DORFMAN

LABORATORIO DE NEUROQUÍMICA RETINIANA Y OFTALMOLOGÍA EXPERIMENTAL, CEFYBO/CONICET

Non-exudative age-related macular degeneration (NE-AMD) is the leading cause of blindness in the elderly. The macular retinal pigment epithelium (RPE) lies in a high oxidative environment

because its high metabolic demand, mitochondria concentration, reactive oxygen species levels, and macular blood flow. It has been suggested that oxidative stress-induced damage to the RPE plays a key role in NE-AMD pathogenesis. The fact that the disease limits to the macular region raises the question as to why this area is particularly susceptible. We have developed a NE-AMD model induced by superior cervical ganglionectomy (SCGx) in C57BL/6J mice, which reproduces the disease hallmarks exclusively circumscribed to the temporal region of the RPE/outer retina and advantages the existing rodent models. The aim of this work was analyzing RPE regional differences that could explain AMD localized susceptibility, the effects of SCGx on the different regions of the RPE/outer retina, and develop new therapeutic strategies for the disease. Histological, ultrastructural and biochemical parameters were studied the retina and RPE from adult male C57Bl/6J mice. Lower melanin content, thicker basal infoldings, higher mitochondrial mass, and higher levels of antioxidant enzymes, were observed in the temporal RPE compared with the nasal region (*p<0.05 vs. nasal RPE, by Student´s t-test). Moreover, SCGx induced a decrease in the antioxidant system, and mitochondria mass, as well as an increase in mitochondria superoxide, lipid peroxidation products, nuclear factor erythroid 2–related factor (Nrf2) and heme oxygenase-1 levels, and in the occurrence of damaged mitochondria exclusively at the temporal RPE (**p<0.01 vs. nasal RPE from sham-treated eyes; a: p<0.01 vs. temporal RPE from sham-treated eyes, by Tukey´s test (F=4.53)). The treatment with melatonin prevented and reversed the functional and structural damage in the RPE induced by SCGx. These findings suggest that the macular RPE histologic and metabolic specific attributes make it more susceptible to choroid alterations leading to a localized RPE oxidative stress, mitochondria dysfunction, and RPE/outer retina damage, and that the treatment with melatonin could both prevent and reverse the NE-AMD-induced damage.

0958 - EFFECT OF ANGIOGENIC THERAPIES IN RABBIT WITH PERIPHERAL ARTERY DISEASE

Fernanda Daniela OLEA

IMETTYB-UNIVERSIDAD FAVALORO-CONICET

Peripheral artery disease (PAD) is a progressively disabling pathology characterized by decreased arterial blood flow to the lower limbs and which lacks effective treatment. Therapeutic angiogenesis induced by gene or cell therapy emerges as an alternative for this disease. Fifteen years ago I started a research line to induce tissue regeneration in PAD, where I have been testing different angiogenic therapies in rabbit with experimental PAD. I started studying the effect of the administration of a plasmid encoding VEGF165 (pVEGF165) in ischemic rabbit muscle. This study showed that to induce increase microvasculature and angiographycally visible collaterals, and decrease ischemic muscle lesions is necessary repeat the treatment. To prolong these effects to a long term without have to repeat the therapy, we proposed to evaluate the effect of injection of adipose stromal cells (ASCs) transfected with pVEGF165 (ASCs-pVEGF). We decided it because multipotent ASCs have been shown to have angiogenic effects through the secretion of several angiogenic factors. In this study, we demonstrated that both ASCs and ASCs-pVEGF groups showed an increase in microvascular density, but only in ASCs-pVEGF165. Although these results showed positive effects, the ASCs-pVEGF group did not show better results due to the low transfection efficiency. Based on these results, we recently proposed a new strategy using baculoviral vectors (BV, which have high transduction efficiency and are safe), and new gene encoding an oxygen-resistant HIF-1α (which is transcription factor of several angiogenic factors including VEGF). In this study, Bv mHIF-1α group showed an increase in microvasculature and angiographically visible collaterals respect to control group beside to be safety. Due to these latest results, our next project is to produce this therapy under GMP conditions and thus be able to design a phase I clinical trial in patients with symptomatic PAD without chances of conventional revascularization.

SAIC AWARD IV

GADOR FOUNDATION'- UNSATISFIED MEDICAL NEEDS

Juries - Jorge Geffner | Paula Heller | Mónica Kotler | Osvaldo Uchitel

0380 - CANCER VERSUS NEURODEGENERATION: CHARACTERIZING THE ROLE OF ALPHA-SYNUCLEIN IN MELANOMA

Florencia MALIZIA (1) | Luciano ANSELMINO(1) | Mauricio MENACHO MÁRQUEZ(2)

INSTITUTO DE INMUNOLOGÍA CLÍNICA Y EXPERIMENTAL DE ROSARIO (IDICER, UNR-CONICET) (1); INSTITUTO DE INMUNOLOGÍA CLÍNICA Y EXPERIMENTAL DE ROSARIO (IDICER, UNR-CONICET); CIPREB (FCM-UNR) (2)

Both cancer and Parkinson disease (PD) are a consequence of the interaction between genes and environmental factors; a key difference is that the biological processes leading to these pathologies occur in different cellular environments, leading to cell division or death. Recent studies suggested that alpha-synuclein (aS), a key regulator in PD, although toxic to dopaminergic neurons, is protective for advanced melanoma. In this work, we began to explore the biological role of aS in processes associated to melanoma development and progression. First, by bioinformatics and Western Blot studies, we confirmed that aS is highly expressed in melanoma samples, being part of high molecular weight aggregates. Indeed, by differential lysis with detergents we found that aS is preferentially present at the cytoplasm or bound to membrane structures in mouse (B16-F0) and human (SK-MEL 28 and A375) melanoma cells. By using shRNA technologies and plasmid expression vectors, we were able to modulate aS expression in melanoma cells. Growth studies based on cells counting indicated that reduced expression of aS leads to proliferative defects (p<0.05), changes in actin cytoskeleton architecture and tubulin fibers organization observed by immunocytochemistry. Interestingly, we noted that melanoma cells were able to uptake different aggregation species of aS exogenously added (p<0.01). These species, although toxic for neuronal cells (SH-SY5Y, p<0.01), failed to trigger toxic effects on melanoma cells. Instead, they promoted proliferation (p<0.05), changes in actin cytoskeleton architecture and migration (p<0.01) of melanoma cells. Altogether, our data indicate a putative role for aS in different processes associated to melanoma growth and development. Indeed, aS expression could have prognostic value for tumor patient outcome.

0473 - ENVIRONMENTAL ENRICHMENT PREVENTS BEHAVIORAL AND CONNECTIVITY ALTERATIONS IN AN EXPERIMENTAL MODEL OF AUTISM SPECTRUM DISORDERS

Nonthué Alejandra UCCELLI (1) | Martín Gabriel CODAGNONE(1) | Nadia LEVANOVICH(2) | Marianela Evelyn TRAETTA(1) | María Victoria ROSATO SIRI(3) | Juan Ventura LACOUR(1) | Leandro URRUTIA(2) | Germán FALASCO(2) | Silvia VÁZQUEZ(2) | Juana PASQUINI(3) | Analía REINÉS(1)

INSTITUTO DE BIOLOGÍA CELULAR Y NEUROCIENCIA "PROF. E. DE ROBERTIS", UBA-CONICET (1); CENTRO DE IMÁGENES MOLECULARES-FLENI (2); INSTITUTO DE QUÍMICA Y FISICOQUÍMICA BIOLOGICA (IQUIFIB) UBA-CONICET (3)

Autism spectrum disorders (ASD) are a group of neurodevelopmental disabilities characterized by social impairments and atypical connectivity. Studies in patients report hypo- and hyper-connectivity and alterations in the corpus callosum (CC), the main structure that connects brain hemispheres. No pharmacological approaches for ASD core symptoms are available and behavioural therapies have shown the best outcomes. The aim of this work was to study brain connectivity in a rat model of ASD and evaluate benefits of environmental enrichment, dissecting the role of social and physical stimuli. For this purpose, pregnant dams were administrated with valproic acid (VPA) or saline and behavioural, functional (PET), ultrastructural and molecular parameters were studied in male pups. VPA animals showed delayed growth, maturation and behavioural development in early stages. Pups were weaned and kept in standard (S), physically plus socially (EE) or physically (PE) enriched environments. In juvenile stages, VPA rats exhibited decreased exploratory activity and sociability. PET showed lower global glucose uptake in VPA brain with greater uptake in local areas related with core symptoms. CC from VPA rats elicited diminished percentage of myelinated axons and G-ratio. Oligodendrocyte lineage was explored: CC1+ cells decreased and PDGFαR+ cells increased in the CC of VPA rats. EE fully prevented behavioural and ultrastructural deficits in juvenile VPA rats and had partial effects on cellular changes. PE had the same behavioural benefits than EE. This work demonstrates brain metabolic alterations in VPA rats that can be related with connectivity deficits. Also, aberrant myelin ultrastructure and alterations in oligodendroglia support the long-distance hypoconnectivity hypothesis for ASD. Our results indicate that social stimulus is not necessary to provide behavioural benefits in VPA rats and postulate PE and EE as valuable tools for the development of therapeutic strategies in ASD.

0505 - ARE PROLACTIN AND ITS RECEPTOR INVOLVED IN THE PATHOGENESIS OF GLIOBLASTOMA?

Antonela Sofía ASAD (1) | Alejandro J. NICOLA CANDIA(1) | Nazareno GONZÁLEZ(2) | Camila Florencia ZUCCATO(1) | Araceli ABT(1) | Santiago Jordi ORRILLO(1) | Yael LASTRA(3) | Emilio DE SIMONE(3) | Florence BOUTILLON(4) | Vincent GOFFIN(4) | Adriana SEILICOVICH(1) | Daniel Alberto PISERA(1) | Jimena FERRARIS(1) | Marianela CANDOLFI(1)

INBIOMED- INSTITUTO DE INVESTIGACIONES BIOMÉDICAS. UBA-CONICET (1); MPLBIOR. MAX PLANCK LABORATORY FOR STRUCTURAL BIOLOGY, CHEMISTRY AND MOLECULAR BIOPHYSICS OF ROSARIO (2); FACULTAD DE CS. VETERINARIAS - UBA (3); U1151, INSTITUT NECKER ENFANTS MALADES (INEM), FACULTY OF MEDICINE, UNIVERSITY PARIS DESCARTES (4)

Gliomas are primary brain tumors derived from glial cells. Glioblastomas (GB) are the most frequent and aggressive gliomas. Prolactin (PRL) and its receptor (PRLR) have been detected in GB biopsies. Although PRL has been involved in the development of hormone-dependent tumors, its role in the pathogenesis of GB remains unclear. Our aim was to explore the contribution of PRL and PRLR in GB progression. We have previously found that GB cells express PRL and PRLR, and that the PRL/PRLR pathway increases GB cell proliferation rate and viability. In the current study, we observed that the overexpression of PRL using plasmid transfection significantly increased GB cell viability and decreased cisplatin cytotoxic effect, whereas the overexpression of PRLR antagonist d1–9-G129R-hPRL (PRLR-A) showed opposite results. The overexpression of PRLR isoforms inhibited the effect of cisplatin on viability and clonogenicity of GB cells. PRLR blockade using PRLR-A decreased GB cell migration, as assessed by the wound assay. Moreover, zymography of conditioned media from GB cells incubated with PRL showed higher activity of MMP-2 and MMP-9, enzymes involved in GB cell invasion. Transcriptomic meta-analysis of human glioma specimens indicated that PRLR mRNA was present in virtually all grade II-III glioma (GII-III) and GB samples. PRL expression was upregulated in GB biopsies when compared to GII-III. While in the general population tumor PRL/PRLR expression did not correlate with median survival, biological sex-stratified analyses revealed that male patients with PRL+/PRLRhigh GB performed worse than PRL+/ PRLRlow GB. In contrast, all male PRL+/PRLRhigh GII-III patients were alive whereas only 30% of PRL+/PRLRlow GII-III patients survived after 100 months. These results suggest that PRLR could constitute a therapeutic target for GB treatment and provides additional evidence that sexual dimorphism should be taken into account to improve the care of GB patients.

0640 - DEVELOPMENT OF "AMI", AN ISOTHERMAL MOLECULAR AMPLIFICATION PLATFORM TO ENABLE NEW DIAGNOSTICS SYSTEMS

Luciana LAROCCA | Fabiana STOLOWICZ | Santiago WERBAJH | Adrian VOJNOV | Carolina CARRILLO

ICT MILSTEIN - CONICET

Infectious diseases have a great impact on health and productivity, particularly in lower income countries. A timely diagnosis can make the difference between health and illness or mortality; but there still are many diseases that do not have a diagnosis method that answer to public health needs. In the case of animals and plants, early infection detection can prevent irreparable losses in the productive sector. Our objective is to develop and transfer kits - and / or know-how - on diagnostic tests (aimed at humans, animals and plants) that are quick, sensitive, economical and easy to use under any condition. We have created "AMI", an Isothermal Molecular Amplification Platform. Applying it, we have developed innovative diagnostic tests, and started to produce and transfer kits and their Standard Operation Procedures (SOPs) to produce and to use them. "NeoKits" do not require complex purification or processing steps, the process takes less than 90 minutes to yield a result and has greater sensitivity than Real Time PCR at a lower cost. The tests can be done using a simple thermal device -of any kind- (constant temperature at 64 °C by one hour); and be performed by an operator with a basic training who can do the analysis at the same place at which the sample was taken (field conditions or Point Of Care: "POC"). The kits are stable for up to 18 months. "ChagasNeoKit", our first product, detects Chagas in neonates. It specifically gives a positive reaction with the 6 discrete typing units (DTU´s) of T. cruzi and does not cross-react with DNA from other organisms. This NeoKit has successfully completed clinical trials in public hospitals (Durand and Ramos Mejía in CABA and Perrando in Resistencia), becoming de FIRST ARGENTINE REAGENT FOR MOLECULAR DETECTION APPROVED by ANMAT. Its Patent is under revision and transference is being mediated through an agreement between CONICET and a Consortium of Public-Private Partnership.

SAFE AWARD I:

PHARMACOLOGY WORK

Juries - Graciela Balerio | Andrea Errasti | Ventura Simonovich

Chair - María Graciela López Ordieres

0171 - PHARMACOLOGICAL STRATEGIES TO OVERCOME MELPHALAN RESISTANCE IN VITRO

María Belén CANCELA (1) | Ursula WINTER(1) | Santiago ZUGBI(1) | Maria Del Rosario ASCHERO(2) | Mariana SGROI(3) | Claudia SAMPOR(4) | Adriana FANDIÑO(3) | Fabiana LUBIENIECKI(2) | Guillermo CHANTADA(5) | Angel CARCABOSO M.(6) | Paula SCHAIQUEVICH(1)

UNIDAD DE TRATAMIENTOS INNOVADORES, SERVICIO MEDICINA DE PRECISIÓN, HOSPITAL DE PEDIATRÍA GARRAHAN (1); SERVICIO DE PATOLOGÍA, HOSPITAL DE PEDIATRÍA GARRAHAN (2); SERVICIO DE OFTALMOLOGÍA, HOSPITAL DE PEDIATRÍA GARRAHAN (3); SERVICIO DE HEMATO-ONCOLOGÍA, HOSPITAL DE PEDIATRÍA GARRAHAN (4); SERVICIO DE MEDICINA DE PRECISIÓN, HOSPITAL DE PEDIATRÍA GARRAHAN (5); DEPARTAMENTO DE HEMATOLOGÍA Y ONCOLOGÍA PEDIÁTRICA, HOSPITAL SANT JOAN DE DEU (6)

Retinoblastoma (Rb) is the most common intraocular tumor in children. Over the last decade, innovations in Rb treatment lead to an improvement in the rate of ocular survival. However, some eyes experience a relapse after initial response that may be related to acquired drug resistance. If resistance is established, the patient may need higher doses of chemotherapy or to switch to other drugs so as to respond. Thus, our aim was to establish a melphalan-resistant (ML-RE) cell subtype as a preclinical model to characterize the resistance to this drug, cross-resistance to other agents commonly used in Rb, and to evaluate alternative treatments that may allow to overcome this phenomenon including metronomic (MT) treatment with melphalan (MLF) and the cytotoxicity to digoxin (DX) as a repositioning drug. A primary cell culture was established from the tumor biopsy of an upfront enucleated patient with intraocular Rb. The parental cell line (HSJD-RB-7) was exposed to 3 weekly doses of MLF at the IC50. Then, MLF, topotecan (TP) and carboplatin (CP) IC50 was determined in the resistant cell subtype (HSJD-RB-7-MLF) to verify resistance and to test for possible cross-resistance using the MTT assay. Also, MT-MLF (7 days) and DX IC50 was determined in HSJD-RBT-7-MLF cells. Mean (range) MLF IC50 in HSJD-RB-7-ML was 1.3 µM (0.8-1.4), 4-fold higher than in HSJD-RB-7 ($p<0.05$). TP and CP IC50 in HSJD-RB-7-MLF was 2-fold and 3-fold higher than that in HSJD-RB-7, respectively ($p<0.05$). MLF IC50 significantly decreased after metronomic treatment of HSJD-RB-7-ML cells ($p<0.05$). Both parental and resistant cells showed similar DX IC50s ($p>0.05$). We were able to establish a primary cell culture of retinoblastoma with acquired resistance to melphalan. These cells showed cross-resistance to TP and CP but were sensitive to DX and metronomic treatment with MLF.

0348 - DISCOVERY OF NOVEL BOVINE VIRAL DIARRHEA ENTRY INHIBITORS

Emilse LEAL (1) | Natalia ADLER(1) | María José PASCUAL(2) | Manuela MARTINEFSKI(1) | María Eugenia MONGE(1) | Diego ÁLVAREZ(2) | Mariela BOLLINI(1)

CENTRO DE INVESTIGACIONES EN BIONANOCIENCIAS (CIBION), CONSEJO NACIONAL DE INVESTIGACIONES (1); INSTITUTO DE INVESTIGACIONES BIOTECNOLÓGICAS (IIB-UNSAM-CONICET) (2)

Bovine viral diarrhea virus (BVDV) is a pathogen of cattle that causes both acute and persistent infections, leading to substantial financial losses to the livestock industry each year. The global prevalence of persistent BVDV infection and the lack of an antiviral therapy have spurred efforts to discover and develop novel therapies in the pharmaceutical industry. Antiviral targeting of virus envelope proteins is an effective strategy for therapeutic intervention. We performed structure based virtual screening (SBVS) to identify molecules that likely bind to the region delimited by domains I and II of the envelope protein E2 of BVDV. Nineteen structurally different compounds were synthesized and evaluated in a reporter-based assay for antiviral activity. Compound PTC12 was the most active antiviral displaying an IC50 of 0.30 ± 0.13 µM against BVDV and selectivity index= 294. Also, PTC12 blocked virus entry at the stage of internalization. In order to validate the target, we performed selection and sequence analysis of drug-resistant mutants and identified R154Q as a candidate substitution associated to resistance. The mutation is located nearby the proposed binding pocket and MD simulations suggested a stable cation-π interaction between the N+ and the aromatic ring of PTC12. MM/PBSA calculations for the wild type and R154Q mutant indicate that PTC12 complexed with wt-BVDV had the most favorable binding energy (ΔGbind= -29.12 kcal/mol) whereas the complex with R154Q mutant caused a significant energetic change (ΔGbind= -21.6 kcal/mol). The physicochemical properties of PTC12 were evaluated in vitro: solubility was tested at three different pH values, and stability studies in media such as PBS, SIF, SGF, mouse or bovine plasma are currently ongoing. Altogether, we uncovered a novel druggable pocket in BVDV E2 that can be effectively targeted to block virus entry. SBVS against this target led to the identification of PTC12 as a potent BVDV inhibitor.

0370 - INTEGRATED PHARMACOLOGICAL EVALUATION OF THE COMBINATION OF SYNTHETIC ANTHELMINTICS AND BIOACTIVE PHYTOCHEMICALS: IN-VITRO AND IN VIVO STUDIES

María Victoria MIRÓ (1) | Sonia LUQUE(2) | Mercedes LLOBERAS(2) | Livio COSTA-JUNIOR(3) | Carlos LANUSSE(1) | Guillermo VIRKEL(1) | Adrián LIFSCHITZ(1)

CIVETAN (CONICET-CICPBA-UNCPBA), FACULTAD DE CIENCIAS VETERINARIAS, UNCPBA, TANDIL (1); LABORATORIO DE PARASITOLOGÍA, INTA, ESTACIÓN EXPERIMENTAL BALCARCE (2); CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE, UNIVERSIDADE FEDERAL DO MARANHÃO (3)

In a context of increasing anthelmintic resistance, combination of synthetic anthelmintics with bioactive phytochemicals may be a pharmacological tool for improving the nematode control in livestock. The coadministration of natural compounds and anthelmintics may lead to kinetic/dynamic interactions. This work evaluated the drug-drug interactions between synthetic anthelmintics and bioactive phytochemicals at the metabolism and drug transport level. The impact of these interactions on drug efficacy was also studied. Trial 1 include in vitro and in vivo the combination of thymol (TML) and albendazole (ABZ). The stability of TML (800 µg/ml) in sheep ruminal content and its effects (5 mM) on the metabolism of ABZ in sheep liver microsomes were evaluated. The in vivo plasma concentrations and efficacy of the combination were studied in lambs naturally infected with resistant nematodes. TML was stable in sheep ruminal content and inhibited ($p<0.05$) the ruminal sulphoreduction of ABZ sulphoxide. Besides, TML markedly inhibited the hepatic FMO-dependent S-oxygenation of ABZ (54.1 ± 11.6 %, $p<0.05$) and the sulphonation of ABZ sulphoxide ($p<0.05$). However, the in vivo pharmacokinetic changes did not improve the efficacy of ABZ after the combined treatment with TML. In trial 2 the combination of carvone (CVN) and abamectin (ABM) was studied. The modulation of CVN on the P-glycoprotein-mediated transport of Rhodamine 123 (Rho-123) was assessed using the intestinal explants model. The in vivo effect of CVN on ABM kinetic disposition and efficacy was evaluated in lambs infected with resistant nematodes. The presence of CVN increased the Rho-123 accumulation in intestinal explants (60 %, $p<0.05$). In vivo, the coadministration of CVN prolong the absorption half-life of ABM (57 %, $p<0.05$) and increased the efficacy from 94.9 to 99.8 %. In-vitro-in vivo trials are necessary to corroborate the clinical relevance of the combinations of bioactive phytochemicals and anthelmintics.

0605 - GA-RXODE METHOD IN BIOEQUIVALENCE STUDIES

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PRIMERA CÁTEDRA DE FARMACOLOGÍA, FACULTAD DE MEDICINA, UBA

Bioequivalence (Beq) studies are simple in design, expensive in execution, and complicated in some ethical aspects. Because their analytical cost and volunteers' exposition are high, perform less determinations could be optimal; but how manage this without substantial informative loss. Since GA-RxODE method allows reconstruction of missing data, it would be useful in this scenario. This work aimed to demonstrate that the GA-RxODE method is capable to build concentration-time (C-T) curves suitable for Beq studies from few determinations. Unpublished Beq studies (original studies or OrSt) on oral formulations of alprazolam, clonazepam and metformin were employed to provide raw data. Each contained 11 or more C-T determinations per volunteer and round. From those determinations, 5 were selected (1 at time zero; 1 during absorption phase; 1 close to Cmax, and 2 during elimination phase) to recalculate through CA-RxODE the complete sim(ulated)-data C-T curves. From these curves new Beq analysis (derived studies or DeSt) were performed using Grizzle's ANOVA and Schuirmann test and then, they compared with OrSt. Beq between reference product and innovator is claimed if 90 % confidence interval (90%CI) of the geometric means' difference (D) of estimate parameters lies inside 0.80-1.25 range. E.g., alprazolam: area under curve 0-t (AUCt) OrSt D (90%CI)= 1.043 (0.896-1.213); AUCt DeSt D (90%CI)= 1.031 (0.877-1.213); clonazepam: AUCt OrSt D (90%CI)= 1.040 (0.958-1.128); AUCt DeSt D (90%CI)= 1.007 (0.920-1.103); metformin: AUCt OrSt D (90%CI)= 0.914 (0.857-0.975); AUCt DeSt D (90%CI)= 0.965 (0.894-1.041). Because OrSt D fall within the DeSt 90%CI and vice versa, it can be said both type of studies are non-different. The obtained results demonstrated, for the mentioned drugs, there were no differences between the original Beq studies and their derived made with sim-data. Thus, with GA-RxODE method it could be possible to plan safer and less expensive Beq studies.

0895 - THE EFFICACY OF VANCOMYCIN AGAINST STAPHYLOCOCCUS AUREUS IS ENHANCED BY THE ACTION OF A CATIONIC POLYMER

Melisa Belen CORTI | Veronica ROMERO | Fabiana ALOVERO

UNITEFA - CONICET - UNIVERSIDAD NACIONAL DE CÓRDOBA

Reports of occurrence of methicillin-resistant S. aureus (MRSA) strains with resistance to the few available drugs such as vancomycin (VAN), daptomycin, linezolid, tigecycline and teicoplanin, prompt the search for novel treatments options. The last decade has witnessed enormous research focused on cationic polymers for various alternative therapeutic applications. We have previously used EudragitE100 (Eu), a cationic polymer, as a carrier of ionizable drugs which exhibited changes in their physicochemical or biological properties. The antimicrobial efficacy of Eu-VAN (VAN-containing Eu aqueous dispersions) against MRSA was evaluated and studies were conducted to understand the mechanisms involved in the observed effects. Eu-VAN bactericidal activity was evaluated by killing curves. VAN and free-drug Eu were assayed for comparison purposes. Eu-VAN at 4xMIC of VAN caused 99.9 % killing within 360 min and bacterial erradication was observed within 24 h, whereas VAN needed 4-fold higher concentration for the same efficacy. Free-drug polymer (Eu) exhibited limited antimicrobial activity as population of bacteria was still viable after 24 h. Eu produces switch in sign of superficial net charge in S. aureus (Z potential measure) and a concentration-dependent membrane depolarization as determined by flow cytometry using DiBAC4, a potential sensitive probe. In addition, morphological changes were observed and these were confirmed by TEM. Fluorescence microscopy using a fluorescent conjugates of VAN (BODIPY-FL®) allowed to demonstrate increased binding of VAN to S. aureus when bacteria is treated with Eu-VAN as compared to free VAN. The difference was statically significant. The interaction of the cationic polymer with the bacterial cell led to improved antimicrobial efficacy of VAN. This result provides a feasible alternative to avoid or combat antimicrobial resistance. Therefore, more studies are needed to define its potential use.

0954 - ALLOPREGNANOLONE DUAL MODULATES THE SEROTONERGIC AND GABAERGIC SYSTEM IN A RAT AGGRESSION MODEL

Maria Belen MULLE BERNEDO | Sebastina GARCIA | Victor ASTORGA | Ricardo Jorge CABRERA

IMBECU

The serotonergic system is involved in a wide variety of physiological and behavioral functions. Serotonergic axons have been shown to target GABAergic inhibitory neurons and vice-versa. Also, the serotonergic system is influenced by changes in plasma and brain levels of neuroactive steroids. Progesterone derivative, allopregnanolone (Allo) enhances GABAA receptors sensibility, acting as an allosteric modulator on the function of GABA. This receptor acts as heteroreceptor in serotoninergic neurons. Allo, also modulates negatively 5-HT3 receptors. This neurosteroid influences a wide range of behaviors, among others, like aggressive behavior in rodents. This work aimed to evaluate modulatory Allo effects in an aggressive behavior rat model. Male Sprague-Dawley rats 60 days old were used. On a postnatal day 60 (PND), the rats were cannulated in the 3rd ventricle (icv). On PND 66, the rats received once pCPA (300 mg/kg, i.p) injection in order to generate aggressive behavior. On PND 72, the rats were divided randomly into groups, 1) Allo; 2) Bicuculine (Bic)+Allo; 3) Bic 4) 5-HT 5) Allo+ 5-HT. Moreover, 30` before resident intruder test (RVI) receive the drug icv. The behavioral activity of all groups was video recorded and was analyzed by the researchers. Aggressive behavior was evaluated as the presence of tromping, bites, attempted mounts, and lateral threats (AB). We also measured non-social interaction (lying and sitting), social interaction (sniffing and grooming) and locomotor activity. All data were expressed as a mean+ SEM and analyzed by ANOVA I and Tukey post hoc test. Allo positively modulates the GABAergic system by decreasing aggressive behavior ($p< 0.01$). This decrease was reversed by the blockage of this system with Bicuculin ($p < 0.01$). The administration of 5HT icv did not modify the aggressive behavior induced by pCPA depletion. Moreover, the previous administration of Allo to 5HT icv significantly increased this behavior ($p< 0.05$). We conclude that Allo is a neurosteroid modulator of aggressive behavior in rats. This modulatory effect would be mediated by GABAergic and serotonergic mechanisms oppositely, thus proposing a duality in its modulatory capacity not described above, for this type of aggressive behavior in rats

SAFE AWARD II

PHARMACOLOGY RESEARCH

Juries - Alicia Fuchs | Adrian Lifschitz | Victoria Lux-lantos | Miriam Wald

Chair - Carlos Reyes Toso

1036 - DIISOPROPYLPHENYL-IMIDAZOLE (DII): A NEW COMPOUND THAT EXERTS ANTHELMINTIC ACTIVITY THROUGH NOVEL MOLECULAR MECHANISMS.

María Gabriela BLANCO (1) | María Soledad VELA GUROVIC(2) | Gustavo Fabián SILBESTRI(3) | Andrés GARELLI(1) | Sebastián GIUNTI(1) | Diego RAYES(1) | María José DE ROSA (1)

INIBIBB-CONICET, DEPTO. BIOLOGÍA, BIOQUÍMICA Y FARMACIA-UNS (1); CERZOS-CONICET, DEPTO. BIOLOGÍA, BIOQUÍMICA Y FARMACIA-UNS (2); INQUISUR, DEPARTAMENTO DE QUÍMICA, UNIVERSIDAD NACIONAL DEL SUR (UNS)-CONICET (3)

Nematode parasites cause infections that affect approximately one-third of the world´s population and considerable losses in livestock and food crops. Paradoxically, the repertoire of effective anthelmintics for treating these parasitoses is very limited, as drug development has been delayed for decades. Moreover, resistance to currently available drugs is a global concern in livestock parasites and is an emerging issue for human helminthiasis. Therefore, anthelmintics with novel mechanisms of action are urgently needed. Taking advantage of Caenorhabditis elegans as an established model system for developing agents, in this project we synthesized and screened the anthelmintic potential of novel imidazolium and imidazole derivatives. We found that one of these derivatives, diisopropylphenyl-imidazole (DII), is lethal to C. elegans at both mature and immature stages. Toxicity appears to be specific because DII concentrations which are lethal to C. elegans do not induce significant lethality on bacteria, Drosophila melanogaster, and HEK-293 cells. Our analysis of DII action on C. elegans mutant strains determined that, in the adult stage, null mutants of unc-29 are resistant to the drug. Muscle expression of this gene completely restores DII sensitivity. UNC-29 was reported as an essential constituent of the levamisole-sensitive muscle nicotinic receptor (L-AChR). Nevertheless, null mutants in unc-63 and lev-8 (essential and non-essential subunits of L-AChRs, respectively) are as sensitive to DII as the wild-type strain. Therefore, our results suggest that DII effects on adult nematodes rely on a previously undescribed AChR. This novel AChR is composed by UNC-29 (a non-alfa subunit incapable of forming homomeric receptors) and other unidentified subunits. To completely elucidate its stoichiometry, we are analyzing DII resistance in different strains containing null mutations in AChR subunits. Since DII mechanism is different from those of currently used anthelmintics, it could constitute a therapeutic option when traditional anthelmintic agents fail. Interestingly, DII targets appear to be different between larvae and adults, as unc-29 null mutant larvae are sensitive to the drug. The existence of more than one target could delay resistance development. The specificity and novel mode of action of DII, which includes differential targeting in larvae and adult nematodes, support its potential as a promising drug candidate to treat helminthiasis.

1037 - ISCHEMIC CARDIOMYOPATHY AND THYROID ALTERATIONS: FROM THE ENERGETICS OF CALCIUM HOMEOSTASIS TO CARDIOPROTECTION IN RAT CARDIAC MODELS.

Matías BAYLEY(1) | Sofía LÓPEZ(1) | **María Inés RAGONE** (1) | COLABORADORES: Alicia CONSOLINI(1) | Patricia BONAZZOLA(2)

CÁTEDRA DE FARMACOLOGÍA; DEPARTAMENTO DE CS BIOLÓGICAS; FACULTAD DE CS. EXACTAS; UNLP (1); INSTITUTO DE INVESTIGACIONES CARDIOLÓGICAS, FACULTAD DE MEDICINA, UBA-CONICET (2)

Thyroid diseases affect cardiac Ca^{2+} homeostasis and induce long-term pathologies. The consequences of cardiac ischemia-reperfusion (I/R) are still worse in a hyper- or a hypothyroid patient. The aim of this project is to characterize the myocardial mechanisms of hyperthyroidism (HypT) and hypothyroidism (HypoT) in the postischemic dysfunction, especially the mitochondrial role in two models of stunning due to I/R. HypT rats were obtained by daily SC injection of triyodothyronine, and HypoT rats by drinking methymazol, both during 15 days. Results were compared with euthyroid rats (EuT). Ventricles were perfused in a flow calorimeter and exposed to one of two models of I/R: moderate (20 min I) or severe (30 min I) followed by 45 min R (mI/R or sI/R, respectively). Intraventricular contractile pressure (P, mmHg), diastolic contracture (ΔLVEDP) and total heat rate (Ht, mW.g-1) were measured, and the total muscle economy (Eco= P/Ht) was calculated. HypT was cardioprotector in mI/R because it increased the post-ischemic contractile recovery (PICR) and Eco with low ΔLVEDP. Clonazepam (Clzp, inhibitor of mitochondrial Na^+/Ca^{2+}-exchanger, mNCX) or 5-hydroxidecanoate (5-HD, blocker of mitochondrial ATP dependent-K^+ channels, mKATP) reduced the PICR and Eco in HypT but not in EuT. Ru-360 (blocker of mitocondrial Ca^{2+} uniporter, UCam) strongly reduced PICR and Eco in both hearts. HypT was not cardioprotector in sI/R. It was reversed by Cys-A (inhibitor of mitochondrial permeability transition pore, mPTP). HypoT was cardioprotector in mI/R and sI/R. In models, mI/R and sI/R, HypoT improved PICR and Eco and reduced ΔLVEDP, but Clzp reversed these effects. In sI/R, 5-HD, wortmanine (PI3K/Akt inhibitor) and chelerythrine (PKC inhibitor) worsened PICR and Eco in HypoT. L-NAME (NOS-inhibitor) was cardioprotector. However, adrenaline reduced the HypoT cardioprotection, and it was prevented by oral 20 mg/kg/day carvedilol (β-blocker). Conclusions: a) The HypT was cardioprotector only in mI/R, and it was due to activation of mNCX and mKATP which reduced Ca2+ overload, while in sI/R the mPTP opening cause dysfunction; b) The HypoT was cardioprotector in both models of I/R. In sI/R, cardioprotection was related to activation of PI3K/Akt and PKC pathways and reduction of Ca^{2+} overload; c) The NOS-activation and adrenaline perfusion avoided cardioprotection, but carvedilol prevents the adrenergic dysfunction.

Supported by UNLP X-795 grant.

1038 - NON-CONVENTIONAL CITOPROTECTIVE EFFECTS OF DRUGS AGAINST EXPERIMENTAL MODELS OF SEPSIS.

Julieta RONCHI | Gabriela OLEA | **Tania STOYANOFF** | COLABORADOR: María Victoria AGUIRRE

LABORATORIO DE INVESTIGACIONES BIOQUÍMICAS, HOSPITAL ESCUELA "JOSÉ DE SAN MARTÍN". UNNE.

Currently, sepsis is defined as an organic dysfunction caused by a deregulated host response to infection and is one of the main causes of morbi-morbility in intensive care units. This pathogenesis is complex, multifactorial and is associated with organic dysfunction and inflammation, hypoxia, microvascular disorders and injury due to apoptosis. This highlights the importance of studying new therapeutic strategies to mitigate these effects. The preliminary work of our group showed that the administration of recombinant Erythropoietin (EPOrh) attenuates cardiac lesions in a murine model of Doxorubicin-induced injury, improving both; the tisular damage and the enzymatic changes. On the other hand, there were several drugs, such as Eporh, that exert histoprotective, anti-apoptotic, anti-inflammatory, proangiogenic and/or antioxidant actions in experimental models of injury. In this sense, our field of research aims to dilucidate the cellular and molecular non-canonical mechanisms of various drugs involved in the histoprotection in pre-clinical models of sepsis. Thus EPOrh, sildenafil and dexmedetomidine (DEX) were used in experimental sepsis induced by lipopolysaccharide (LPS) and cecal ligation and puncture (CLP). We have demonstrated that the administration of EPOrh has renoprotective effects in an LPS-induced AKI model through several mechanisms: a) anti-

apoptotic: by modulating the intrinsic and extrinsic pathways; b) pro-angiogenic: increasing the VEGF/VEGFR-2 pair and the expression of PeCAM-1; c) anti-inflammatory/antioxidants: by decreasing iNOS expression and inflammatory infiltration and d) attenuation of tisular hypoxia by decreasing the expression of HIF-1α. These effects are associated with the overexpression of EPO-R in the hypoxic context of the injured renal tissue. Likewise, EPOrh attenuates both pulmonary and renal injury in an experimental CLP model through the modulation of the EPO/EPO-R and VEGF/VEGF-R2 systems. Concurrently, we determined that sildenafil also mitigates pulmonary injury (ALI) and acute renal injury (AKI). Furthermore, we have established the protective dose of DEX and verified significant improvements in renal and hepatic functionality post- endotoxemia. In addition, DEX attenuates histopathological alterations in kidney, lung and liver, through the modulation of Bax/BclxL expressions. Currently, we are focused in the study of the additional molecular mechanisms involved in the histoprotection by DEX. Our next purpose in this field is the study of flavonoid's cytoprotective effects in these experimental sepsis models from native species of the Northeastern of Argentine for their potential application in phytomedicine.

RESÚMENES DE LAS COMUNICACIONES

Cardiovascular y Respiratorio / Cardiovascular and Respiratory I

Chairs: Bruno Buchholz | Nicolás Kouyoumdzian | Ana María Puyó

0165 - EFFECT OF THE INFUSION OF LIGARIA CUNEIFOLIA OR ARGENTINE MISTLETOE ON THE LIPID PROFILE AND HEMORRHEOLOGICAL PARAMETERS IN PATIENTS WITH HYPERCHOLESTEROLEMIA

Mariana Paula FERRERO (1) | Maria José SVETAZ(2) | Constanza GIACOSA(1) | Marcelo WAGNER(3) | Juan BELOSCAR(4) | Cristina Ester CARNOVALE(5) | Alejandra Nora LUQUITA(1)

CÁTEDRA DE BIOFÍSICA, DEPARTAMENTO DE CIENCIAS FISIOLÓGICAS, FACULTAD DE CIENCIAS MÉDICAS, UNR (1); LABORATORIO CENTRAL HOSPITAL PROVINCIAL DEL CENTENARIO (2); CÁTEDRA DE FARMACOBOTÁNICA. FACULTAD DE FARMACIA Y BIOQUÍMIA. UNIVERSISAS DE BUENOS AIRES. (3); SERVICIO DE CARDIOLOGÍA. HOSPITAL PROVINCIAL DEL CENTENARIO. (4); INSTITUTO DE FISIOLOGÍA EXPERIMENTAL. FACULTAD DE FARMACIA Y BIOQUÍMICA. UNIVERSIDAD NACIONAL DE ROS (5)

Ligaria cuneifolia (Lc) is a plant used in Argentine folk medicine to lower excess cholesterol and increase blood flow, improving the hemorrological profile. Previously we observed that intraperitoneal Lc-treatment in Wistar rats, leads to a decrease in plasma cholesterol levels (Cho) and has a dose-dependent effect on blood viscosity. The objective was to analyze the effect of the infusion of Lc on the lipid profile and hemorrheological parameters in patients with hypercholesterolemia. Eleven patients of both sexes (6 females, 5 males) were studied, prior to signing an informed consent. All received envelopes with dried extract of leaves and stems of Lc (2.6 gr each) and instructive to prepare the infusion to be ingested three times a week, for two months. Determinations made in blood: Total Cho, ChoHDL and ChoLDL, triglycerides (TG), hepatogram (glutamic-oxalacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) (all by autoanalizer), relative blood viscosity (with rotational viscometer), and erythrocyte rigidity index (RI) (by filtration method), at 0 (Baseline) and 60 days (post-treatment with Lc). Results: Mean ± SEM, n= 11, B: baseline, TLc: post-treatment with Lc, *p<0.05 vs. B, ns= no significant vs. B (t-Test for paired data). Cho (mg %): B: 257 ± 11; TLc: 241 ± 8*. ChoLDL (mg %): B: 168 ± 13; TLc: 158 ± 10*. ChoHDL (mg %): B: 70 ± 7; TLc: 67 ± 7, ns. Triglycerides (mg %): B: 114 ± 18; TLc: 106 ± 15, ns. Relative blood viscosity (centipoisse): B: 3.5 ± 0.3; TLc: 3.6 ± 0.4, ns. RI: B: 7.8 ± 1.1; TLc: 8.1 ± 1.2, ns. GOT (IU/l): B: 19 ± 2; TLc: 19 ± 1, ns. GPT (IU/l): B: 20 ± 2; TLc: 18 ± 2, ns. No differences between sexes. In the patients evaluated, with the analyzed dose, there were no changes in the hemorrheological parameters or alterations in transaminases. Besides, we obseved a decrease in both Cho (-10 %) and ChoLDL (-8.4 %), suggesting that an increase in the dose of Lc could lead to a marked decrease in both parameters, which would support the Lc treatment as an important tool in lipid lowering.

0185 - ACUTE TREATMENT WITH TRIIODOTHYRONINE (T3) ATTENUATES POSTISCHEMIC MITOCHONDRIAL INJURY: A RESPONSE ASSOCIATED WITH ENHANCED AMP-ACTIVATED PROTEIN KINASE (AMPK) ACTIVATION

Romina HERMANN | María de Las Mercedes FERNÁNDEZ PAZOS | Federico REZNIK | Mailen CÓRDOBA | Victoria MESTRE CORDERO | Débora VÉLEZ | Andrea FELLET | María Gabriela MARINA PRENDES

CÁTEDRA DE FISIOLOGÍA, FACULTAD DE FARMACIA Y BIOQUÍMICA, UBA - IQUIMEFA (CONICET-UBA)

Recent studies have provided evidence that acute treatment with T3 could enhance the recovery of ischemic myocardium through the preservation of mitochondrial function and the improvement of energy substrate metabolism. To this respect, our previous results showed that T3 enhanced the activation of AMPK, a key enzyme that regulates the cellular energy metabolism, during Is-Rs, which was prevented by AMPK pharmacological inhibitor, Compound C (CC; 10 µM). During reperfusion, T3 increased contractile function recovery, mitochondrial ATP production and tissue ATP, effects that were reverted by CC. The aim of the present study was to investigate the effects produced by the acute treatment with T3 (60 nM) and AMPK inhibitor, in mitochondria of isolated rat left atria subjected to 75 min simulated ischemia (Is)–75 min reperfusion (Rs). ANOVA, followed by Tukey's test was used, n= 8/group. The results showed that mitochondrial ultrastructure, analyzed by electron microscopy, was better preserved in the atria subjected to Is-Rs in the presence T3, effect that was reverted by CC. Moreover, calcium retention capacity (CRC), defined as the amount of Ca^{2+} required to trigger a massive Ca^{2+} release by isolated mitochondria, was increased by acute treatment with T3, effect that was reverted by CC (Is-Rs: 77 ± 9, Is-Rs+T3: 114 ± 12*, Is-Rs+T3+CC: 82 ± 8 nmol/mg protein; *p<0.05). As accumulating evidence suggests that the phosphorylation and inhibition of GSK-3ß; acts as a master switch to limit the mPTP opening, improving mitochondrial recovery function, the relation between phosphorilated/total GSK-3ß was assessed. Results showed that T3 increased this relation, which was prevented by CC (Is-Rs: 1.5 ± 0.2, Is-Rs+T3: 2.2 ± 0.1*, Is-Rs+T3+CC: 1.6 ± 0.2 AU; *p<0.05). The results suggest that AMPK is involved, at least in part, in the protective effects exerted by T3 at mitochondrial level in the myocardium subjected to ischemia-reperfusion, contributing to mitochondrial structure and function preservation.

0244 - ANGIOGENESIS REGULATION: DIFFERENT MECHANISM OF ACTION ELICITED BY PROGESTERONE (PG) AND MEDROXYPROGESTERONE ACETATE (MPA)

Pablo Hernán CUTINI | Virginia MASSHEIMER

INSTITUTO DE CIENCIAS BIOLÓGICAS Y BIOMÉDICAS DEL SUR (UNS-CONICET)

In atherosclerosis, the generation of microvessels within the plaques represents a survival option for damaged tissue but would also be associated with the instability of the plaque. The risk/benefit of hormone replacement therapy using natural or synthetic progestins such MPA as an alternative to prevent cardiovascular diseases in menopausal women is controversial. The aim of this work was to evaluate the mechanism of action by which Pg and MPA regulate angiogenesis. Tube formation assay and endothelial cell (EC) culture derived from murine aorta were used to evaluate angiogenesis. Total tube length of vessel segments was quantified using ImageJ software. Both progestogens significantly enhanced the number of tube structures (26 %; 46% above control, 100 nM Pg; 100 nM MPA respectively, p<0.05). Firstly, we tested the participation of Pg receptor (PgR). Pre-tre atment of EC with RU486, an antagonist of PgR, completely inhibited the proangiogenic effect of Pg and MPA. Considering that VEGF is the main regulator of angiogenesis, we neutralize its action with a VEGF antibody (a-VEGF). Besides, we used the compound genistein to block the tyrosine kinase activity of VEGF receptor (VEGFR). The presence of a-VEGF or genistein abrogates the proangiogenic action of Pg. Meanwhile, the effect of MPA was not modified. Nitric oxide synthase (NOS) is involved in VEGFR downstream signaling pathway. In the presence of L-NAME, a NOS inhibitor, the stimulation of tube formation induced by Pg was blunted. Meanwhile, MPA action was not affected. The proangiogenic action of Pg was not altered by the presence of platelet-rich-plasma (PRP)-derived plasma. Instead, the MPA action was potentiated (29 % vs.

100 nM MPA, p<0.05). We demonstrated that 100 nM Pg markedly increased VEGF synthesis (39 % vs. control, p<0.05). In contrast, MPA (100 nM) did not affect VEGF production. In conclusion, both progestogens promote angiogenesis with a slight different mechanism of action elicited by each steroid.

0301 - IDENTIFICATION OF CARDIOPULMONARY DISEASES BY THE PERFUSION INDEX IN THE 6-MINUTE WALK DISTANCE TEST

Carmen Beatriz GÓMEZ (1) | Elena Catalina LASCANO(2) | Miriam DI LORETTO(1) | Alejandro M BERTOLOTTI(1) | Jorge Antonio NEGRONI(2)

HOSPITAL UNIVERSITARIO FUNDACIÓN FAVALORO (1); IMETYB, UNIVERSIDAD FAVALORO (2)

The distance walked in the 6-minute walk distance test (6MWD) identifies healthy subjects (HS) from patients with cardiopulmonary diseases, but it can be influenced by factors independent of the underlying pathology, such as musculoskeletal disorders, frailty and lack of training. The perfusion index (PI) measures the pulsatile force at the control site and is an indirect assessment of peripheral perfusion, providing a more reliable evaluation of the response to the 6MWD. The objective of this study was to assess the ability of PI to detect patients with heart failure (HF, n= 37), pulmonary hypertension (PHT, n= 36) and chronic obstructive pulmonary disease (COPD, n= 93) from HS (n =36) in the 6MWD. O_2 saturation (O2Sat), heart rate (HR), the Borg scale (BS) and PI were measured at baseline, and during the 6MWD and 3 min recovery, and the total distance walked at the end of the exercise test. PI was calculated as the absorbed light amplitude of arterial blood (AC)-to-non-pulsatile blood and other tissues ratio (DC) (PI= AC/DC*100). The study was approved by the institutional Ethics Committee. In baseline conditions PI was significantly different between HS and COPD (p<0.01, ANOVA), whereas HR and O2Sat did not present clinically relevant differences between HS vs. any of the other groups. In the 6MWD, HF, PHT and COPD patients walked approximately 25 % less than HS with progressively worse BS (p<0.01, respectively). O2Sat changed <3 % between groups, though significantly between PHTand HS (p<0.01) during the 6MWD, while HR was significantly different only between HF and HS (p<0.01). PI was the only index that provided a physiological insight of the 6MWD response between healthy and pathological individuals with the progression of exercise and during recovery (p<0.01 vs. HF, PHT and COPD). A lower response of the microvascular system to exertion might explain the lower distance walked and worse BS of patients with cardiopulmonary diseases.

0475 - COMPLEX I, NO AND H2O2 AS EARLY MARKERS OF HEART MITOCHONDRIAL DYSFUNCTION IN A TYPE 1 DIABETES MELLITUS MODEL

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Sustained hyperglycemia (25 days) leads to heart mitochondrial dysfunction in the absence of changes in resting cardiac performance (Bombicino et al., 2016, 2017) suggesting that mitochondrial impairment precedes the onset of diabetic cardiac failure. Our aim was to study the early events that take place in heart mitochondrial dysfunction in a model of Type 1 Diabetes Mellitus (DM), in which hyperglycemia happens without the presence of insulin resistance, obesity, hypercholesterolemia and hypertension. Diabetes was induced by a single dose of Streptozoticin (STZ, 60 mg/kg, i.p.) in male rats. Glycemia (mg/dl) was determined after 72 h (C: 130 ± 5; DM: 415 ± 23). The animals were sacrificed at day 10 or 14 after injection. Oxygen consumption, respiratory complexes activities, hydrogen peroxide (H_2O_2) and nitric oxide (NO) production, and NOS expression were determined in heart mitochondrial fraction. PGC-1alpha expression was measured in heart homogenate. State 3 oxygen consumption sustained by malate-glutamate (22 %) or by succinate (16 %), and complexes I-III (26 %), II-III (24 %) and IV (20 %) activities were lower in DM group, when animals were sacrificed at day 14 (11 days of hyperglycemia). These results were similar to those obtained after 25 days of hyperglycemia. In contrast, when animals were sacrificed at day 10 (7 days of hyperglycemia), only the state 3 respiration sustained by malate-glutamate (22 %) and its corresponding respiratory control (39 %) were lower in diabetic rats, in accordance with complex I-III activity reduction (19 %). Moreover, mitochondrial H_2O_2 (96 %) and NO (25 %) production rates and mtNOS expression (79 %) were higher in DM group after 7 days of hyperglycemia. While PGC-1alpha expression increased in diabetic animals (76 %) when the hyperglycemia was sustained by 25 days, it was similar between groups after 7 days of hyperglycemia, suggesting that mitochondrial biogenesis was not triggered yet. Complex I, NO and H_2O_2 could be considered early markers of cardiac mitochondrial dysfunction. In addition, NO and H_2O_2 appear to be molecules located upstream de novo synthesis of mitochondria, in response to hyperglycemia.

0527 - POSTNATAL HYPOTHYROIDISM: ALTERATIONS IN NITRIC OXIDE SYSTEM IN LEFT VENTRICLE CARDIOMYOCYTES

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We previously demonstrated that hypothyroidism reduced cardiac contractility decreasing Ca^{2+} transient amplitude and sarcoplasmic-reticulum Ca^{2+} content in isolated cardiomyocytes. This negative inotropic effect was associated with an increased cardiomyocyte relaxation as revealed by a reduction in the time to 50 % relengthening. The aim of this study was to examine whether these cardiac function alterations involve changes in left ventricle nitric oxide system, caveolins 1/3 and /or Akt protein levels. Male Sprague–Dawley rats weighing approximately 50 g were randomly assigned to one of the two experimental groups: (1) euthyroid rats (received s.c. injections of 0.9 NaCl (0.1 ml/100 g body weight) or (2) hypothyroid rats (received 0.02 % methimazole in drinking water during 60 days). Heart function was evaluated by echocardiography. Measurements of arterial blood pressure, heart rate, nitric oxide synthase (NOS) activity and protein levels of NOS, Cav 1-3 and Akt were performed. Hypothyroid animals had decreased fractional shortening and ejection fraction and increased left ventricle internal diameter. While perinatal hypothyroidism increased total NOS activity, the protein levels of the different isoenzymes were not modified. Both total Akt and phosphorylated Akt protein levels were increased in hypothyroid rats. Caveolin-1 and 3 protein content were not affected by the treatment. We speculate that the reduction in contractility and relaxation time observed in hypo rats might be attributed to the increase in NO production, explaining the decrement of the EF observed in hypo rats. Akt pathway could be related to the rise of NO synthase activity, altering cardiac function during this thyroid disorder.

0577 - THE ANTIARRYTHMIC AMIODARONE PROTECTS EUTHYROID HEARTS AGAINST CARDIAC STUNNING BY ISCHEMIA/REPERFUSION, BUT NOT THE HYPOTHYROID ONES: ENERGETIC STUDY.

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CÁTEDRA DE FARMACOLOGÍA; DEPARTAMENTO DE CS BIOLÓGICAS; FACULTAD DE CS. EXACTAS; UNLP (1);

CÁTEDRA DE FARMACOLOGÍA; DEPARTAMENTO DE CS. BIOLÓGICAS; FACULTAD DE CS. EXACTAS; UNLP/CONICET (2)

We previously showed that hypothyroidism reduced the stunning in two cardiac ischemia-reperfusion (I/R) models. It is associated with the reduction of mitochondrial Ca^{2+} overload due to activation of the mKATP channels and the role of the PI3K/Akt and PKC antiapoptotic pathways (SAFE 2018). It is known that the antiarrhythmic amiodarone (Amd) clinically worsens the hypothyroid state (HypoT), by which we expected that it affects the postischemic cardiac dysfunction in HypoT, a point not previously studied. The current aim was to evaluate whether Amd either orally administered or ex vivo perfused, prevents the mechano-energetic dysfunction of hearts from euthyroid (EuT) and HypoT rats under I/R (30 min I/45 min R). Rats made HypoT by drinking methimazole (0.02 %) for 15 days. Isolated hearts were perfused in a flow calorimeter and stimulated at 3 Hz. Contractile intraventricular pressure (P, mmHg), total heat flow (Ht, mW/g) and diastolic pressure (LVEDP) were measured. In EuT, oral Amd 30 mg/kg/day for 7 days successfully improved the post-ischemic contractile recovery (PICR) to 59.3 ± 7.5 % of initial P ($p<0.05$ vs. 11.5 ± 4.7 % in EuT, n= 5-5) and muscle economy (P/Ht) to 3.4 ± 1.2 mmHg.g/mW ($p<0.05$ vs. 1.0 ± 0.4 in EuT). But in HypoT oral Amd did not modify the HypoT cardioprotection (PICR of 59.2 ± 16.9 vs. 53.9 ± 4.8 % in HypoT, and P/Ht of 2.8 ± 0.8 vs. 2.8 ± 0.35 mmHg.g/mW, n= 5-5). However, when Amd was perfused ex vivo at 5 µg/ml, the PICR was reduced in HypoT hearts to 20.5 ± 4.1 % (n= 5) and P/Ht to 0.88 ± 0.3 mmHg.g/mW ($p<0.05$ vs. HypoT). But in EuT hearts perfused Amd did not induce postischemic changes. In both types of administration, Amd increased LVEDP in EuT and HypoT hearts. Summing up a) Oral Amd prevents severe I/R dysfunction only in EuT hearts, not adding more protection to HypoT ones; b) Amd directly perfused only evidenced its Ca^{2+} channels inhibition; c) Cardioprotection of oral Amd could be due to induction of a "cardiac hypothyroid effect".

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0901 - SEX DIFFERENCES IN BLOOD PRESSURE RESPONSE TO CONTINUOUS ANG II INFUSION: ARE SEX HORMONES THE ONLY ONES TO BLAME FOR SUCH DIFFERENCES?

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INSTITUTO DE INVESTIGACIÓN MEDICA MERCEDES Y MARTÍN FERREYRA - INIMEC-CONICET-UNC (1); CÁTEDRA DE FISIOLOGÍA ANIMAL. FCEFYN - UNIVERSIDAD NACIONAL DE CÓRDOBA (2)

Evidence demonstrate that the pressor response to Ang II infusion is sexually dimorphic under physiological and pathophysiological circumstances. But why do male and female show differences in rennin angiotensin system (RAS) activation and inhibition? Sex steroids can induce organizational (long-lasting or permanent) effect during critical periods of development but can also impart (temporary or reversible) activational effects. Furthermore, males and females also carry different sex chromosome complements (SCC:XY/XX) and thus are influenced throughout life by different genomes. Previous evidence demonstrates a modulatory effect of SCC in RAS receptor expression (brain and renal), as well as in the Ang II sexually dimorphic bradycardic barroreflex and hypertensive responses. In the present study we evaluated the involvement of SCC, organizational and activational hormone effect on changes in mean arterial pressure (MAP) in a 10 min Ang II infusion protocol. For this purpose, we used gonadectomized (Gdx) mice of the "four core genotype" model, in which the effect of gonadal sex and SCC is dissociated, allowing comparisons of sexually dimorphic traits between XX and XY females as well as in XX and XY males. For hormonal replacement experiments Gdx mice were daily injected with ß-estradiol or testosterone propionate (2 ug/g) for a 4 day period. The statistical analysis reveals an interaction of SCC, organizational and activational hormonal effect during Ang II infusion {$F(7.39=2.60, p<0.01)$}. Our results indicate that in absence of the activational hormonal effects an interaction between the SCC and the organizational hormonal action differently modulate changes in the arterial pressure. Furthermore, estrogen and testosterone exert important activational effects on changes in MAP during Ang II acute continous infusion. Thus, our data demonstrate the contribution and interaction of SCC, activational and organizational hormonal effects in sex differences in blood pressure regulation.

Neurociencias / Neurosciences I

Chairs: Fernando Correa | Flavia Saravia

0027 - INSULIN RECEPTOR ACTIVATION EFFECTS ON SYNAPTOSOMAL 2-AG HYDROLYSIS IN AN AMYLOIDOSIS MODEL INDUCED BY Aß OLIGOMERS

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INIBIBB-CONICET, DEPTO. BIOLOGÍA, BIOQUÍMICA Y FARMACIA-UNS

Insulin (Ins) plays an important role in synaptic plasticity and is tightly related to Alzheimer´s disease (AD). Aß oligomers (OAß), which are responsible for synaptic dysfunction in AD, can bind to Ins receptor (IR) and can therefore be internalized into neurons. OAß also disrupt the synaptic membrane and diminish 2-AG availability, the main neuroprotective cannabinoid. Ins can prevent OAß binding to IR, thus attenuating its neurotoxicity. Here, we hypothesized that Ins prevent OAß deleterious effects on 2-AG metabolism. To this end, we isolated cerebral cortex synaptosomes (syn) by differential centrifugation purified in ficoll gradients and preincubated them with 10 µM LY294002 (phosphatidylinositol-3-kinase -PI3K- inhibitor) or 100 µM genistein (tyrosine kinase inhibitor) for 10 min, and subsequently incubated with 0.2 mM vanadate (protein-tyrosine phosphatase inhibitor), 100 nM Ins, or 0.2 mM vanadate plus 100 nM Ins, for 30 min. Syn were then incubated for 10 min with or without 0.1 µM OAß. After this incubation, activation of IR signaling by Western blot, released LDH activity, and 2-AG hydrolysis activity were evaluated. It was observed that a 30 min incubation with Ins and vanadate activated IR and Akt ($p<0.05$). The subsequent incubation with OAß did not alter IR activation ($p>0.05$). As to syn membrane damage, neither of the pretreatments could prevent OAß effect on LDH release ($p>0.05$). On the other hand, Ins and vanadate decreased 2-AG hydrolysis ($p<0.01$) and their effect was not observed if syn were preincubated with LY ($p>0.05$). However, in the presence of OAß, Ins and vanadate failed to alter 2-AG hydrolysis ($p>0.05$) and LY increased this activity ($p<0.001$). Our results show a regulation of 2-AG hydrolysis by Ins, possibly increasing its availability via IR and involving PI3K pathway, which is abolished by OAß. The effect of OAß appears to be independent of IR and to involve PI3K activity. Ins also failed in preventing OAß damage in synaptic membrane.

0037 - SPINAL CORD INJURY DRIVES CHRONIC HIPPOCAMPAL CHANGES

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After spinal cord injury (SCI), patients exhibit cognitive deficits that could be related to hippocampal alterations. The objectives of this work were:1) to determine which step in the neurogenic process was altered after chronic SCI; 2) to explore the role of acute glucocorticoids (GC) and transneuronal degeneration in chronic neurogenesis reduction after SCI; 3) To evaluate cognitive hippocampal dependent-tasks after chronic SCI. In order to

perform the first objective, we used Nestin-GFP mice combined with multiple immunolabeling (BrdU, GFAP, doublecortin DCX) and confocal microscopy. Survival and mitotic capability of neural stem cells (NSCs, Nestin-GFP+/GFAP+) and amplifying progenitors (ANP, Nestin-GFP+/GFAP-) were assessed by labeling these cells with BrdU. The number of DCX + cells together with mitotic NSCs and ANPs was downregulated after 60 days post-injury (dpi) ($p<0.05$, SCI vs. sham). To comply with the second objective, GC action was blocked using the GC receptor antagonist, RU-486 during the acute phase and neurogenesis was measured 60dpi. This result implied acute GC in chronic neurogenesis reduction since the number of DCX + cells was restored after RU-486-treatment ($p<0.01$, SCI vs. SCI+RU486). On the other hand, spinal cord hemisection was performed in order to lacerate axons of only one side. Afterwards, neurogenesis 60 dpi was measured in the ipsilateral and contralateral hippocampus. Neurogenesis decreased in the contralateral side with respect to the ipsilateral side ($p<0.05$), which would involve transneuronal degeneration in this downregulation. To achieve the last objective, cognitive hippocampal dependent-tasks were evaluated using the novel object recognition and Y-maze test. After SCI, animals showed deficits in recognition ($p<0.01$, SCI vs. Sham) and spatial working memory ($p<0.01$, SCI vs. sham) 60 dpi. These results support that acute GC and transneuronal degeneration caused chronic neurogenesis reduction that could lead to cognitive impairments

0041 - GENISTEIN RESTORES HIPPOCAMPAL ABNORMALITIES AND CONGNITIVE IMPAIRMENT IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

Santiago RONCHETTI | Florencia LABOMBARDA | Paulina ROIG | Analia LIMA | Alejandro F. DE NICOLA | Luciana PIETRANERA

INSTITUTO DE BIOLOGÍA Y MEDICINA EXPERIMENTAL (IBYME-CONICET)

Hippocampal neuropathology is a recognized feature of the spontaneously hypertensive rat (SHR). Previous studies have found abnormalities in the hippocampus of SHR consisting of decreased neurogenesis and astroglial and microglial reactivity. These abnormalities are reversed by exogenous administration of estradiol, an active neuroprotective and hypotensive factor. Also, these hippocampal alterations associate with cognitive impairment. We have recently demonstrated that both types of estradiol classical receptors (ERα and ERß) and the GPER membrane receptor are involved in the neuroprotective action of estradiol. Genistein is a phytoestrogen which binds to ERß and GPER and it is known to have neuroprotective actions. To elucidate if this phytoestrogen exerts neuroprotective effects in hypertensive encephalopathy, we treated 5 month old SHR during 2 weeks with 10 mg/kg daily s.c. injections of genistein. We measured the expression of DCX+ neuronal progenitors and GFAP+ astrocytes in the hippocampus by immunocytochemistry. Furthermore, we evaluated hippocampal dependent memory using the novel object recognition test (NOR). We found that SHR showed decreased number of DCX+ neural progenitors in the dentate gyrus and treatment with genistein increased this parameter ($p<0.05$). Expression of GFAP was increased in the hilus and genistein decreased astrogliosis ($p<0.05$). Time exploring the novel object in the NOR was increased in SHR and treatment with genistein decreased the elapsed time ($p<0.05$). These data indicate that genistein was able to exert neuroprotective actions increasing neurogenesis and decreasing astrogliosis. Furthermore, the phytoestrogen improved hippocampal dependent memory in SHR. Given the side effects due to estradiol treatment, much effort is made to look for alternative treatments that mimic estradiol neuroprotective actions avoiding the undesirable effects. Genistein opens an interesting possibility in this direction.

0053 - IN VITRO EFFECTS OF ATRAZINE AND DIAMINO-CHLORO TRIAZINE ON MITOCHONDRIAL FUNCTIONALITY AND NITRIC OXIDE METABOLISM IN STRIATUM.

Barbara PAEZ | **Analia KARADAYIAN** | Silvia LORES-ARNAIZ | Analia CZERNICZYNIEC

INSTITUTO DE BIOQUIMICA Y MEDICINA MOLECULAR (IBIMOL-CONICET)

Atrazine (ATZ) is an herbicide frequently used in Argentina and it is metabolized primarily by cytochrome P450. Diamino-chloro triazine (DACT) is the main metabolite detected in plasma and rat urine after in vivo atrazine exposure. Considering that both ATZ and DACT can cross the blood brain barrier, the aim of this work was to evaluate the in vitro effect of those compounds on striatal mitochondrial function and nitric oxide (NO) metabolism. Striatal mitochondria were isolated from SD rats (180-200 g) and exposed to 10 µM ATZ or 100 µM DACT. Respiratory rates, H_2O2 production, respiratory complexes activity, monoamine oxidase, membrane potential, superoxide anion production and NO levels were evaluated. Results showed that ATZ did not modify respiratory rates. However, DACT increased O2 consumption in state 4 (55 %) and decreased respiratory control in striatal mitochondria. Production of H_2O_2 was increased after exposure of both ATZ (25 %) and DACT (38 %), probably due to the inhibition of complex I-III activity (30 and 17%, respectively). No significant changes were observed in the activity of complex II-III or monoamine oxidase in striatal mitochondria in the presence of the herbicide or its metabolite. The results showed a depolarization of 15 and 19% in striatal mitochondria exposed to ATZ and DACT, respectively. The evaluation of superoxide anion production showed a 13 % increase after atrazine treatment and no significant changes were observed in the presence of DACT. Meanwhile, an increase in NO levels (11 %) was observed after exposure of striatal mitochondria to atrazine, without changes in those exposed to DACT. Obtained results suggested that in vitro ATZ and DACT affect striatal mitochondrial function and nitric oxide metabolism through different mechanisms.

0113 - BEHAVIORAL PATTERN SEPARATION PERFORMANCE AND HIPPOCAMPAL DOUBLECORTIN NEURON ANALYSIS IN VERY OLD FEMALE RATS

Martina CANATELLI MALLAT | Priscila CHIAVELLINI | Marianne LEHMANN | Gustavo MOREL | Rodolfo GOYA

INIBIOLP

Aging is associated with impaired performance in behavioral pattern separation (PS) tasks based on similarities in object features and in object location. Hippocampal dentate gyrus (DG) is required for PS. DG is thought to preprocess information, which facilitates pattern completion in the CA3 region. In order to evaluate the effect of aging on discrimination of overlapping memories of object location or features, we assessed three groups of rats: Young (Y, 4-5 mo., n= 25), Middle Aged (MA, 18 mo., n= 32) and Senile (S, 28 mo., n= 13). Experiment 1: spontaneous location recognition (SLR) and Experiment 2: spontaneous object recognition (SOR). The testing chamber for SLR was a black circular arena (90 cm diameter) and for SOR was a white triangular arena (60 cm per side). On the SLR sample phase, rats were exposed to three identical objects (A1, A2, and A3). On the choice phase, rats were exposed to two identical objects (A4 and A5). A4 was placed in a familiar location and A5 was placed in a new location. On the SOR sample phase, three different objects were assembled (AB, BC, and DE). On the choice phase, a novel object was assembled in AC (two non-shared features of the objects in the sample phase), and the familiar object was a copy of DE. Morphometric analysis revealed a significant age-related reduction in doublecortin (DCX) cell number (MA and S) but only the S rats presented DCX neuron diameter reduction in the DG. In the SLR, the discriminatory performance was markedly

deteriorated in the MA and S rats versus Y rats. In the SOR, only S rats presented deterioration in memory disambiguation. However, MA rats showed a preserved memory as compared with Y rats. These findings reveal the existence of a significant vulnerability of DCX neurons to age in rats and a deficient DG neurogenesis-related object location discrimination. Furthermore, the overlapping memories involving object location are more sensitive to age than overlapping memories involving features.

Inmunología e Inflamación / Immunology and Inflammation I

Chairs: Verónica García | Silvina Lompardia

0160 - OXYTOCIN AS ANTI-INFLAMMATORY AGENT FOR PERIODONTAL DISEASE

Ganna DMYTRENKO (1) | Pablo Nicolás SURKIN(2) | Bernardo MIRAMÓN(3) | Javier FERNANDEZ - SOLARI(4) | Andrea DE LAURENTIIS(1)

CENTRO DE ESTUDIOS FARMACOLÓGICOS Y BOTÁNICOS (CEFYBO-CONICET), FACULTAD DE MEDICINA, UBA (1); IBYME-CONICET (2); CÁTEDRA DE ENDODONCIA, FACULTAD DE ODONTOLOGÍA, UNIVERSIDAD DE BUENOS AIRES (3); CÁTEDRA DE FISIOLOGÍA, FACULTAD DE ODONTOLOGÍA, UNIVERSIDAD DE BUENOS AIRES (4)

Periodontitis (P) is an oral multifactorial infectious disease triggered by bacteria which affects the teeth-support tissue. The penetration of bacterial products causes an immuno-inflammatory response leading to the destruction of gingival tissue and alveolar bone loss. We previously demonstrated an anti-inflammatory role of Oxytocin (Oxy) that reduces systemic levels and hypothalamic expression of pro-inflammatory cytokines. Furthermore, atosiban, a competitive antagonist of Oxy receptors, induces per se a slight increase in these cytokines expression and blocked Oxy anti-inflammatory effect. Endocannabinoids (ECs) are endogenous lipid signaling molecules that can modulate the neuro-immune-endocrine response, are present in the gingival tissue and participate in the regulation of gingival inflammation and bone metabolism. Since we previously determined a closed bidirectional relationship between ECs and Oxy production and release, we could speculate that both participate in the inflammatory response in P. Given that Oxy could act as an anti-inflammatory agent, in this work we study the effect of this hormone on gingival inflammatory parameters in a rat model of experimental P, as well as the participation of gingival cannabinoid receptors in this effect. The experimental P was induced in male adult rats by bilateral linen ligature around the first lower molar during one week. After that, the gingival tissue was removed and jaws were cleaned and stained with methylene blue at 0.1% to visualize the amelocementary limit. We demonstrate for the first time the mRNA expression (PCR) for the Oxy receptor in rat gingiva, also P significantly ($p<0.01$) increased its expression. Daily Oxy (1000 µg/kg s.c.) administration significantly reduces bone loss in P ($p<0.001$). We also demonstrate that Oxy reduces the mRNA expression for the cytokines IL-1 beta and IL-6 ($p<0.05$ vs. P) in the gingiva. We determined that rat normal gingiva expresses both cannabinoid receptors, CB1 and CB2 proteins (Western blot). The protein expression of both is significantly increased ($p<0.05$) in gingiva during P. Oxy only prevented CB2 receptors increased expression, but not of CB1. In conclusion, Oxy acts as an anti-inflammatory in P since prevent bone loss and decrease pro-inflammatory cytokines expression in the gingival tissue. Moreover, these effects could be mediated at least by cannabinoid CB2 receptors.

0389 - CIRCULATING CYTOKINE LEVELS IN CHAGAS PATIENTS WITH AND WITHOUT DILATED CARDIOMYOPATHY

Silvia Esther MIRANDA (1) | Gustavo SOSA(1) | María Gabriela LOMBARDI(2) | Alejandra VON WULFFEN(3) | Natalia CIAMPI(3) | Analía PAOLUCCI(3) | Mario PRINCIPATO(3) | Justo CARBAJALES(3) | Guillermo DI GIROLAMO(1)

INSTITUTO ALBERTO C. TAQUINI DE INVESTIGACIONES EN MEDICINA TRASLACIONAL (IATIMET), UBA-CONICET (1); CENTRO DE ESTUDIOS FARMACOLÓGICOS Y BOTÁNICOS (CEFYBO), UNIVERSIDAD DE BUENOS AIRES-CONICET (2); CENTRO DE CARDIOGENÓMICA. HOSPITAL RAMOS MEJIA (3)

Dilated cardiomyopathy (DC) represents the most severe manifestation that affects around 30 % of individuals along 10-30 years after T. cruzi infection. To study the underlying mechanisms, circulating levels of different cytokines/chemokines were investigated in patients with DC or not (NC) and compared with healthy control donors (C). Chagas´ patients were recruited by the Cardiogenetic Center, Ramos Mejía Hospital, Bs. As., after signing the informed consent approved by the local Ethics Committee. All patients came from northwestern Argentina and southern Bolivia. No patient presented another etiology of dilated cardiomyopathy except Chagas. Enrolled patients were submitted to a standard follow up and clinical treatment. The NC group (n= 22) included asymptomatic individuals, with ejection fraction (EF) more than 50 % and DC group (n= 22) showed EF less than 35 %. Normal healthy volunteers showing negative serological tests for the infection were included as the (C) group (n= 14). Fasting plasma levels of IFNÿ, IL-1ß, IL-6, IL-10, IL-12 (p70), IL-15, IL-17A, MCP-1/CCL2, MIP-1alpha/CCL3, TNFalpha and IL-2 were assessed using a Magnetic Bead Multiplex Assay in a Magpix® equipment (Merck Millipore). Concentrations were determined using the xPONENT software version 4.2. Our results showed that: 1- DC, in contrast to NC, had higher levels of TNFalpha than C (p= 0.010), 2- IL-6 concentration increased in DC with respect to NC (p= 0.033), 3- NC showed a trend to higher MCP-1 levels respect to DC (p= 0.119), 4-no differences were observed in the rest of the analyzed cytokines. These data suggest that IL-17A producing cells (IL3 and Th17) and INFý producers (NK, Tc, Th1 cells) have a little contribution in this phase of the disease. The present observations agree with the similar levels of IL-2, IL-12p70 and IL-15 found in all groups. In contrast, IL-6 and TNFalpha, likely derived from monocytes/macrophages, would contribute to cardiac inflammation and dysfunction in Chagas disease.

0544 - MODULATION OF ROS AND INTERLEUKIN-1ß PRODUCTION BY ARSENIC TRIOXIDE AND DRUGS THAT INFLUENCE MITOCHONDRIAL BIOGENESIS AND MITOPHAGY IN HUMAN KERATINOCYTES

Luciana SALAVERRY (1) | Marco BESSONE(2) | Andrea Cecilia PARRADO(1) | Franco Mauricio MANGONE(1) | Agustina Daniela SOTELO(2) | Tomás LOMBARDO(1) | Andrea CANELLADA(1) | Estela REY- ROLDÁN(1) | Guillermo BLANCO(3)

UNIVERSIDAD DE BUENOS AIRES, FACULTAD DE FARMACIA Y BIOQUÍMICA, INMUNOLOGÍA, IDEHU (UBA-CONICET) (1); UNIVERSIDAD DE BUENOS AIRES, FACULTAD DE FARMACIA Y BIOQUÍMICA. CÁTEDRA DE INMUNOLOGÍA. (2); INSTITUTO DE ESTUDIOS DE LA INMUNIDAD HUMORAL DR. R.A. MARGNI (IDEHU). UBA-CONICET (3)

Chronic exposure to arsenicals is associated to a skin disease known a HACRE. Arsenic increases mitochondrial ROS (mtROS) and compromises mitochondrial function. Mitophagy (MF) and mitochondrial biogenesis (MB) are two critical processes in maintaining mitochondrial homeostasis. In addition, high levels of ROS may induce pro-inflammatory mediators like interleukin (IL)-1ß. Here we explored the effect of drugs that modulate MB (valproic acid, VPA) and MF (vincristine, VCR) on mitochondrial mass (MM), superoxide anion production (O_2^-) and IL-1ß levels, in HaCaT keratinocytes exposed to sublethal doses of arsenic trioxide (ATO). HaCaT cells were exposed to 1 µM ATO, 2 mM VPA and 0.75 µM VCR in 48h assays under several combinations, and stained with

nonyl-acridine orange (NAO) and dihydroethidium (HE) to evaluate changes in MM and O_2^- respectively by flow cytometry. IL-1ß levels were quantified by ELISA in cell culture supernatants. A p <0.05 was considered statistically significant. ATO caused a significant increase in MM. VPA, a known inducer of MB, caused a significant increase in MM and O_2^- in HaCaT cells either alone or in combination with ATO. By contrast, the pro-inflammatory cytokine IL-1ß levels were decreased when ATO was combined with VPA. When keratinocytes were exposed to ATO and/or the MF blocker VCR, a significant increase in MM, O_2^- and IL-1ß production was observed. Remarkably, when VPA was combined with ATO and/or VCR, IL-1ß was decreased below control values. In conclusion, ATO might exerted a pro-inflammatory effect raising IL-1ß levels in keratinocytes, while VPA raised both MM and O_2^- in ATO-treated cells, but decreased IL-1ß to baseline values, denoting a profound anti-inflammatory effect. In contrast, the increase in MM and O_2^- observed with MF blockage by VCR was consistent with accumulation of ATO-damaged mitochondria, increased IL-1ß levels and enhanced pro-inflammatory effects.

0570 - ROLE OF PI3K PATHWAY IN THE ANTI-INFLAMMATORY EFFECT OF BENZNIDAZOLE

Ágata Carolina CEVEY | Azul Victoria PIERALISI | Aldana Soledad SEQUEYRA | Rocío Antonella COMITO | Federico Nicolás PENAS | Maria Jimena RADA | Gerardo Ariel MIRKIN | Nora Beatriz GOREN

INBIRS, CONICET, FAC. MEDICINA-UBA

Chagas disease is the main cause of dilated cardiomyopathy in Latin America. During the acute infection, the inflammatory response is critical for the control of parasite proliferation and disease evolution. Benznidazole, one of the antiparasitic drugs currently used for its treatment, also exerts anti-inflammatory effects. Previously, we described that benznidazole inhibits the activation of the NF-kappaB pathway by increasing the expression of SOCS3 through the IL-10/STAT3/SOCS3 pathway. It has been reported that PI3K is a negative regulator of inflammation, partly through SOCS3. To deepen the knowledge of the mechanism of action of benznidazole, we used a primary culture of mouse neonatal cardiomyocytes. Heart cells were pretreated with 15 µM of benznidazole and then stimulated with 10 µg/ml of LPS, to assess the anti-inflammatory effect of benznidazole, regardless of its antiparasitic effect. The treatments were performed, in parallel, in the presence of LY294002, a specific inhibitor of PI3K activity. Under these conditions, we found that benznidazole could not increase SOCS3 after 24 h of stimulation, evaluated by RT-qPCR ($p<0.05$). In addition, preliminary tests show that when PI3K is inhibited, benznidazole does not inhibit the expression of IL-6 or NOS2, evaluated by RT-qPCR. These preliminary results support the hypothesis that PI3K would participate in the anti-inflammatory effect of benznidazole.

0597 - PARTICIPATION OF HEART MACROPHAGES IN THE EFFECTS OF FENOFIBRATE IN T. CRUZI-INFECTED MICE

Federico Nicolás PENAS (1) | Azul Victoria PIERALISI(1) | Rocio Antonella COMITO(1) | Martin DONATO(2) | Ágata Carolina CEVEY(1) | Maria Jimena RADA(1) | Ricardo J. GELPI(2) | Gerardo A. MIRKIN(3) | Nora Beatriz GOREN(1)

INBIRS, CONICET, FAC. MEDICINA-UBA (1); INSTITUTO DE FISIOPATOLOGÍA CARDIOVASCULAR, DEPARTAMENTO DE PATOLOGÍA, FACULTAD DE MEDICINA, UBA (2); IMPAM (UBA-CONICET) (3)

Macrophages (Mø) are one of the main infiltrating leukocytes in response to heart infection in Chagas disease. Due to their functional and phenotypic plasticity, manipulating specific Mø subsets can be crucial in collaborating with vital cardiovascular functions, such as tissue repair and defense against the infection. Previous works of our group showed that fenofibrate, a PPARalpha; ligand, improves cardiac function and inflammatory parameters in a murine model of T. cruzi (Tc) infection. In this work we tested the hypothesis that heart Mø participate in the effects of fenofibrate in Tc-infected mice. To test this hypothesis, we depleted circulating Mø (with clodronate liposomes treatment) in Tc-infected mice. The results show that Mø depletion impedes the beneficial effects of fenofibrate on different ventricular parameters: ejection and shortening fraction, left diastolic diameter and left final systolic diameter, measured by echocardiography ($p<0.05$). Consistent with this, Tc-infected and clodronate-fenofibrate treated mice showed 60 % survival, whereas that Tc-infected and fenofibrate treated mice showed 100 % survival ($p<0.05$). Then, the expression of M2 profile markers in the heart was evaluated, by RTq-PCR. The depletion of circulating Mø decreased the expression of CCR2, IL-10, CD206 and Arginase I in heart tissue ($p<0.05$), while increased the expression of NOS2 analyzed by western blot and RTq-PCR, in comparison with Tc-infected and fenofibrate treated mice ($p<0.05$). The data presented here suggest that both resident and infiltrated Mø participate in the effects of fenofibrate in heart of Tc-infected mice. The in-depth knowledge of the mechanisms of action of fenofibrate could provide a rational framework for the therapeutic approach of chagasic cardiomyopathy by combination of an anti-inflammatory therapy together with the classical parasiticidal treatment.

Oncología/ Oncology I

Chairs: Guillermo Dalton | Mariano Gabri

0036 - COMBINATION OF PALBOCICLIB WITH ANTIPROGESTINS IN EXPERIMENTAL BREAST CANCER MODELS WITH DIFFERENT PROGESTERONE RECEPTOR ISOFORM RATIOS

Gabriela PATACCINI (1) | Gonzalo SEQUEIRA(1) | Silvia VANZULLI(2) | Claudia LANARI(1) | Sebastián GIULIANELLI(3)

INSTITUTO DE BIOLOGÍA Y MEDICINA EXPERIMENTAL (IBYME-CONICET) (1); ACADEMIA NACIONAL DE MEDICINA DE BUENOS AIRES (2); INSTITUTO DE BIOLOGÍA DE ORGANISMOS MARINOS, IBIOMAR-CCT CENPAT-CONICET, ARGENTIN (3)

Luminal breast cancers represent more than 70% of all breast cancer patients and they are susceptible to an endocrine therapy. Palbociclib (PALBO), an oral CDK 4/6 inhibitor, is currently used in combination with endocrine therapy to treat advanced hormone receptor-positive breast cancer. However with time almost all patients acquire resistance. Therefore, alternative therapies are required to reduce breast cancer mortality. We have recently reported that breast cancer patients with tumors expressing higher levels of isoform A of the progesterone receptor (PRA) than isoform B (PRB) may benefit from an antiprogestin treatment. The aim of this study was to evaluate the effect of PALBO in combination with the antirpogestin mifepristone (MFP) in luminal experimental breast cancer models. We first tested the effects of MFP (10 nM) and/or PALBO (100 nM) on cell proliferation in human T47D cells and in T47D-YA and T47D-YB cells expressing only PRA or PRB respectively. Cells were incubated in the presence of 5 % steroid stripped fetal calf serum and FGF2 (50 ng/ml) to have a basal high proliferative state. T47D and T47D-YA FGF2-treated cells were inhibited by MFP, or PALBO ($p<0.001$) and in both cases the drug combination induced a higher inhibitory effect as compared with single treatments ($p<0.001$). In T47D-YB cells, PALBO induced a significant inhibitory effect ($p<0.001$) that was similar in the presence or absence of MFP. To test the effect of the combination of drugs in vivo we used the murine 59-2-HI and C4-HI mammary carcinomas which are inhibited by MFP and have a high PRA/PRB ratio. Whereas PALBO (20 mg/kg/day; sc) inhibited the growth of 59-2-HI tumors ($p<0.05$), C4-HI tumors displayed PALBO resistance. The drug combination only proved to be effective in the 59-2-HI tumor. In conclusion PALBO improves the efficacy of antiprogestins in a subgroup of tumors with higher levels of PRA than PRB.

0058 - RUNX2 ABROGATES FGFR BLOCKADE IN HUMAN BREAST CANCER MODELS

María Sol RODRÍGUEZ | Caroline Ana LAMB | Isabel LÜTHY | Claudia LANARI | Cecilia PÉREZ PIÑERO

IBYME-CONICET

We have previously shown an interaction between the FGF2/FGFR2 axis and RUNX2 transcription factor. We have shown that FGFR2 or RUNX2 silencing in luminal IBH6 cells give rise to small tumors with a less aggressive phenotype and a lower proliferation index compared with control tumors. On the contrary, IBH6 or T47D cells overexpressing RUNX2 (RUNX2-IBH6 and RUNX2-T47D, respectively) develop lung metastases and express high levels of FGFR2 and FGF2, showing a more aggressive phenotype than controls. This evidence supports the hypothesis that FGF2 increases RUNX2, and RUNX2 in turn increases FGF2 expression, maintaining a positive loop. The aim of this work was to evaluate if RUNX2 overexpressing tumors are sensitive to the FGFRs inhibitor PD173074. RUNX2-IBH6 or RUNX2-T47D cells and their respective controls (empty vector transfected cells) were injected into the flank of NSG mice. When the tumor size reached 30 mm^2 animals were treated for 10 days with PD173074 (0.5 mg/day i.p.). Control treated tumors showed a significant inhibition of size ($p<0.001$), a lower Ki67 index ($p<0.01$) and higher stromal remodeling compared with untreated tumors. RUNX2-IBH6 and RUNX2-T47D tumors were resistant to the therapy. Remarkably, IBH6-RUNX2 treated tumors were significantly larger than the untreated counterparts ($p<0.001$). Our results show that RUNX2 induces breast cancer progression and these effects remain even after blocking the FGF2/FGFR2 axis, suggesting that RUNX2 is hierarchically more important than FGFR2 in tumor progression. These results suggest that RUNX2 inhibitors may have a better performance inhibiting the FGF2-FGFR axis than FGFR inhibitors.

0078 - COMBINED TREATMENT OF MENADIONE PLUS CALCITRIOL ON CACO-2 CANCER CELLS: ANTIPROLIFERATIVE EFFECTS

Romina KOHAN | Alejandro COLLIN | Nori TOLOSA DE TALAMONI | **Gabriela PICOTTO**

CÁTEDRA DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR, FACULTAD DE CIENCIAS MÉDICAS; UNC

Colorectal cancer (CRC) is the third most frequent and the fourth leading cause of cancer-associated mortalities worldwide. Effective targeted therapies based on the current knowledge of CRC are essential to achieve a successful treatment of this pathology. We have previously reported that oxidant drugs as menadione (MEN) increased tumour cell sensibility. Calcitriol (D) has well known antineoplastic actions on different tumor cells. However, the doses employed to reach this effect in vivo have undesirable hypercalcemic consequences. The aim of this study was to evaluate the effects of a combined MEN and D therapy on the viability of Caco-2 colon cancer cells and to study reactive oxygen species as possible inductors of oxidative stress (OS). Cells were treated with MEN, D, both or vehicle (ethanol). Crystal violet staining and microscopy evaluated antiproliferative effects. Cell migration was estimated by wound healing assay. Superoxide anion content, catalase (CAT) activity and cellular adhesion were also determined. One way ANOVA and Bonferroni as a post-hoc test were used as statistical methods. MEN and D inhibited Caco-2 growth in a time and dose-dependent manner. The antiproliferative effect began at 48 h being higher at 96 h. The selected concentration was 20 µM MEN/200 nM D. The combined treatment caused a dramatic reduction of viability (>80%) and a complete inhibition of cell migration. Morphological nuclear changes resulted compatible with cell death. Changes in cell adhesion were not observed. Superoxide anion content increased by the combined treatment concomitant with modifications in CAT activity. The antiproliferative effect of the combination was partially rescued exposing cells to the flavonoid naringin, a natural antioxidant. In conclusion, D enhances the antiproliferative effect of MEN on Caco-2 cells probably via the induction of OS. The study of this drug combination will continue in order to analyse future therapeutics applications.

0092 - EFFECTS OF PERITUMORAL ADIPOCYTE-SECRETED FACTORS ON LIPOLYTIC ACTIVITY AND MITOCHONDRIAL FUNCTION OF MATURE 3T3-L1 ADIPOCYTES.

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Adipose microenvironment is involved in signaling pathways that influence breast cancer progression. However, adipocyte characteristics involved in this process remain poorly understood. We have previously demonstrated that adipocytes exposed to conditioned media from human adipose tissue explants of tumor breasts (hATT-CMs) displayed characteristics that morphologically resembled brown adipocytes with high expression of mitochondrial UCP1 and other thermogenic proteins. Lipolysis is a prerequisite for thermogenesis in brown and beige adipocytes. Hence, our next aim was to examine the possibility that hATT induced changes in mitochondrial architecture as well as expression and subcellular localization of lipolytic proteins that could contribute to beige thermogenesis. Small and round-shaped mitochondria, partially swelled, were observed in adipocytes 3T3-L1 exposed to hATT-CMs, in comparison to treated with hATN-CMs, which displayed a mix of tubular and spherical mitochondria like control adipocytes. Interestingly, adipocytes exposed to hATT-CMs showed increased HSL and perilipin expression levels, and the number of micro-lipid droplets (LDs), which were stained for a discontinue intensity signal of perilipin in a similar manner to adipocytes treated with rosiglitazone. Contrarily, adipocytes exposed to hATN-CMs increased LDs size, without affecting HSL and perilipin expression levels and perilipin subcellular localization. Importantly, the activated form of HSL was able to translocate to the LD surface in response to the activation of lipolysis when adipocytes 3T3-L1 were treated with hATN-CMs or hATT-CMs. In summary, these findings suggest that hATT secrete a different set of factors compared to hATN, which may induce mitochondrial fission and modify the lipolytic activity of mature adipocytes. This would indicate a loss of normal functions in mature adipocytes (such as energy storage) attached to the tumor, in support of others that might favor tumor growth.

0283 - MULTIAPPROACH FOR REPOSITIONING DRUGS FOR COLORECTAL CANCER TREATMENT

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INSTITUTO DE INMUNOLOGÍA CLÍNICA Y EXPERIMENTAL DE ROSARIO (IDICER, UNR-CONICET) (1); INSTITUTO DE GENETICA EXPERIMENTAL (IGE-UNR) (2)

Drug repositioning in oncology refers to the use of drugs indicated for the treatment of certain pathologies that have proved some anticancer effects. There are two main ways to select drugs for repositioning: "text mining-based" and "in silico-based" approaches. The first one uses prior knowledge of the mode of action of a drug, while the second one detects putative target genes from genetic expression data using computational tools to propose an active drug. In this work we have used these two approaches to select a group of candidate drugs to reposition for colorectal cancer (CRC) treatment. First, by MTT-based proliferation assays we evaluated the effect of a group of drugs, either individually or in a combined manner on HCT116 and HT29 cell lines. Form all the

combination tested, we focused on metformin (M; diabetes treatment) and propranolol (P; hypertension treatment) combination because it showed a strong growth inhibition even combining low doses of both drugs ($p<0.001$). We found that M+P treatment not only affected CRC cells proliferation, but it also produced an inhibition of metastasis-related events like migration ($p<0.01$) and modulation of epithelial-mesenchymal transitions (reduced E-cadherin and B-catenin expression by Western blot; $p<0.05$). We also observed in two different CRC in vivo models that M+P combination was able to prevent the development ($p<0.05$) and decrease the growth ($p<0.01$) of this type of tumor, with no associated symptoms of toxicity. Alternatively, by differential expression analysis of public gene expression datasets we detected a group of genes differentially expressed (FDR <0.01) in CRC recurrent to conventional chemotherapies and proposed a group of putative active drugs for these targets. Altogether, our results suggest that therapy with repositioned drugs might be of interest for colon cancer treatment and, in particular, the combination of M+P could inhibit colon cancer development and metastasis.

0707 - SUSTAINED RET EXPRESSION DURING MAMMARY GLAND POST-LACTATION INDUCES PREMATURE INVOLUTION ENHANCING TUMORIGENESIS

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IFIBYNE, CONICET-UBA (1); CEDIE (2); CENTER FOR GENOMIC PATHOLOGY, SCHOOL OF MEDICINE, UNIVERSITY OF CALIFORNIA (3); DEPARTMENT OF CANCER BIOLOGY, PERELMAN SCHOOL OF MEDICINE, UNIVERSITY OF PENNSYLVANI (4); FRIEDRICH MIESCHER INSTITUTE FOR BIOMEDICAL RESEARCH (5)

Ret is a receptor tyrosine kinase with oncogenic potential in the mammary epithelium. Several receptors described as oncogenes have been shown to play important roles during normal mammary gland development. We found that Ret is normally expressed in lactation and its deregulation alters the efficient transition to involution. Involution is the period which returns the lactating gland to a quiescent state after weaning (post-lactation stage). Inhibition of Ret activity in vivo does not alter lactation, however impacts in the involution process. Nevertheless, Ret overexpression promotes factors that drive involution, including premature Stat3 activation. In addition, sustained expression of Ret during the post-lactation produces defective cell recycling and enhances cancer potential disrupting Stat3 signaling through SOCS3, a key regulator of Stat pathway. These data demonstrate that Ret has a key role in mammary gland post-lactation stage.

0767 - THYROID HORMONES EFFECTS IN BEXAROTENE TREATMENT OF BREAST CANCER CELLS.

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INSTITUTO DE INVESTIGACIONES BIOMEDICAS (UCA - CONICET)

Chemoresistance is a major cause of cancer treatment failure. Many breast cancer (BC) cells develop chemoresistance by diminishing intracellular drug accumulation, upregulating protein levels or activating transporters like MDR1. Previously we demonstrated that thyroid hormones (THs) modulate chemotherapy response in T cell lymphoma (TCL) cells. However, in BC cells little is known about these mechanisms that lead to tumor chemotherapy resistance and are crucial to assure the success of treatment. Bexarotene (Bex) is an oral retinoid-X-receptor agonist that is effective for the treatment of early and advanced-stage in cutaneous TCL and there are ongoing clinical trials to determine its role in both BC treatment and prevention. However thyroid dysfunction is recognized as an important side effect of such therapies, potentially manageable by THs administration. To study how THs affect MDA-MB-231 drug transport, rhodamine 123 (RHO123) incorporation assay was performed. Our results shown that MDA-MB-231 cells incorporate RHO123 in a time dependent manner, reaching to a plateau at 3 h, and this effect was not affected by THs treatment. On the other hand, we found that THs increase RHO123 exclusion at 1 h in BC cells ($p<0.01$). We evaluate if Bex can induce MDR associated protein clearance from MDA-MB-231 cells by secreting extracellular lipid vesicles (EVs). We found that Bex treatment reduces intracellular RHO123 accumulation and THs reverts this effect. Also, by electron microscopy we found that Bex induces EVs release and THs increases this effect. Our data suggest that the requirement of THs replacement therapy may affect Bex treatment in BC.

0838 - ANDROGEN RECEPTOR AND NOTCH SIGNALING: POTENTIAL TARGETS FOR COMBINED TREATMENT IN PROSTATE CANCER

Agustina CHIMENTO (1) | Sofía PERRONE(1) | Nadia BONADEO(1) | María Lucia ROMANO(1) | Licina TESSONE(2) | Kurt VILLALBA(3) | Fernanda PARENTI(4) | Carolina CRISTINA(1)

CENTRO DE INVESTIGACIONES BÁSICAS Y APLICADAS (CIBA) – CITNOBA (UNNOBA – CONICET) (1); LABORATORIO DE PATOLOGÍA DR ALBERTO PETRAGLIA (2); CLÍNICA CENTRO (3); CENTRO MEDICO FAMYL (4)

Prostate cancer is the most common cancer in males. The second–generation anti–androgens, such as Enzalutamide, have been used to treat advanced prostate cancer leading to improvement patient lifespan. Aberrant Notch signaling has been widely demonstrated to be associated with tumor progression and therapeutic resistance in many types of cancers. However, the role in prostate cancer is poorly understood. In this work, we aimed to study the role of Notch pathway in the development of prostate cancer and its regulation under Enzalutamide treatment. We first determined the expression of Notch1 and Notch4 receptors and the proliferation marker PCNA by IHQ in prostate tumor samples obtained by surgery. In prostate cancer PC3 cells under Notch pathway inhibition with DAPT, the expression of TMPRSS2, an androgen dependent gene, showed lower levels after 24 h of treatment (10 and 30 μM) by RT-qPCR ($n= 2$; $p= 0.02$). In addition, we observed by WB a trend of reduction in PCNA expression with DAPT (30 μM) after 24 and 48 h ($n= 3$). In turn, Enzalutamide treatment (30 and 50 μM) reduced the levels of HES1, a target gene of the Notch pathway, determined by RT-qPCR ($n= 3$). Cell viability was measured using MTS assay and we observed significantly reduced viability of prostate cancer PC3 cells both with DAPT ($p= 0.0027$; $n= 3$) and with Enzalutamide isolated treatment ($p= 0.0018$; $n= 3$). Importantly, the combined treatment with DAPT (10 μM) and Enzalutamide (50 μM) significantly reduced PC3 viability at 48 and 72 h ($p= 0.0043$; $n= 3$). Our results show Notch signaling activation in prostate PC3 cells and prostate tumor samples. Interestingly, DAPT treatment decreased the levels of TMPRSS2, and Enzalutamide decreased the levels of HES1. Moreover, we observed a significantly reduced viability both with DAPT and Enzalutamide isolated treatment and with the combined treatment. Our results suggest an interconnection between Notch and Androgen receptor pathways in prostate cancer.

0951 - THERAPEUTIC EFFECT OF CURCUMIN PLUS OXALIPLATIN IN CHEMORESISTANT COLORRECTAL CANCER

Rodrigo LLOYD(1) | Elena María SANMARCO(2) | Dailenys ESPINOSA MARTINEZ(1) | Julia GALLINO(2) | Florencia GIANNONI(2) | **Lucia POLICASTRO** (2)

LABORATORIO DE NANOMEDICINAS, COMISIÓN NACIONAL DE ENERGÍA ATÓMICA-ANPCYT (1); LABORATORIO DE NANOMEDICINAS, COMISIÓN NACIONAL DE ENERGÍA ATÓMICA-CONICET (2)

Colorectal cancer is the third most common cancer constituting 10% of new cancer cases in men and 11 % in women. Despite the use of surgical resection and chemotherapy, nearly 50 % of patients with colorectal carcinoma develop recurrent disease, highlighting the need for improved therapies. In this context curcumin (Cur), the major active ingredient of turmeric (Curcuma longa), inhibits the growth of transformed cells, has also been related with tumor regression in colon carcinogenesis in rodent models, and was found to be effective in targeting drug resistant cancer cell or cancer stem cell (CSC). This background makes this compound interesting to be combined with chemotherapeutic drugs such as oxaliplatin (Oxp) in order to improve the treatment of resistant colorectal cancer. In our laboratory, we have developed a colorectal oxaliplatin chemoresitant cell lines and oxaliplatin chemorresistant tumor generate in vivo. Thus, the aim of this work was to evaluate the effect of Cur combined with Oxp in our chemoresistant in vitro and in vivo models. We performed toxicity in-vitro assays in Oxp resistant T-84 colorectal cancer cell line developed by subculture in presence of incremental doses of Oxp. Besides we generate an in-vivo chemoresistant model by serial passaging of sensible T84 subcutaneous tumor xenografts in nude mice treated with Oxp and re-derived at least by four times. Oxp and Cur were administrated by intraperitoneal injection. We found that Cur can inhibit the proliferation in-vitro increasing the cytotoxic effect in combination with Oxp, furthermore the combination can inhibit the tumor growth in-vivo but increasing the toxic effect in healthy tissues. In conclusion we believe that the Oxp and Cur combination has a therapeutic potential but it is necessary the optimization of the drug delivery system in order to increase the dose in tumor site and decrease the dose in healthy tissues.

Toxicología / Toxicology I

Chairs: Paola Ingaramo | Enrique Sánchez Pozzi

0067 - CONTINUOUS EXPOSURE TO URBAN AIR POLLUTION INDUCES BRAIN OXIDATIVE STRESS, INFLAMMATION AND IMPAIRED MITOCHONDRIAL FUNCTION IN MICE.

Valeria CALABRÓ (1) | Mariana GARCÉS(2) | Timoteo MARCHINI(1) | Natalia MAGNANI(1) | Lourdes CÁCERES(1) | Agustina FREIRE(1) | Tamara VICO(1) | Virginia VANASCO(1) | Clara BERDASCO(3) | Nahuel MÉNDEZ DIODATI(1) | Manuela MARTINEFSKI(4) | Valeria TRIPODI(5) | Jorge GOLDSTEIN(3) | Ricardo J GELPI(1) | Alejandro BERRA(6) | Silvia ALVAREZ(1) | Pablo EVELSON(1)

CONICET-UNIVERSIDAD DE BUENOS AIRES. INSTITUTO DE BIOQUÍMICA Y MEDICINA MOLECULAR (IBIMOL). (1); UNIVERSIDAD DE BUENOS AIRES, FACULTAD DE FARMACIA Y BIOQUÍMICA, QUÍMICA GENERAL E INORGÁNICA. IBIMOL (2); UNIVERSIDAD DE BUENOS AIRES, CONICET. IFIBIO. FACULTAD DE MEDICINA (3); UNIVERSIDAD DE BUENOS, CONICET. DEPARTAMENTO DE TECNOLOGIA FARMACÉUTICA (4); UNIVERSIDAD DE BUENOS AIRES, CONICET. DEPARTAMENTO DE TECNOLOGÍA FARMACÉUTICA (5); UBA, FACULTAD DE MEDICINA, DEPTO DE PATOLOGÍA, CENTRO DE PATOLOGÍA EXPERIMENTAL Y APLICADA (6)

Increasing evidence indicates that the central nervous system (CNS) is a target of air pollution, which might lead to oxidative stress and neuroinflammation. However, the mechanisms mediating these effects have not been fully elucidated. The aim of this work was to study the effects of chronic exposure to air pollution, on mice brain cortex (CX) and olfactory bulb (OB), focusing on oxidative and inflammatory markers, and mitochondrial function. Male 8-week-old BALB/c mice were exposed to filtered air (FA, control) or urban air (UA) inside whole-body inhalation chambers located in a highly polluted area of Buenos Aires City, for up to 4 weeks. Glutathione levels, assessed as GSH/GSSG ratio, were decreased in CX after 1 and 2 w of exposure to UA, and after 4 w in the case of the OB (26 and 60 % respectively; $p<0.05$). NADPH oxidase and GPx activities were increased in all of the studied time points, while this increment was observed only after 4 w for SOD and GR activities, in CX of UA group ($p<0.05$). After 4 w, increased GFAP expression levels showed reactive astrocytes in OB, probably associated with the altered olfactory function observed by a behavioral test, in UA compared to FA mice ($p<0.05$). Also, UA mice showed impaired mitochondrial function due to a 50% reduction in O2 consumption in active state (state 3) ($p<0.05$), a 65% decrease in ATP production rate ($p<0.01$) and a 30 % increase of H_2O_2 production ($p<0.01$). Moreover, respiratory complexes I-III and II-III activities were decreased in UA group (30 and 36 %, respectively; vs. FA, $p<0.05$). Taken together, UA exposed mice showed alterations in mice olfaction and mitochondrial function, increased oxidants production, along with an inflammatory process evidenced by astrocyte activation. These data indicate that oxidative stress and inflammation may play a key role in CNS damage mechanisms, triggered by air pollution.

0068 - BEHAVIORAL DISORDERS CAUSED BY ACUTE CARBON MONOXIDE POISONING AND ITS RELATIONSHIP WITH PROGNOSTIC BIOMARKERS

Analia CORTEZ (1) | Rocío A GALARZA(1) | Sonia MOLINA(1) | Maria Agustina MENEGHINI(1) | Analía G KARADAYIAN(2) | Alicia Graciela FALETTI(1)

CENTRO DE ESTUDIOS FARMACOLÓGICOS Y BOTÁNICOS (CEFYBO-CONICET), FACULTAD DE MEDICINA, UBA (1); INSTITUTO DE BIOQUIMICA Y MEDICINA MOLECULAR (IBIMOL-CONICET) (2)

Carbon monoxide poisoning (CO) is preventable and avoidable. Hundreds of people die by acute intoxication to CO and many of them suffer from the well-known “late neurological syndrome” (LNS) with irreversible consequences. The aims of the present work were to assess the effects of an acute exposure to CO on the behavior, memory, anxiety and gait dynamics and to relate these changes to some prognostic biomarkers. To this end, adult rats were exposed to CO at acute doses (350 ppm for 20 seconds) capable of causing deterioration of the sensorium and different test were performed seven days post-intoxication. Compared with control animals (C), rats exposed to CO showed changes in the i) footprint test, compatible with ataxia, by exhibiting a greater maximum difference in the stride length, expressed as cm, (C: 6.5 ± 0.9, CO: 9.1 ± 0.7; $p<0.05$) and left overlap (C: 1.1 ± 0.1, CO: 1.8 ± 0.2; $p<0.01$); ii) open field test by exhibiting a greater exploratory activity and memory deficit ($p<0.05$); iii) elevated pluz maze test, by manifesting a greater degree of anxiety ($p<0.05$); and iv) inhibitory advance test by displaying lower latency to enter the dark compartment ($p<0.01$). By histological sections, at hippocampal level, we found a decreased thickness in areas CA1 ($p<0.01$), CA3 ($p<0.05$) and Subiculum zones ($p<0.05$), but not in the dentate gyrus. To search for some prognostic biomarkers, we evaluated the genetic damage in different cells using the comet assay. Compared with C, CO-exposed rats exhibited a higher genetic damage, expressed as tail DNA %, in i) peripheral blood (C: 5.1 ± 0.6, CO: 14 ± 3; $p<0.05$) and bone marrow (BM, C: 15 ± 2, CO: 31 ± 3; $p<0.01$) at 1 h post intoxication; and ii) in BM (C: 10 ± 2, CO: 27 ± 2; $p<0.001$) and brain (C: 27 ± 3, CO: 36 ± 3; $p<0.05$) at 7 days post-intoxication. These results suggest that the evaluation of behavior, gait, together with the presence of morphological changes in hippocampus and the detection of genotoxicity may inform early disorders before the development of LNS.

0102 - OVERNUTRITION INCREASES THE ADVERSE EFFECTS INDUCED BY REPETITIVE EXPOSURE TO 3-METHYLCHOLANTHRENE ON SPERM QUALITY

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3-Methylcholanthrene (3MC), a polyaromatic hydrocarbon, is an environmental pollutant that causes reproductive toxicity and is considered an obesogen by altering the lipid homeostasis, increasing the adipogenesis and causing metabolic disbalances. Previously, we found that 3MC impair the ovarian function by affecting the oocyte integrity and follicular development. Now, the aim of the present work was to study the effect of repetitive exposure to low doses of 3MC on the sperm quality in rats and to assess its relationship with overnutrition. To this end, male prepubertal rats fed standard (SD) or cafeteria (CD) diet were exposed to vehicle or 3MC (0.1 mg/kg) three times a week for 40 days. Rats were euthanized at 61 days of age and spermatozoa were collected by dissecting the caudal region of the epididymis. Sperm count (millions of spermatozoa/ml), motility (percentage), morphology by staining with Giemsa, and the presence of abnormal chromosomes by cytogenetic assay, were examined. CD rats treated with 3MC (CD3M) exhibited lower body weight ($p<0.01$) than SD rats treated with vehicle (SDV). No differences were found in the sperm morphology. However, SD rats treated with 3MC (SD3M; 22.9 ± 0.5), CD rats treated with vehicle (CDV, 16.5 ± 0.5) and CD3M (15.2 ± 1) had lower sperm count than SDV rats (35.6 ± 0.8; $p<0.001$). Likewise, and compared with SDV group (63 ± 2), both diet-fed rats and 3MC-treated rats exhibited decreased percentage of the sperm motility (CDV: 27 ± 2; SD3M: 43 ± 1; CD3M: 21 ± 3; $p<0.001$). Preliminary analyzes showed some chromosomal abnormalities in the 3MC-exposed rats. In addition, CD3M group showed a greater toxic effect on both the sperm count and motility when compared to the SD3M group. These results indicate that either overnutrition or exposures to low doses of 3MC affect the quality of male germ cells, and suggest that overnutrition makes organisms more susceptible to the toxic effect induced by 3MC, particularly when the exposure is repetitive.

0183 - SOY-BASED DIET MODIFIES THE INFILTRATION PROCESS IN CADMIUM INTOXICATED LUNGS.

Gabriel BOLDRINI (1) | Glenda Daniela MARTIN MOLINERO(1) | Eloy SALINAS(2) | Maria Eugenia CIMINARI(2) | Verónica Silvina BIAGGIO(1) | Silvina Monica ALVAREZ(1) | Nidia Noemi GOMEZ(1)

INSTITUTO MULTIDISCIPLINARIO DE INVESTIGACIONES BIOLOGICAS (IMIBIO-SL) (1); UNIVERSIDAD NACIONAL DE SAN LUIS (2)

Cadmium (Cd) is a toxic metal and also is an important environmental contaminant. Previously we observed by optical microscopy that Cd induces lung infiltration, so that we studied Cd intoxication and the parallels effects on haematological parameters and bronchoalveolar lavages (BAL) under different diets. Four lots of female Wistar rats were used: 2 received casein (Cas) and 2 received soybean (Soy) as protein sources. Within each group: 1 lot received regular water (control-Co) and the other 15 ppm of Cd in drinking water for 60 days. Blood samples were collected and they were analyzed using a hemocytometer ADVIA 360 (SIEMENS) and autoanalyzer A15 (BIOSYSTEMS). BAL was performed, cells were counted using a Neubauer's chamber and LDH activity was measured. Total RNA was isolated from lungs using Trizol and cDNA was obtained: VCAM-1 and Nrf2 were determined by PCR, using S28 as control. ANOVA test was used for statistical analysis. White blood cells decreased in both intoxicated groups ($p<0.05$). No significant differences among the percentages of lymphocytes (LYM), monocytes (MID) and granulocytes (GRA) were found. Nevertheless, MID showed a trend to increase in CasCd group and GRA showed the same trend in SoCd group. Hematocrit was lower in Soy vs. Cas groups ($p<0.05$). While total red blood cells were decreased in SoCo vs. CasCo ($p<0.01$), MCV was diminished in SoCd vs. CasCd ($p<0.01$). RDW decreased in SoCo vs. CasCo ($p<0.05$). Hemoglobin decreased in SoCo vs. CasCo ($p<0.05$). MCHC was significantly augmented in SoCd vs. CasCd ($p<0.05$). Uric acid was increased in CasCd vs. CasCo ($p<0.05$) and decreased in SoCd vs. CasCd ($p<0.01$). On the other hand, LYM increased in BAL of both Cd-intoxicated groups and GRA augmented in SoCd. LDH activity increased in CasCd vs. CasCo ($p<0.001$) and decreased in SoCd vs. CasCd ($p<0.0001$). Concerning to lung tissue, VCAM-1 expression increased in SoCd vs. SoCo ($p<0.05$) and showed the same trend in CasCd vs. CasCo, while Nrf-2 expression augmented only in SoCd vs. SoCo ($p<0.01$) and vs. CasCd ($p<0.01$). Based on these results we concluded that drinking water Cd induces oxidative stress on lungs and modifies hematological parameters. Uric acid levels showed a systemic oxidative stress in Cas-fed animals in presence of Cd. On the other hand, Nrf-2 expression showed an oxidative stress response only in Soy-fed animals. Changes on hematological parameters, especially WBC, could be related with changes in BAL cells population through modifications on VCAM-1 expression.

0775 - EFFECTS OF HYDROCARBONS ON CELL SURVIVAL AND ANTIOXIDANT SYSTEM OF BREAST CANCER CELLS.

Mariana MARDIROSIAN(1) | Mariel NÚÑEZ(2) | Marianela Soledad LASAGNA(1) | Clara VENTURA(2) | **Claudia COCCA** (1) | Andres VENTURINO(3)

INSTITUTO DE QUÍMICA Y FISICOQUÍMICA BIOLÓGICAS "PROF. ALEJANDRO C. PALADINI" (UBA-CONICET) (1); LAB RADIOISÓTOPOS, DEPTO DE FISICOMATEMÁTICA, FAC DE FARMACIA Y BIOQUÍMICA, UBA (2); CITAAC - CONICET - UNIVERSIDAD NACIONAL DEL COMAHUE (3)

The main productive activity of the Province of Neuquén is the extraction of oil and gas. Although the extractive process is controlled, incidents of spills and contamination of soils and water may occur. The aim of this work was to evaluate the content of the water-soluble fraction of petroleum (WSF) and to study the effects of hydrocarbons (HC) on cell survival and its antioxidant system. The chemical composition of the WSF was analyzed by gas chromatography. The total concentration of HC present in the WSF was 3.66 ± 0.24 mg/L and the main components were HC between C9 and C33. Polyaromatic hydorcarbons (PAH) concentration was 0.019 ± 0.003 mg/L. To study the effects of HC, MCF-7 and MDA-MB-231 mammary tumour cell lines were treated for 7 d with dilutions of the WSF (control, 1/500, 1/250, 1/100, 1/50 and 1/25) to perform clonogenic assays and exposed for 72 h to study viability (MTT assay), glutathione S-transferase (GST) and catalase (CAT) activities. No significant differences in clonogenicity were observed between controls and WSF-exposed cells. However, a significant decrease was observed in the viability of MCF-7 cells exposed to the lowest dilutions of the WSF and of MDA-MB-231 cells exposed to intermediate dilutions of the WSF ($p<0.05$). CAT activity was significantly increased in MCF-7 cells exposed to the lowest concentrations ($p<0.05$) and in MDA-MB-231 cells exposed to a 1/250 dilution of the WSF compared to the control group ($p<0.05$). No significant effects of the WSF were observed on GST activity in either cell line. The results suggest that GST may not participate in HC detoxification and that CAT would be induced only at low concentrations, being a possible biomarker of HC exposure. Alternatively, the WSF would induce predominant cytotoxic effects at low concentrations, triggering an antioxidant response through CAT. This hypothesis will be evaluated and the effects of the main PAH (anthracene and naphthalene) present in the WSF will be studied independently.

0914 - BIOMONITORING HORMONAL ACTIVITY IN COMPLEX SAMPLES: YEAST ESTROGEN AND ANDROGEN SCREEN ASSAY OPTIMIZATION

Sofia Candela MORA | Yamil TAVALIERI | Mariana FRITZ | Germán GALOPPO | Enrique Hugo LUQUE | Laura KASS | Mónica MUÑOZ-DE-TORO

INSTITUTO DE SALUD Y AMBIENTE DEL LITORAL (ISAL, UNL-CONICET),

Lack of control in the treatment of urban and industrial effluents, and the increasing use of agrochemicals lead to an increased exposure to hormonal active chemicals also known as endocrine disruptors (EDC). EDC are hormonally active substances that mimic or antagonize endogenous hormones that can be found worldwide and cause adverse effects in humans and wildlife. The yeast estrogen screen (YES) and yeast androgen screen (YAS) assays are used to evaluate hormonal activity in complex samples. The assays are performed using two strains of Saccharomyces cerevisiae stably transfected with human androgen receptor (AR) and estrogen receptor alpha (ER) respectively, together with reporter plasmids containing hormone response elements upstream of reporter gene LacZ. Each assay is carried out using serial dilutions of 17Estradiol (E2), tamoxifen (TMX), dihydrotestosterone (DTH) or flutamide (FLU) to build standard curves. Anti-hormonal activity is evaluated in the presence of TMX and FLU. Incubated with the yeasts and a chromogenic substrate, samples with hormonal activity develop a color changing reaction. Previously we reproduced the assay as described by Sohoni and Sumpter (1998). Reliable results were obtained using that method but sensitivity was lower than expected. The aim of this study was to optimize the methods of the YES and YAS assays. An alternative method was designed and compared to the original one. Parameters changed include incubation times with the chromogenic substrate, data analysis and incorporation of a lysis buffer. Solutions with different concentrations of BPA and water samples from the Ecological Reserve of the Universidad Nacional del Litoral (UNL), Santa Fe were evaluated to compare both methods. The new method presents advantages: reduced assay time, greater sensitivity to working dilutions, and reduced ranges of absorbance allowing better measurement discrimination and enhancing data analysis. Therefore, is our choice.

0923 - EFFECTS EVOKED BY PRENATAL EXPOSURE TO ATRAZINE ON CAIMAN LATIROSTRIS THYROID GLAND ARE ORGANIZATIONAL AND SEXUALLY DIMORPHIC.

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INSTITUTO DE SALUD Y AMBIENTE DEL LITORAL (ISAL, UNL-CONICET),

Increasing thyroid disorders, mainly in women, raised the hypothesis that exposure to endocrine disruptor compounds (EDCs) and sex-related factors could influence thyroid disease epidemiology. Exposure to EDCs can cause organizational or activational effects. Caiman latirostris is a crocodilian species highly sensitive to EDCs exposure. Atrazine (ATZ) is an herbicide suspected to cause thyroid disruption. Our aims were to describe thyroid histoarchitecture, to assess sexual dimorphic features and to determine the long term effects of prenatal exposure to ATZ on thyroid gland. Male and female caimans prenatally exposed to vehicle or to 0.2ppm of ATZ were raised, biometric parameters were recorded and, at prepubertal juvenile stage, caimans were sacrificed, thyroid were excised, weighed and processed until paraffin embedding. Thyrosomatic (TI) and condition indexes (CI) were calculated. The percentage of the gland occupied by stroma, epithelium or colloid; follicular hyperplasia, follicular epithelial height (FEH) and expression of ERα and Androgen Receptor (AR) were assessed. ANOVA followed by Tukey or Dunn´s post tests were used. No sexual dimorphism was observed in control caimans. Whereas in those ATZ exposed, sexually dimorphic responses were observed. In ATZ-females CIs were higher than in control while the opposite was observed in males. In females, ATZ exposure caused colloid depletion ($p= 0.0077$), increased FEH ($p= 0.0261$) and TI ($p= 0.0468$). In males, ATZ increased ERα expression ($p<0.0001$). In males and females, exposure to ATZ increased the gland area occupied by stroma ($p<0.0001$) and the percentage of hyperplastic follicles ($p= 0.0003$) and decreased the expression of AR ($p<0.0001$). Our findings demonstrate that prenatal exposure to ATZ cause caiman thyroid disruption; the effects were organizational and observed long after exposure ended, female higher vulnerability was evidenced. These findings alert on ATZ effects on exposed organisms, particularly females.

Reproducción / Reproduction I

Chairs: Andreina Cesari | Mariel Núñez

0107 - LUTEOTROPHIC EFFECT OF GNRH OR HCG ON CORPUS LUTEUM FUNCTIONALITY DURING THE LUTEAL PHASE IN EWES

Jimena FERNANDEZ (1) | Macarena BRUNO GALARRAGA(1) | Andres SOTO(2) | Luzbel DE LA SOTA(2) | Marcela CUETO(1) | Ulises NOTARO(3) | Natalia SALVETTI(3) | Hugo ORTEGA(3) | Alejandro GIBBONS(1) | Isabel LACAU-MENGIDO(4)

INSTITUTO FORESTAL AGROPECUARIO (IFAB- INTA CONICET) (1); INSTITUTO DE INVESTIGACIONES EN REPRODUCCION ANIMAL, FCV, UNLP (2); INSTITUTO DE CIENCIAS VETERINARIAS DEL LITORAL (ICIVET-LITORAL) (UNL-CONICET) (3); INSTITUTO DE BIOLOGÍA Y MEDICINA EXPERIMENTAL (IBYME-CONICET) (4)

Different therapeutic strategies have been used with the objective of increasing the concentration of progesterone (P4) and improving luteal function in order to reduce embryonic losses. The objective of the study was to determine the effect of gonadotrophin releasing hormone (GnRH) or human chorionic gonadotrophin (hCG) treatment at 4 days after timed artificial insemination (TAI) on the induction of accessory corpora lutea (acc-CL), the production and synthesis of P4. A total of 27 adult Merino ewes were randomly assigned to three groups on day 4 post TAI: GnRH group (n= 9; 4 µg IM of GnRH analogue, Receptal®, Intervet, Argentine), hCG group (n= 9; 300 IU IM of hCG, Gonacor®, Ferring, Argentine) and Control group (n= 9; 1 ml IM of saline solution). Laparoscopic observations of the ovaries on days 4 and 10 post TAI were performed to determine the presence of ovulatory CL (o-CL) and acc-CL, respectively. Serum P4 concentration was assessed by chemiluminescence on days 4, 7 and 14 post TAI. On day 14 post TAI, o-CL and acc-CL were removed (n= 5 ewes per each treatment) to determine STAR and 3ß-hydroxysteroid dehydrogenase (3ß-HSD) expressions by immunohistochemistry. The results were analyzed by ANOVA. The GnRH and hCG treated groups had a higher number of acc-CL compared to the control group (1.3 ± 0.1 vs. 0.0 ± 0.0, $p<0.01$). The administration of hCG increased serum P4 concentrations on days 7 and 14 post TAI (4.2 ± 0.4, 12.1 ± 1.4 ng/ml) compared to GnRH group (3.1 ± 0.4, 8.5 ± 1.4 ng/ml) and the Control group (3.5 ± 0.4, 6.7 ± 1.4 ng/ml; $p<0.05$). The STAR and 3ß-HSD positive cell/area tissue of both o-CL and acc-CL were higher in the hCG group than GnRH and Control groups ($p<0.05$). In conclusion, administration of hCG or GnRH on day 4 post TAI induced the formation of an acc-CL. However, serum concentration of P4 and some members of the progesterone synthesis pathway increased significantly only in the hCG group, evidencing different steroidogenic capacity of this hormone.

0223 - INVOLVEMENT AUTOPHAGY IN THE OVARY OF PREGNANT FEMALE VIZCACHA (LAGOSTOMUS MAXIMUS) WITH EMPHASIS ON THE CORPUS LUTEUM REGRESSION

Noelia LEOPARDO | Daira KARAM | Candela Rocío GONZÁLEZ | Alfredo VITULLO

CEBBAD, UNIVERSIDAD MAIMÓNIDES

Germ cell loss (follicular atresia) and corpus luteum (CL) regression (luteólisis) have been mainly associated to apoptosis although other cell death mechanisms, such as autophagy, could be also at play. Lagostomus maximus (L.m) is the only mammal which shows a very low or suppressed apoptosis-dependent follicular atresia and CL regression both in fetal and adult ovary. In this scenario, we

investigated autophagy protein expression during Lm pregnancy aimed to discern a possible role for autophagy proteins in germ cell and luteal cell death. Ovaries were obtained from female vizcachas at early (n= 5), mid (n= 5) and late (n= 5) phase of the pregnancy. The ovaries were screened for the expression of BECLIN1, LC3B, LAMP1 by Inmunohistochemistry and Western blot. The results of autophagy-associated protein LC3B and LAMP1 (lysosomes) showed that autophagy was significantly increased in CL during the late luteal phase, which was further confirmed by the protein expression changes of the autophagy related protein, BECLIN1. These results show that autophagy may play an important role in luteolysis. On the other hand, the expression of BECLIN 1, LAMP1, LC3B in oocytes and granulosa cells of all follicle types was observed only in isolated atretic follicles. These preliminary results suggest that autophagy as a cell death mechanism is induced mainly in atretic follicles and contributes to luteolysis of CL during the late luteal phase in pregnant animals. To our knowledge, this provides a new insight into the mechanism regulating the luteolysis in pregnant mammals. In conclusion, we propose autophagy as a mechanism of cell death and removal of corpora lutea to provide the necessary space for the formation and maturation of new follicles that sustain the massive ovulation and rebuilding of ovary architecture at each reproductive cycle that characterizes L. maximus.

0548 - ENDOGENOUS LIPID AND AMINOACID METABOLISM DURING BOVINE OOCYTE MATURATION IN A DEFINED MEDIA WITHOUT OXIDATIVE SUBSTRATES

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UNIVERSIDAD DE BUENOS AIRES, FACULTAD DE CIENCIAS VETERINARIAS, INITRA (UBA), INPA (UBA-CONICET)

Metabolic studies carried out in cumulus oocytes-complexes (COCs) usually refer to carbohydrates as they are the main substrates in the culture media. There is little information about endogen lipids (EL) and aminoacids (AA) as oxidative substrates. The aim of this work was to evaluate the nuclear maturation in a defined media lacking other oxidative substrates. Maturation was performed in a media without oxidative substrates, SOFm (without pyruvate and lactate) supplemented with FSH, LH, EGF, insulin and PVA, under mineral oil at 39 ºC in humidified atmosphere for 22 h. For the EL evaluation oocytes were randomly divided in 4 groups: positive control1 (+C1): SOF+glucose 5.5 mM, positive control2 (+C2): SOF+L-Carnitine (β-oxidation fatty acid stimulator), negative control (-C): SOF+etomoxir (β-oxidation fatty acid inhibitor) and treatment (T): without any supplementation and for the AA evaluation: positive control (+C): SOF+glucose+AA, treatment (T): SOF+AA, negative control1 (-C1): SOF+ sodium salicylate (inhibitor of glutamate dehydrogenase) and negative control2 (-C2): without any supplementation. The proportion of matured oocyte was evaluated by the presence of metaphase II after staining with Hoechst 33342. For the EL evaluation, the presence of carnitine (+C2) increased the nuclear maturation rate respect to the -C and the T groups ($p<0.05$). For the AA evaluation, the presence of AA (T) increased nuclear maturation rate respect to both negative controls (-C1 and -C2). EL and AA can in part support nuclear maturation during bovine oocyte in vitro maturation in defined media without other oxidative substrates.

0639 - ENERGETIC SOURCES REQUIRED TO MAINTAIN HUMAN SPERM HYPERACTIVATION IN VITRO

Clara MARIN BRIGGILER (1) | Guillermina María LUQUE(1) | Darío KRAPF(2) | Pablo VISCONTI(3) | Mariano BUFFONE(1)

IBYME-CONICET (1); IBR-CONICET, UNR (2); DEPARTMENT OF VETERINARY AND ANIMAL SCIENCES-University of Massachusetts (3)

To gain fertilization competence, mammalian sperm must undergo a complex process called capacitation, which is associated with the development of a distinct type of non-progressive motility (hyperactivation, HA). In spite of its relevance, the molecular mechanisms underlying HA, including its ionic and metabolic requirements remain elusive. The objective of the present work was to analyze the metabolic substrates necessary to maintain human sperm HA. Motile sperm obtained from healthy donors, were incubated for 4 h in BWW media containing glycolysable (Glucose, Glu) or non-glycolysable substrates (Pyruvate/Lactate, Pyr/Lact), all these nutrients (Complete medium, Comp), or none of them (Incomplete medium, Incomp). Motility parameters were measured by computer-assisted sperm analysis (SCA system). ATP content was analyzed by luminescence (kit by Cayman Chemical), and PKA-related phosphorylation (pPKA) and tyrosine phosphorylation (pY) were evaluated by Western blotting. Only sperm incubated in Comp medium showed maximum percentages of HA, and significantly lower HA levels were obtained in Incomp, Pyr/Lact ($p<0.01$) or Glu media ($p<0.05$, n= 4). Reduced ATP and pY levels were observed in Incomp, Pyr/Lact or Glu compared to Comp condition ($p<0.05$, $n > 4$); however, similar pPKA levels were obtained in all conditions. When incubated for 4 h in Incomp medium, the addition of Glu and Pyr/Lact for 1 min allowed the development of HA, which was accompanied by an increase in ATP content, but not in pY levels. This study indicates that both glycolysable and non-glycolysable substrates are required to support human sperm HA, and provides a model for the study of signaling mechanisms underlying this process.

0747 - MECHANISM OF ACTION OF COENZYME Q10 IN IMPROVING OVARIAN FUNCTION IN OBESITY

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Obesity has grown to epidemic proportions worldwide. It is associated with multiple adverse reproductive outcomes such as infertility and ovulation dysfunction. Coenzyme Q10 (CoQ10) is applied for the treatment of several pathologies, improving the reproductive outcome. Cafeteria diet (CAF) is the animal model used for the study of obesity that more closely reflects the western diet habits. However, the effects of CoQ10 on reproduction under obesity conditions are still little known. The aim of this work was to evaluate the effect of Coq10 on the ovarian function in obesity. For that purpose, 22 day-old female Wistar rats were divided in 2 groups that were fed ad libitum with standard rodent chow diet (Control groups) and CAF (Obese groups). After 7 weeks of diet protocol, half rats of each group received CoQ10 (5 mg/kg) orally for 13 days; while the others received vehicle. Animals (Ctrl, Ctrl+CoQ10, Obese and Obese+Coq10) were sacrificed on estrus phase; blood and ovaries were collected. Results: Estrous cycles were altered by obesity. Moreover, ovarian function was altered by obesity since: the number of corpora lutea was lower in obese rats compared to controls ($p<0.05$), as well as the number of antral follicles synthesizing Anti-Müllerian hormone ($p<0.01$). Serum estradiol levels were increased by obesity ($p<0.001$) without modifying those of estrone and progesterone. The ovarian mRNA expression Cytochrome P450 Aromatase was not altered in any group. All these alterations were normalized in obese rats after been treated with CoQ10. CAF diet-induced obesity alters the ovarian function by increasing estradiol synthesis and reducing the ovulation rates and the ovarian reserve. CoQ10 normalizes all of this parameters. Further studies are needed to clarify the effect and mechanisms of action of CoQ10.

0814 - MEMBRANE POTENTIAL HYPERPOLARIZATION INDUCES CYTOPLASMIC ALKALINIZATION OF MOUSE SPERM.

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IBYME-CONICET (1); IBR-CONICET, UNR (2)

To gain fertilization ability sperm must undergo a series of physiological modification in the female tract called capacitation, which leads to the acquisition of hyperactivated motility (HA) and the ability to undergo acrosome reaction (AR). Membrane potential (Em) hyperpolarization and alkalinization of the intracellular pH are necessary for HA and AR to take place. It has been reported either pharmacologically or genetically (potassium channel Slo3 knockout mice) that hyperpolarization is necessary and sufficient for sperm acrosomal responsiveness. In addition, intracellular alkalinization allows the opening of CatSper channels which lead to an increase in intracellular calcium that is fundamental for the HA of sperm. The aim of this work was to elucidate the interplay between Em and pH in mouse sperm. We used BCECF-AM and DISC3(5), probes that measures changes in cytoplasmic pH and Em, respectively. First, we studied the dependence of pH and Em, by co-incubating sperm with both probes followed by Flow Cytometry analysis. We observed that all the cells were grouped into two populations: 1) low pH and depolarized Em or 2) high pH and hyperpolarized Em. We also determined that sperm incubated under non-capacitating conditions with the addition of 1 µM valinomycin displayed a robust increase in pH suggesting that changes in Em are necessary for the rise in pH observed during capacitation. This increase was abrogated in the presence of high K^+ in the extracellular space. In addition, we studied the kinetics of this changes by assessing Em and pH at different time points of capacitation (0 to 60 min). Our results showed that there is a rapid pH alkalinization during the first 15 min of incubation in capacitating medium that is followed by a transient acidification at 30 min. Alkalinization is then restored at 45 min and remain unchanged. Thus, our results suggested that changes in the Em induces modifications in intracellular pH during mouse sperm capacitation.

Bioinformática, genoma, proteoma y nuevas tecnologías/ Bioinformatic I

Chairs - Francisca Ebel Barrera | Macarena Sarli

0192 - AQUEOUS MICELLAR TWO-PHASE SYSTEMS AS NOVEL TOOLS TO RECOVER PEPSIN-LIKE SURUBÍ PROTEASES: EFFECT OF SURFACTANTS ON ENZYME ACTIVITY

Antonella Valeria ACEVEDO GOMEZ (1) | Guillermo ESCOBAR(1) | Gabriela Noemí GOMEZ(1) | Laura LEIVA(1) | Soledad BUSTILLO(1) | Bibiana NERLI(2)

LABORATORIO DE INVESTIGACIÓN EN PROTEÍNAS. IQUIBA-NEA, UNNE, CONICET, FACENA. UNNE (1); INSTITUTO DE PROCESOS BIOTECNOLÓGICOS Y QUÍMICOS (IPROBYQ), UNR, CONICET. FCBYF (2)

Surubí is farmed in the northeast of Argentina. It is of interest to valorize the current disposal of fish processing through the use of viscera waste, source of enzymes such as Pepsin. These enzymes have been recovered mainly using conventional methods (e.g. salting out, chromatography), however the use of liquid-liquid extraction using surfactants have not been fully explored. Aqueous micellar two-phase systems (AMTPS) are an extractive method based on the ability of some surfactants to form two immiscible aqueous phases, a rich and a poor micelle phases to recover the product of interest. Thus, the objective of this work was to firstly evaluate the stability of surubí crude extracts under different concentrations of Genapol (GX080) and Tergitol (Tg7) to estimate the feasibility of their use as potential micellar extractants of surubí pepsin. Enzymatic extracts (ERPi) were recovered from stomachs homogenates using salting-out procedure and pepsin activity was estimated by acid hemoglobin method. ERPi were first incubated with various concentrations of surfactants (1, 3 and 5% GX080/Tg7) in 100 mM NaCit, pH= 5, for different times (0, 1, 2 and 3 h) and then enzymatic activity was measured. Results showed that any surfactant concentration tested affected ERPi enzymatic activity moderately and this effect was dose dependent. ERPi retained 92, 65 and 60% of its initial activity when were incubated with 1, 3 and 5 % of Tg7. After incubating with GX080 (1, 3 and 5 %), the ERPi exhibited 80, 70 and 60 % of its initial activity, respectively. The time variable in these assays had not significant influence on enzymatic activity decrease. Pepsin, a hydrophilic protein, is expected to be preferentially distributed into the aqueous phase of AMTPS.Considering that the surfactant concentration at this phase is always below 1 %, the obtained results suggest that AMTPS formed with either GX080 or Tg7 could be viable tools in the primary recovery of pepsin-like proteases from fishing waste.

0199 - EFFECTS OF A DIETARY SUPPLEMENT BASED ON ABSCISIC ACID, COUMARIC ACID, OMEGA ACIDS 3-6 AND AMINO ACIDS IN APIS MELLIFERA COLONIES

Facundo RAMOS | Nicolas SZAWARSKI | Facundo René MEROI ARCERITO | Azucena Elizabeth IGLESIAS | Giulia MITTON | Pablo Darian GIMENEZ MARTINEZ | Fiorella Giselle DE PIANO | Martín EGUARAS | Matías MAGGI

LABORATORIO DE ARTRÓPODOS, CENTRO DE INVESTIGACIÓN EN ABEJAS SOCIALES (CIAS). FCEYN. DPTO. BIOLOGÍA.

Currently, the majority of General Pueyrredón landscapes are dominated by the modern agriculture, characterized by high percentages of monocultives and a considerable amount of agrochemicals application. In this context, the regional apiculture has been affected by a drastic reduction in floral resources, leading to a diet deterioration and health weakening in Apis mellifera. In an attempt to improve parameters of strength in bee colonies, the present study assessed the effectiveness of a dietary supplement (COMP), made of abscisic acid (ABA) 10 µM, coumaric acid (CUM) 600 µM, protein hydrolyzate (HID) 14 µl/ml, omega acids 3-6 (O3/O6) 6 µl/ml and sugar syrup 2:1 (sugar:water). First, in vitro toxicity of COMP, and its individual components, were evaluated by laboratory bioassays. For this purpose, nurse bees were fed with these compounds for 96 hours. Survival rates were calculated, using a Kaplan-Meier test, and compared with those calculated from bees fed with syrup 2:1 (sugar: water) as controls. On the other hand, a field trial was performed, consisting in beehives fed (4 colonies per treatment) with COMP for 30 days during summer season. Every 15 days and at the end of the trial, the adult bee population, the amount of cells covered by brood, honey and pollen from each treated colony were recorded and compared with the respective controls, using two- way ANOVA test. The results obtained showed that COMP is palatable and non-toxic to A. mellifera (mean ± SE: 94.23 ± 3.65 % survival), producing an increase in the open-breeding population (p= 0.00825), with a consequent growth in pollen reserves (p= 0.0504). Thus, COMP could become a new nutritional tool in apiculture development under current stressful factors. Based on previous works, likely COMP induces immunological and detoxification mechanism in bee health.

0286 - PRELIMINARY RESULTS OF A COMPETITION ELISA TO DETECT ANAPLASMA MARGINALE ANTIBODIES.

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CONSEJO NACIONAL DE INVESTIGACIONES CIENTÍFICAS Y TÉCNICAS (CONICET) (1); INSTITUTO NACIONAL DE TECNOLOGIA AGROPECUARIA (2)

Bovine anaplasmosis is caused by the intraerythrocytic rickettsia Anaplasma marginale. It is an arthropod-borne hemolytic disease characterized by high lethality in adults. The disease is expanding and it is a threat to cattle industry. Live vaccine based on A. centrale, a less pathogenic species, is used to prevent acute anaplasmosis. Competition ELISA (cELISA) is used to assess rodeo epidemiological status through the detection of anti-MSP5 antibodies. A. marginale MSP5 (MSP5m) and A. centrale MSP5 (MSP5c) have 91 % of identity and cELISA does not differentiate A. marginale-infected from A. centrale-vaccinated cattle. In this work, a cELISA (cELISAm) was developed to detected specific A. marginale antibodies. cELISAm is based in the antigen A. marginale MSP5 and the Am6 monoclonal antibody. Am6 recognized an A. marginale specific epitope, absent in A. centrale. ELISA plates were coated with MSP5m (1 µg/well) overnight at 4 ºC. After blocked, the plates were incubated with serum samples diluted 1:2 in PBST/10 % fat-free dried milk containing 35 µg/ml MSP5c. Then, Am6 1/2500 and anti-mouse IgG peroxidase conjugate 1/3000 in PBS were added consecutively. The reaction was revealed with ABTS/H_2O_2. Results were expressed as inhibition percentage (%I). A total of 744 serum samples, previously classified by cELISA and nPCR, were analyzed by cELISAm: 497 negative, 185 A. marginale positive and 62 A. centrale positive serum samples. Sensitivity and specificity of the cELISAm were 97 and 95 %, respectively; with a cut off >18 %I. The 65 % of A. centrale-vaccinated cattle were not detected by cELISAm. Preliminary results showed that the cELISAm developed, allows us to detect with high sensitivity A. marginale-infected cattle decreasing the cross reaction for A. centrale. The identification of the specific epitope recognized for Am6 and the use of the polypeptide of this epitope in a cELISA with Am6, could enhance the performance of this specific cELISAm in the future.

0398 - HUMORAL IMMUNITY CONFERRED BY FOUR RECOMBINANT PROTEINS FROM NEOSPORA CANINUM ADJUVANTED WITH LIPOSOMES AND CPG-ODN IN CATTLE.

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EEA RAFAELA, INTA - CONICET (1); LABORATORIO DE INMUNOLOGÍA EXPERIMENTAL, FACULTAD DE BIOQUÍMICA Y CIENCIAS BIOLÓGICAS, UNL (2); EEA RAFAELA, INTA (3)

The apicomplexan protozoan Neospora caninum is a major cause of abortion in cattle with the consequent severe economic losses to production. It persists for life in tissue cysts with periodic reactivations. The present study was designed to evaluate the immunity generated by the recombinant proteins NcMIC1, NcMIC3 (micronemes), NcSRS2 (tachyzoites surface) and NcGRA7 (dense granules), adjuvanted with liposomes (Lip) and CpG oligodeoxynucleotides (CpG-ODN) against N. caninum infection and persistence. Recombinant His-tagged proteins expressed in Escherichia coli were purified by Ni-NTA affinity chromatography. Eighteen 3-year-old steers were divided in three groups (G) of six steers each. GA animals were inoculated with 100 µg of each recombinant protein and Lip+CpG-ODN. GB received Lip+CpG-ODN adjuvant without antigen and GC received sterile phosphate buffered saline (PBS). Steers were inoculated twice (days 0 and 21) and were challenged with 1 million tachyzoites of NC-1 strain at day 56 after the first dose (afd). Serum samples were collected weekly until day 102 afd. Indirect ELISA (iELISA) based on the four recombinant proteins (iELISAr) or N. caninum tachyzoites lysate (iELISAtach) were used to measure specific antibodies induced by the proteins. An increase in the level of antibodies against the four proteins were detected by iELISAr at day at day 14 afd and after challenge at day 75 afd in steers from GA compared with those from GB and GC ($p<0.001$). The iELISAtach detected antibodies against N. caninum 12 days after challenge. The antibody titer was higher in steers from GA than in steers from GB and GC ($p<0.001$). In conclusion, NcMIC1, NcMIC3, NcSRS2 and NcGRA7 recombinant proteins were immunogenic. The presence of tissue cyst in the brain of all cattle after slaughter is under analyzes.

0762 - ABILITY OF CATIONIC AND NEUTRAL HYDROGELS BASED ON N-ISOPROPYLACRYLAMIDE TO BIND EQUINE SPERMATOZOA.

Francisca Ebel (1) | Ana Cecilia LIAUDAT(1) | Nancy RODRIGUEZ(1)| Claudia Rosana RIVAROLA(2) | Pablo BOSCH(1)

INBIAS/CONICET. DEPARTAMENTO DE BIOLOGÍA MOLECULAR-UNIVERSIDAD NACIONAL DE RIO CUARTO (1); IITEMA/CONICET. DEPARTAMENTO DE QUIMICA/UNIVERSIDAD NACIONAL DE RIO CUARTO (2)

PNIPAm (poli(N-isopropylacrylamide) based hydrogels are biocompatible materials extensively used in biomedicine. The aim of this study was to evaluate the ability of PNIPAm hydrogels to attach stallion spermatozoa. PNIPAm was co-polymerized with cationic 3-(acrylamido propyl) trimethylammonium chloride (APTA) or neutral N-[Tris (hydroxymethyl) methyl] acrylamide (HMA) monomers. Studied copolymers contained 5 % APTA, 10 % APTA, 15 % APTA, 20 % HMA and 20 % HMA semi-interpenetrated with hyaluronic acid (20 % HMA-HA, 1 mg/mL). Each hydrogel was placed in a culture dish containing TALP medium and fresh stallion sperm suspension was added (1×10^6). The percentage of sperm attached to the surfaces was determined from the difference between the number of sperm initially added into the culture dish and the recovered non-bound cells after incubation (30 min at 37 °C in 5 % CO_2). Sperm that were exposed to -20 °C for 60 min to render them non-viable were incubated with 15 % APTA and 20 % HMA hydrogels-containing dishes, which served as negative controls. Data was analyzed by one way ANOVA; a $p<0.05$ was considered to be significant. Higher percentage of spermatozoa was attached to the surface with APTA compared to the HMA hydrogels ($p<0.05$). The percentage of sperm bound to APTA hydrogels did not differ among the cationic monomer concentrations (5 % APTA: 94.0 ± 4.0 %; 10 % APTA: 79.6 ± 13.2 %; 15 % APTA: 84.7 ± 14.7 %; $p>0.05$). The semi-interpenetration of 20 %HMA hydrogels with HA did not increase the percentage of spermatozoa bound to the surface (20 % HMA: 43.4 ± 7.9 %; 20 % HMA-HA: 50.5 ± 4.9 %; $p>0.05$). Few non-viable spermatozoa were observed attached to hydrogels (20 % HMA: 16.1 ± 8.3 %; 15 % APTA: 15.3 ± 4.8 %). In conclusion, cationic PNIPAm hydrogels could be an efficient support and binding substrate to adhere viable equine sperm, since stallion spermatozoa attaches more efficiently on them. It is expected that this strategy will allow the development of a technique for sperm selection, which isolates the cell subpopulation with high structural and functional quality characteristics, improving the efficiency of assisted reproduction techniques in horses.

This work was supported by grants from UNRC and MinCyT, Córdoba.

Biología celular y molecular de procesos fisiológicos y patológicos / Biology I

Chairs: Alejandra Erlejman | Gabriela Lombardi

0057 - 20-HYDROXYEICOSATETRAENOIC ACID (20-HETE) PROMOTES A MALIGNANT PHENOTYPE IN HUMAN CASTRATION-RESISTANT PROSTATE CANCER CELLS THROUGH STIMULATION OF THE G PROTEIN-COUPLED RECEPTOR GPR75.

Sofia CARDENAS (1) | Cecilia COLOMBERO(1) | Laura PANELO(2) | Rambabu DAKARAPU(3) | John FALCK(3) | Mónica COSTAS(2) | Susana NOWICKI(1)

CENTRO DE INVESTIGACIONES ENDOCRINOLÓGICAS "DR. CÉSAR BERGADÁ" (CEDIE)-CONICET (1); INSTITUTO DE INVESTIGACIONES MÉDICAS ALFREDO LANARI - UNIVERSIDAD DE BUENOS AIRES (2); UT SOUTHWESTERN MEDICAL CENTER (3)

20-Hydroxyeicosatetraenoic acid (20-HETE), the product of 20-hydroxylation of arachidonic acid by cytochrome P450 isoforms (CYP4F2 and CYP4A11), has a role in the oncogenesis of several human tumors. Recently, the GPR75 receptor has been identified as the target for 20-HETE. We have shown that androgen independent prostate cancer cells (PC-3) express GPR75. The aim of this study was to assess in vitro if 20-HETE/GPR75 modify the metastatic features of PC-3 cells. Cells were incubated with 20-HETE or its stable analog 5,14-HEDGE (both 0.1 nM) in the presence or absence of two different antagonists of the 20-HETE receptor, AAA or 19-HEDE (both 5 or 10 uM). The following assays were performed: e-cadherin and vimentin protein expression (epithelial-mesenchymal transition), zymography (release of matrix metalloproteinase-2 (MMP-2)), immunofluorescence and p-FAK (changes of cytoskeleton), scratch wound healing (migration), and soft agar colony formation (anchorage-independent growth). Results were analyzed using one-way ANOVA followed by Dunnet's. 20-HETE (24 h) increased by 150 % the expression of vimentin ($p<0.0001$, n= 3) and diminished by 40 % the expression of e-cadherin ($p<0.0001$, n= 3), whereas these effects were reversed by AAA ($p<0.0001$ and $p<0.05$, respectively). 20-HETE increased by 52 % the release of MMP-2 ($p<0.05$, n= 3), and this was also inhibited by AAA ($p<0.001$). AAA disorganized the actin filaments throughout PC-3 cells, while tubulin filaments remained unchanged. Also, 20-HETE increased by 89 % FAK phosphorylation (Y397) ($p<0.0001$, n= 3). 20-HETE increased by 147 % cell migration rate ($p<0.0001$, n= 3) and this effect was reverted by both antagonists, AAA or 19-HEDE ($p<0.05$ and $p<0.0001$, respectively), or by knockdown of GPR75 ($p<0.0001$). Finally, 5,14-HEDGE (21 days) formed twice the number of colonies vs. control ($p<0.05$, n= 2) and this was abolished by AAA ($p<0.05$). These results strongly suggest a role for GPR75 in 20-HETE-mediated metastatic features in PC-3 cells.

0072 - INTRACELLULAR CL- MODULATION OF IL-1ß SECRETION AND THE NLRP3 INFLAMMASOME EXPRESSION/ACTIVITY REQUIRE SGK1

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INSTITUTO DE INVESTIGACIONES BIOMEDICAS (UCA - CONICET)

The impairment of the CFTR activity induces intracellular chloride $[Cl^-]_i$ accumulation and consequently, as a second messenger, stimulates the secretion of interleukin-1ß (IL-1ß). We have previously described that this secretion starts an autocrine positive feedback loop. Moreover, the expression of two subunits of the inflammasome complex: NLR family pyrin domain containing 3 (NLRP3) and caspase-1 (CASP1), that are involved in the IL-1ß maturation, are indirectly modulated by the $[Cl^-]_i$. On the other hand, cellular and mitochondrial ROS (reactive oxygen species) also are regulated by $[Cl^-]_i$. Recently, other authors found that differences in $[Cl^-]_i$ modulates SGK1 (serum-glucocorticoid kinase 1) phosphorylation and subsequently regulates NF-kB activation in airway epithelial cells. Therefore, we decided to study the effects of SGK1 on IL-1ß expression at different $[Cl^-]_i$. In this study we used IB3-1 cells (a bronchial cell line derived from a cystic fibrosis patient with a DF508/W1282X CFTR genotype) and Caco-2 cells (transfected with CFTR-shRNA). The cells were incubated for 1 h at 5 or 75 mM Cl^-, in presence of ionophores tributyltin (10 µM) and nigericin (5 µM) to equilibrate $[Cl^-]_e$ and $[Cl^-]_i$. To explore if SGK1 was also involved in the IL-1ß response to $[Cl^-]_i$, we used the SGK1 inhibitor GSK650394 at 0, 0.1, 1 and 10 µM. After, we determine IL-1ß expression by quantitative real-time RT-PCR and ELISA quantification in culture media. To analyze the ROS response, we determined DCF fluorescence and MitoSOX fluorescence by microplate reader and/or flow cytometry. The results showed that SGK1 inhibitor diminished the response of IL-1ß mRNA to changes in the $[Cl^-]_i$ from 5 to 75 mM; GSK650394, at 10 µM, completely abrogated the IL-1ß mRNA response to Cl^- 75 mM ($p<0.05$, n= 3). Similar results were obtained on the secreted IL-1ß. On the other hand, SGK1 inhibitor, significatively reduced both, cellular and mitochondrial ROS levels at 75 mM Cl^- ($p<0.05$, n =3), suggesting that both the IL-1ß loop and the ROS response to Cl- were blocked by GSK650394. Similar results were found in Caco-2 with CFTR-shRNA. The results suggest that Cl^- effects are indirectly mediated by SGK1, which under Cl^- modulation stimulates the secretion of mature IL-1ß, in turn responsible for the observed upregulation of ROS and CASP1, NLRP3, and IL-1ß itself. The exact point of SGK1 action is still unknown.

0074 - INTRACELLULAR SIGNALING PATHWAYS TRIGGERED BY THE STIMULATION OF THE G-COUPLED PROTEIN RECEPTOR GPR75 BY 20-HYDROXYEICOSATETRAENOIC ACID (20-HETE) IN ANDROGEN INDEPENDENT PROSTATE CANCER CELLS.

Sofia CARDENAS (1) | Cecilia COLOMBERO(1) | Laura PANELO(2) | Rambabu DAKARAPU(3) | John FALCK(3) | Mónica COSTAS(2) | Susana NOWICKI(1)

CENTRO DE INVESTIGACIONES ENDOCRINOLÓGICAS "DR. CÉSAR BERGADÁ" (CEDIE)-CONICET (1); INSTITUTO DE INVESTIGACIONES MÉDICAS ALFREDO LANARI - UNIVERSIDAD DE BUENOS AIRES (2); UT SOUTHWESTERN MEDICAL CENTER (3)

20-HETE, the product of 20-hydroxylation of arachidonic acid by cytochrome P450 isoforms (CYP4F2 and CYP4A11), has a role in the oncogenesis of several human tumors. Recently, the GPR75 receptor has been identified as the target for 20-HETE. We have shown that androgen independent prostate cancer cells (PC-3) express GPR75. The aim of this study was to identify intracellular signaling molecules activated upon GPR75 stimulation by 20-HETE in PC-3 cells. Cells were incubated with 20-HETE (0.1 nM) in the presence or absence of the antagonist of the 20-HETE receptor, AAA (5 or 10 µM). Protein expression of the inducible focal adhesion protein Hydrogen Peroxide Inducible Clone-5 (HIC-5), the phosphorylated and total form of NF-kB, AKT, p38 MAP-Kinase (p38) and EGFR were assessed by Western blot. Intracellular localization of p-AKT, NF-kB and PKCa were determined by immunofluorescence and subcellular fractionation. Results were analyzed using one-way ANOVA followed by Dunnet's. Incubation with 20-HETE (2 h) increased the phosphorylation of EGFR, NF-kB and AKT by 146, 172 and 219 %, respectively (vs. control, $p<0.01$ for NF-kB, and $p<0.001$ for EGFR and AKT, n= 3), and this was inhibited by AAA (vs. 20-HETE alone, $p<0.05$ for NF-kB, $p<0.01$ for AKT and $p<0.001$ for EGFR). AAA alone increased p-38 phosphorylation by 248 % ($p<0.001$ vs. control, n= 3). 20-HETE (1 h) induced the translocation of p-AKT to the nuclei ($p<0.001$, n= 3) and promoted the redistribution of PKCa out of the nuclei ($p<0.05$, n= 3) to the plasma membrane ($p<0.001$). Both effects were inhibited by AAA (vs. 20-HETE, $p<0.01$ for AKT and $p<0.05$ for PKCa). AAA alone reduced the nuclear signal of p-AKT and NF-kB, usually activated in tumoral cells ($p<0.001$ for both, n= 3). Additionally, 20-HETE (12 h) increased by 150 % the protein expression of Hic-5 ($p<0.0001$, n= 5) and this was abolished by AAA ($p<0.001$). Our results show that 20-HETE modulates signaling pathways known to be deregulated in malignant cells through the GPR75-axis.

0076 - OPTIMIZATION OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR THE DETECTION OF TRITRICHOMONAS FOETUS

Mariana Paula MORERO(1) | Franco PARADISO LANGHOFF(1) | **Maria RAMIREZ** (2) | Jorge Anibal OYHENART(1)

INCITAP - CONICET – FCEN, UNIVERSIDAD NACIONAL DE LA PAMPA. (1); CONICET- INSTITUTO UNIVERSITARIO DE CIENCIAS DE LA SALUD. FUNDACIÓN H. A. BARCELÓ. (2)

Trichomonosis is a sexually transmitted disease of cattle caused by the parasite Tritrichomonas foetus (Rhyan et al., 1988). The control of the disease is limited to the identification and exclusion of the herd of infected breeding males. The diagnosis is based on microbiological culture, low cost but low sensitivity and specificity, or quantitative PCR, more sensitive and specific but more expensive (Parker et al., 2003; BonDurant et al., 2003; Cobo et al., 2007). Isothermal loop-mediated DNA amplification (LAMP) has great advantages over these diagnostic techniques (rapid, requires no equipment, more sensitive and specific), although it requires an adequate optimization process so that it can be carried out. The objective of this work was to optimize the conditions for amplification by LAMP of a fragment of the T. foetus ef1a gene recently developed using a Taguchi scheme (Cobb & Ciarkson, 1994). The optimization results were carried out by varying the concentration of dNTPs (0.06 - 0.5 mM), betaine (0 – 2 M) and $MgSO_4$ (2 – 18 mM), the results obtained were classified into 3 categories: 1 positive, 2 false positive and 3 negative. The grouping of the results was mainly due to the concentration of Mg^{2+} ions. In categories 2 and 3, regardless of the amount of betaine or dNTPs added, the result was a false positive or negative respectively. Within category 1, the optimized final reaction contained 9 mM Mg^{2+} ions, 1.7 M betaine and 0.125 mM dNTPs. Based on these data, we can conclude that LAMP could be used as an alternative tool to expensive molecular diagnostic techniques for the detection of T. foetus.

0237 - PIAS4 E3 LIGASE PROMOTES TAU ACCUMULATION

María Clara SOKN | Romina GOBBINI | Maia Ludmila BUDZIÑSKI | Belén UGO | Alejandra ATTORRESI | Sergio SENIN | Mariana ERDOCIA | Eduardo ARZT | Ana Clara LIBERMAN

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Tau proteins bind strongly to microtubules and are abundant in neurons. In these cells, they play a key role in the modulation of tubulin dynamics and axonal transport, among others. Tau deregulation leads to neurodegenerative diseases known as taupathies which are characterized by the formation of intracellular tau deposits. These aggregates are composed mainly of hyperphosphorylated tau. Hsp90 is a major cellular chaperone that forms large complexes with a variety of co-chaperones like the inmunophilin FKBP51. This complex has been described as a potential enhancer of abnormal tau stability by inhibiting its proteasomal degradation. Our group has described that FKBP51 SUMOylation, which is enhanced by the SUMO E3 ligase PIAS4, is essential in order to interact with Hsp90. Taking this into account, we hypothesize that PIAS4 could regulate tau stability. In this work we used HEK293 and N2a cells overexpressing PIAS4 SUMO ligase and analysed its effect on tau biology by western blot, cicloheximide assay and confocal microscopy of tau BifC dimerization fluorescent biosensor. Our results show that PIAS4 promotes tau and phospho tau accumulation (total tau: 59 %, $p<0.0001$; PSer396 -tau: 110 %, $p= 0.0004$; PSer214-tau: 310 %, $p<0.0001$; PSer202-tau: 280 %, $p<0.0001$, PSer396/404: 140 %, $p<0.0001$). This is dependent on PIAS4 E3-ligase activity. Interestingly, the increase in tau stability mediated by PIAS4 does not depend on tau SUMOylation, because this enzyme is unable to induce SUMO conjugation to tau. Accordingly, PIAS4 also enhances tau K340R (SUMOylation mutant) stability (45 %, $p<0.001$). PIAS4 reduces tau – microtubules binding as shown by the loss of network distribution in the confocal images of cells cotransfected with tau-BifC plasmids. This could be the consequence of an increase in phosphorylated tau driven by the ligase. Finally, the BifC assay suggests that PIAS4 enhances the interactions between tau proteins, a process that has been linked to tau pathological deregulation.

Supported by ANPCyT, CONICET, UBA and FOCEM (COF 03/11) grants.

0245 - CONTRIBUTION OF NEURAL CREST DERIVED-CELLS AND BONE MARROW GLAST+ CELLS TO THE LIVER

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IIMT (1); INSTITUTO MILSTEIN (2); INSTITUTO MILSTEIN (3)

Cirrhosis, the last stage of liver fibrosis, results from repeated cycles of liver damage and scar formation and is the first cause of liver transplant. Little is known regarding the contribution of neural crest-derived cells and bone marrow (BM) to the liver in health and disease. Objective: The aim of this work was to analyze the role of neural crest-derived and/or BM-GLAST+ cells to liver fibrogenesis and regeneration. Wnt1Cre2 and GLASTCreERT(2);Rosa26Tomato mice were used. Two models of liver cirrhosis were generated: 1) repeated applications of thioacetamide and 2) bile duct ligation. In addition, a model of partial hepatectomy was applied. The phenotype of BM, peripheral blood and liver traced-cells was analyzed by flow cytometry and immunostainings. Data from in vivo studies suggest an activation of liver glia during fibrogenesis as well as increased numbers of traced endothelial cells. In both transgenic mice, some hepatocytes were traced and they increase in numbers in the fibrotic liver. Stromal cells within the BM were also traced in both mouse lines. BM cells were mobilized during fibrogenesis and Wnt1-traced cells formed pure stromal colonies when attached to plastic. Application of IMT504 was able to restore Wnt1-traced CFUs in the context of liver fibrogenesis. The incidence of CD44+ HNF4α+ GLAST-traced hepatocytes increased in numbers during liver regeneration. Bone marrow GLAST- and/or Wnt1-traced cells likely contribute with liver regeneration in models of liver fibrogenesis and partial hepatectomy.

0532 - HOMOLOGY MODELING AND IN SILICO DOCKING ANALYSIS OF A MURINE IL-12 ENGINEERED TO BLOCK ITS HEPARIN BINDING ACTIVITY AND FUSED TO A THERAPEUTIC MONOCLONAL ANTIBODY

Sol FERRERO(1) | Rosendo LURIA-PÉREZ(2) | Pierre CANDELARIA(3) | Tracy DANIELS-WELLS(3) | José RODRÍGUEZ(3) | Manuel PENICHET(3) | **Gustavo HELGUERA** (1)

IBYME-CONICET (1); HOSPITAL DE NIÑOS "FEDERICO GÓMEZ" (2); UNIVERSITY OF CALIFORNIA, LOS ANGELES (UCLA) (3)

The cytokine Interleukin-12 (IL-12) is a heterodimeric immune-modulator with heparin binding activity. This property favors the binding of the cytokine to the cell surface on glycosaminoglycans (GAGs) at the extracellular matrix and has been implicated in modulating IL-12 bioactivity. We have constructed an antibody-cytokine fusion protein with murine single-chain IL-12 genetically fused to a human IgG3 specific for the human tumor-associated antigen HER2/neu. It maintains cytokine bioactivity, antigen binding, anti-tumor activity, and IL-12 heparin-binding activity. Previous studies indicate that the domain responsible of the heparin-binding activity is at the p40 subunit of human and murine IL-12, but the absence of X-ray crystallography studies of the heparin-p40 complex precludes the structural analysis of this interaction. Here we used an in silico docking analysis in order to identify the pocket responsible of this interaction. Using as a template the structure of the human p40 subunit (PDB ID 3DUH), we generated a homology model of mouse p40 using SWISS-MODEL and performed the in silico docking to the heparin molecule using ClusPro. In both cases the analysis of predicted contacts of

best models placed the heparin binding pocket in a conserved cluster of basic arginine (R) and lysine (K) residues (a.a. 254–260 RKKEKMK) of the murine p40, which is partially conserved in human p40. A homology model replacing the basic amino acids by the neutral non-polar amino acid alanine (A), predicted a significant reduction of heparin atom contacts in this pocket. Moreover, after generation of the corresponding mutant fusion protein, ELISA and flow cytometry studies showed that it lacks heparin-binding activity but retains antigen binding. Therefore, these studies suggest that in silico docking analysis tools could be useful to guide the rational design of antibody-cytokine fusion proteins with tailored properties to improve their specificity, efficacy and safety profile.

Infectología y Parasitología / Infectology and Parasitology I

Chairs: Carlos Laino | Andrea Maglioco

0077 - CULTIVATION OF TRITRICHOMONAS FOETUS IN BOVINE CERVICAL FLUID.

Franco PARADISO LANGHOFF(1) | Mariana MORERO(1) | **Maria RAMIREZ** (2) | Jorge Anibal OYHENART(1)

INCITAP - CONICET – FCEN, UNIVERSIDAD NACIONAL DE LA PAMPA. (1); CONICET- INSTITUTO UNIVERSITARIO DE CIENCIAS DE LA SALUD. FUNDACIÓN H. A. BARCELÓ. (2)

Tritrichomonas foetus is a flagellated protozoan bovine parasite. Although it can colonize other species there are no known intermediate hosts, forms of free life or resistance. T. foetus is the causative agent of sexually transmitted disease known as bovine trichomoniasis (TB). TB is common in countries where extensive bovine breeding and natural service are practiced, causing losses in calf production. In the bull the presence of T. foetus is asymptomatic while in the female it is recognized for a prolonged period between services, early abortions, vaginitis, among other aspects. In this study, the objectives were in principle the synchronization and subsequent extraction of vaginal cervical fluid (FCV) belonging to the different stages of the bovine estrous cycle; subsequently the culture of T. foetus cells in the aforementioned flow samples with the purpose of finding differences that allow us to identify an “optimal culture moment”, if this exists. After the analysis of the data, it was possible to observe that the FCV allows cell growth, counted at 48 hours, and does so differentially according to the stage of the estrous cycle to which the sample corresponds. Based on these results, it is possible to conclude that the proposed model is appropriate for the initially proposed objectives.

0137 - HUMAN MESENCHYMAL STEM CELL-DERIVED CONDITIONED MEDIA (MSC-CM) PROTECTS HUMAN MICROVASCULAR ENDOTHELIAL CELLS FROM SHIGA TOXIN TYPE 2 CYTOTOXICITY.

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Hemolytic uremic syndrome (HUS) is the clinical triad of thrombocytopenia, anemia, and acute renal failure (ARF). HUS is classically associated with Shiga toxin–producing Escherichia coli infection and affects mainly children under 5 years old. Argentina exhibits the highest incidence rate in the world. HUS lacks a specific treatment and many patients develop chronic kidney disease. Recently, mesenchymal stem/stromal cells (MSCs) have been proposed to treat the ARF. MSCs release several antiapoptotic and proliferative mediators that could mitigate the cytotoxic effects caused by Stx2 on renal cells. The objectives of this work were to isolate human MSCs and to analyze if whether the human mesenchymal stem cell-derived conditioned media (MSC-CM) would be able to protect human glomerular endothelial cells (HGEC) from the detrimental effects of Stx2. MSCs were isolated by culturing explants of Warthon’s Jelly from human umbilical cord. Then, cells were subcultured and MSC-CM was collected after 24 h of incubation. HGEC were treated with Stx2 (0.5 ng/ml) and in the presence or not of MSC-CM. After 72 h, cell viability was analyzed by neutral red uptake. Cell morphology analysis was evaluated by light microscopy after staining with H&E. Cell counts were performed on four fields and cell area values were obtained using Image J software. Results are expressed as percentage respect to controls (100 %). MSC-CM significantly protected in about 30 % the HGEC viability ($p<0.05$). Also, prevented cell morphologic disturbances, since Stx2 in presence of MSC-CM caused less swelling and retraction. HGEC cell area was preserved in about 62 % ($p<0.05$) and cell detachment was reduced in 46 % ($p<0.05$). In conclusion, MSC-CM were able to avoid the injury caused by Stx2 on HGEC. Therefore, MSCs could be considered as a therapeutic strategy to prevent the renal damage caused by Stx2.

Supported by ANPCyT (PICT 0617), CONICET (PUE2017-IFIBIO Houssay) and UBA (UBACyT 2017) grants.

0224 - PRESENCE OF MICROVESICLES CARRIED SHIGA TOXIN TYPE 2 IN PATIENTS WITH POST-DIARRHEAL HEMOLYTIC UREMIC SYNDROME.

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Shiga toxin (Stx) producing Escherichia coli (STEC) is responsible for bloody diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS). HUS mainly affects children under 5 years old and it is characterized by kidney and brain damage caused by Stx. So far, there is no a clinical marker for establish an early diagnosis of this pathology. Microvesicles (MVs) are small (<1 µm), pro-inflammatory vesicles shed by host cells during activation and apoptosis. We previously were able to detect the presence of circulating MVs bound to Stx2 (MVs-Stx2) in a rat model of sublethal HUS (Sacerdoti et al. Medicina 77 (supl I): 558 (a 1404), 2017). The objective of this work was to analyze whether the finding of MVs-Stx2, in HUS patient’s blood samples, could be useful as an early clinical biomarker to diagnose this disease. Two children who developed bloody diarrhea were admitted to the hospital (P1 and P2). Two days after admission, blood samples were obtained and sequentially ultracentrifuged in order to obtain a MVs-enriched suspension samples. Five age-matched healthy controls (Ctrl) were recruited. Then, MVs were labeled with Annexin V-FITC and MVs-Stx2 were detected by a mouse monoclonal anti-Stx2 antibody and a secondary antibody labeled with Alexa Fluor 647. Finally, MVs were analyzed by flow cytometry. Data are expressed as the percentage of positive MVs-Stx2. From the controls, a cut-off point for MVs-Stx2 was established (1.02 - 1.90 %, n= 5). A significant higher percentage of MVs-Stx2 in both patients was detected (P1: 3.63 %, P2: 5.20 %, $p<0.05$). These results indicate that MVs-Stx2 could be a clinical biomaker for the diagnosis of HUS in the early stage. The detection of MVs-Stx2 in combination with STEC and free fecal Stx2 in stool culture considerably can improve diagnosis. Supported by ANPCyT (PICT 0617), CONICET (PUE2017-IFIBIO Houssay) and Universidad de Buenos Aires (UBACyT 2017) grants.

0231 - ELIGLUSTAT, AN INHIBITOR OF GB3 RECEPTOR SYNTHESIS, PROTECTS HUMAN MICROVASCULAR ENDOTHELIAL CELLS FROM SHIGA TOXIN TYPE 2 CYTOTOXICITY.

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Hemolytic Uremic Syndrome associated to Shiga toxin (Stx)-producing E. coli infection is the most common cause of acute renal failure (ARF) in children in Argentina. Stx2 binds the globotriaosylceramide (Gb3) receptor and causes direct damages on human renal microvascular endothelial cells (HGEC). In this work, we assayed the action of a Gb3 synthesis inhibitor, Eliglustat (EG), to prevent the Stx2 cytotoxicity on human renal cells. Cell viability was analyzed by neutral red uptake and data are expressed as mean ± SEM. Cell morphology analysis was evaluated by light microscopy after staining with H&E. Cell counts were performed on five fields and cell area values were obtained using Image J software. Necrosis and apoptosis were detected by flow cytometry after Annexin V-FITC/PI double staining assay. Non-cytotoxic concentrations of EG were established on HGEC treated with EG (0.05 – 50 µM) for 120 h. While EG (50 µM) caused a significant decreased of cell viability (8.3 ± 0.9% vs. Ctrl: 100 ± 2.7 %, n= 3, $p<0.05$), EG (0.05 – 25 µM) did not exhibit any cytotoxic effect. Next, HGEC were pre-treated with non-cytotoxic EG concentrations at different times (2, 4, 6, 24 and 48 h) and then incubated with Stx2 (0.5 ng/ml) for 72 h and in the presence of EG. At all the times, EG (0.5 - 10 µM) prevented the decrease in HGEC viability caused by Stx2 (n= 5, $p<0.05$) and pre-incubation with EG (5 µM) for only 2 h was enough to protect the HGEC viability in about 73 %. The maximum protection (100 %) was obtained after 24 and 48 h of pre-treatment with 5 µM EG (EG 24 and 48 h: 100.0 ± 2.6 vs. Stx2: 49.0 ± 7.9 %, n= 5, $p<0.05$). Furthermore, EG (5 µM, 24 h) prevented the cell detachment in 80 % and the swelling in 81 %. Finally, a significant prevention (86 %) of necrosis induced by Stx2 was obtained with EG (1 µM, 24 h). We propose EG as a therapy to avoid the renal damage and the consequent ARF.

Supported by ANPCyT (PICT 0617), CONICET (PUE2017-IFIBIO Houssay) and UBA (UBACyT 2017) grants.

0376 - POTENTIAL ROLE OF INTERLEUKIN 10 ON THE EFFECTS OF FENOFIBRATE IN AN EXPERIMENTAL MODEL OF CHAGAS DISEASE.

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Chagas disease, caused by Trypanosoma cruzi (Tc) infection, is conditioned by the presence of the parasite and the development of an inflammatory response. PPARa ligands, such as fenofibrate (fen) modulate inflammation and restore ventricular function. Interleukin 10 (IL10) is produced by a variety of cells and plays an important role in the resolution of inflammatory processes. We evaluated the potential role of IL10 in the effects of fen on the modulation of the immune response and on cardiac remodeling and function, in BALB/c IL10 knockout (IL10-/-) mice infected with a non-lethal Tc strain. The ejection fraction (EF) and the shortening fraction (SF) were measured by echocardiography, which were diminished in Tc in comparison with uninfected mice ($p<0.05$) at 4 weeks post infection (wpi). Fen treatment was given from the 5th to 9th wpi. When the treatment finished, EF and SF were evaluated again, fen restored them to levels of uninfected mice ($p<0.05$). The expression of IL6, TNFa and NOS2 was analyzed at 9 wpi in the heart, using RTq-PCR. Infection increased the expression of IL6, TNFa and NOS2 ($p<0.05$), and fen inhibited them ($p<0.05$). While Tc infection induced the release of IL17, IL6 and TNFa to serum (ELISA $p<0.05$), fen inhibited it ($p<0.05$). The inflammatory reaction was also studied in heart sections. Fen was not capable to decrease the inflammatory infiltrates neither fibrosis (p= NS) in IL10-/- mice. Furthermore, expression of the M2 profile markers was evaluated in the heart of IL10-/- mice. Fen did not modify the expression of Mannose Receptor, FIZZ and YMI (RTq-PCR p= NS) unlike what had been observed for these markers in wild type mice. These results suggest that IL10 is required to induce an M2 profile and modulation of fibrosis and inflammatory infiltrates by fen. On the other hand, fen effects on heart function and modulation of proinflammatory mediators seem to be IL10-independent. Therefore, fen exerts IL10-dependent and IL10-independent effects.

0384 - A TRANSCELLULAR GB3 DEPENDENT PATHWAY IS MAINLY RESPONSIBLE FOR SHIGA TOXIN-2 CYTOTOXICITY AND TRANSLOCATION ACROSS HUMAN INTESTINAL EPITHELIAL CELLS INFECTED WITH E. COLI O157:H7

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Shiga toxin-2 (Stx2) is produced and released by E. coli O157:H7 (O157:H7) into the intestinal lumen after colonization, and is able to translocate to the circulatory system and reach target cells causing hemolytic uremic syndrome. Our aim was to elucidate which pathways were involved in Stx2 endocytosis and translocation across intestinal cells infected with STEC. HCT-8 cells grown on 96-well plates were preincubated with specific endocytosis inhibitors such as Eliglustat (EG), Dynasore (DY), MßCD or Amiloride (AM). Then, cells were washed and incubated for 4 h with 100 ng/ml Stx2 alone or in the presence of O157:H7 mutant lacking stx2 gene (O157:H7Ðstx2). Stx2 uptake was measured by flow cytometry and its cytotoxic effect by neutral red uptake assay. Translocation of Stx2 was evaluated by inhibitor preincubation of HCT-8, grown as monolayers on Millicell inserts, and incubated with O157:H7Ðstx2+ Stx2. Then Stx2 cytotoxicity was quantified in lower chamber media by neutral red uptake, To analyze inhibitors effect on bacteria attachment, bacterial adherence assays were performed on HCT-8 monolayers cultured on 24-wells plates. EG caused the maximum decrease of Stx2 cytotoxic activity, followed by MßCD. AM and DY significantly neutralized Stx2 cytotoxicity but only in presence of O157:H7Ðstx2. Furthermore, Stx2 uptake was reduced when cells were pre-incubated with EG or MßCD, compared to DY or AM ($p<0.05$), indicating that Stx2 uptake may depend on Gb3 receptor, and, to a lesser extent, on cholesterol, which is consistent with a necessary interaction between Stx2 and its receptor to cause cytotoxicity. Moreover, both dynamin-dependent endocytosis and Gb3-independent macropinocytosis became relevant only when bacteria were present, suggesting that these mechanisms are sensible to bacterial infection. Taken together, our study suggests that the mechanisms responsible for enhanced cytotoxicity and transcytosis during infection may have the same endocytic origin.

0397 - ANTIBACTERIAL ACTIVITY OF EXTRACTS OF ILEX PARAGUARIENSIS ST. HIL AGAINST METHICILLIN-RESISTANT AND METHICILLIN-SENSITIVE STAPHYLOCOCCUS AUREUS

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Methicillin-resistant Staphylococcus aureus (MRSA) is a major nosocomial pathogen which causes severe morbidity and mortality worldwide. The emergence of resistance against classical pharmacological treatments generates interest in the development of new alternatives. Ilex paraguariensis St. Hil is a plant species of South American origin and source of bioactive principles useful for both the food and pharmaceutical industry. The aim of this work was to search for antimicrobial activity of leaf extracts of Ilex paraguariensis against methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-sensitive Staphylococcus aureus (MSSA). Staphylococcus aureus ATCC 43300 (MRSA) and Staphylococcus aureus ATCC 25923 (MSSA) were analyzed with aqueous extracts obtained from Ilex praguariensis leaves dried at room temperature and at 80 °C. The extracts were obtained by controlled digestion at 37 °C for 24 h with water. The Minimum Inhibitory Doses (MID) was determined by plate disc diffusion test inoculating a 0.5 McFarland turbidity bacterial suspension on Mueller-Hinton agar. Fifteen, 10, 5, 1 and 0.5 mg of each extract were loaded on the discs. Distilled water was used as control. The tests were done in triplicate. Only the aqueous extract obtained from leaves dried at room temperature showed antibacterial activity against both strains with a MID of 5 mg disc-1. Whereas the aqueous extract obtained from leaves dried at 80 °C did not show inhibitory activity at all. We conclude that the aqueous extract of this plant may be an effective potential candidate for the development of new strategies to treat Staphylococcus aureus infections. It is necessary to continue with studies to identify and characterize the metabolites responsible for antibacterial activity considering that temperatures above 80 °C inhibit their functionality.

0427 - COMPARATIVE STUDY OF THE HOST GENOTYPE-PARASITE INTERACTION IN RESPONSE TO INFECTION WITH INCREASING DOSES OF TRICHINELLA SPIRALIS (TS) OR TRICHINELLA PATAGONIENSIS (TP) IN THE CBI-IGE MURINE MODEL

Paula A. INDELMAN(1) | Guillermina SPREAFICHI(1) | Ma. Agustina FERNÁNDEZ(1) | Ana Victoria CODINA(2) | **María Delia VASCONI** (2) | Lucila I. HINRICHSEN(2)

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Trichinellosis is a parasitic zoonosis caused by the consumption of meat and its products from domestic pigs and wild animals infected by the parasite Trichinella spp. Recently, a new species of Trichinella, Trichinella patagoniensis, associated with human consumption of puma meat, was discovered in Argentina. Host-parasite interactions define the onset, progression, and outcome of an infection with the host and parasite genotypes ultimately determining these interactions. This work aimed to compare the effect of the host genotype on response to infection with increasing doses of T. patagoniensis or T. spiralis in genetically defined lines of the CBi-IGE colony. CBi+, CBi-, CBi, and CBi/L males (n= 8 per line and infective dose) were used. Mice were divided into three groups and infected with 1, 2 or 4 Ts or Tp L1 larvae/g of body weight, and were sacrificed at 40 ± 2 days post-infection, in the chronic stage of the parasite cycle. The degree of infection of each mouse was estimated by the number of larvae/g of fresh tissue recovered from the tongue (rPB, relative muscle parasitic burden). rPB was significantly higher in Ts infected mice in the dose range analyzed, indicating that, in this model, Tp infectivity is low compared with Ts ($p<0.01$). The infective dose/host genotype interaction was significant in Ts ($p= 0.002$) and non-significant in Tp infected animals ($p= 0.26$). The latter showed dose ($p<0.001$) and host genotype ($p= 0.001$) effect. The infective dose/parasite genotype interaction was only significant in CBi+ mice ($p= 0.005$). These analyses showed that genotype CBi/L was always the most resistant host, whereas CBi+ degree of susceptibility depended on the parasite genotype. CBi and CBi-, on the other hand, had a similar response to increasing doses of the two Trichinella species. In this model, the interaction between the host defense mechanisms and the pathogen's ability to avoid or modify such defenses determines the success of a parasitic infection.

Cardiovascular y Respiratorio / Cardiovascular and Respiratory II

Chairs: Rocío Castilla| Natalia Rukavina

0096 - THE CARDIOPROTECTION MEDIATED BY ISCHEMIC POSTCONDITIONING AND TRX1 OVEREXPRESSION RESTORE MITOCHONDRIAL FUNCTION IN MICE HEART.

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Thioredoxin-1 (Trx1) maintains the cellular redox status and decreases the infarct size in ischemia/reperfusion injury (I/R). However, it is not fully understood the role of Trx1in ischemic postconditioning (PostC) in young and middle-aged mice and its relation with mitochondrial function. The aim was to study if Trx1is involved in the PostC cardioprotection mechanism and can restore mitochondrial function and if the age can modify this. Wild type mice hearts (Wt), transgenic mice hearts overexpressing Trx1, and a dominant negative mutant (DN-Trx1) of Trx1 were used, mice were divided in young group (4 months) and middle-aged group (12 months). The mice hearts were subjected to 30 min of I and 120 min of R (Langendorff technique) (I/R group). In the PostC group, after I, a protocol of 6 cycles of R/I was performed. The assessment of the infarct size was performed using TTC. Also, it was measure mitochondrial function (polarographically with a Clark-type electrode). Data are expressed as mean±SEM, n= 6 each group and $p<0.05$ was considered statistically significant. Previously, we showed that Trx1 is involved in cardioprotection conferred by PostC in young mice but in middle-aged mice, the cardioprotection was abolished in groups with PostC. This infarct size behaviour was accompanied by a lack of survival proteins phosphorylation (Akt and GSK3B) and changes in Trx1 expression (in Wt group). Trx1 activity was diminished, H_2O_2 production and protein nitration were increased in all middle-aged groups. Our results showed that Trx1 plays a key role in the PostC protection mechanism in young mice but in middle-aged mice, this cardioprotective mechanism was abolished. Recently, we performed the same groups and observed no changes in state 4 oxygen uptake in middle-aged mice and young mice neither. In young groups, state 3 oxygen uptake was significantly lower in Wt-I/R group than Wt-Nx group (Wt-Nx: 137.4 ± 6.0 vs. Wt-I/R: 97.1 ± 14.2) and in Wt-PostC group the value tended to recover normoxic values (113.9 ± 11.7). In middle-aged groups, state 3 oxygen uptake was lower in Wt-I/R and Wt-PostC groups than Wt-Nx group (Wt-Nx: 176.0 ± 9.4 vs. Wt-I/R: 135.5 ± 9.6 and Wt-PostC: 113.9 ± 11.7). No differences appeared between Trx1 groups and the same was observed in DN-Trx1 groups. In conclusion, we found the cardioprotection mediated by PostC and Trx1 overexpression restore mitochondrial function in young mice but not in middle-aged mice.

0187 - CARDIOPROTECTIVE EFFECTS OF NEBIVOLOL ON ISCHEMIA/REPERFUSION IN HYPERTHYROID RATS: MECHANIC AND ENERGETIC STUDY

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Nebivolol (Nbv) is a third-generation ß-blocker with high selectivity for ß1-adrenergic receptors and it induces vasodilation by stimulating endothelial nitric oxide synthase (NOS). These effects could be cardioprotective in hyperthyroidism. Now, we studied the mechanic and energetic effects of Nbv in euthyroid (EuT) and hyperthyroid (HypT) rat hearts exposed to severe ischemia-reperfusion (sI/R) without infarct. Rats were daily injected with 20 µg/kg triiodothyronine s.c. for 15 days. Isolated ventricles from either HypT or EuT rats were perfused in a calorimeter and exposed to sI/R (30 min I/ 45 min R). Left intraventricular pressure (P, mmHg) and total heat release (Ht, $mW.g^{-1}$) were measured. Perfusion with 0.03 µmol/l Nbv improved the postischemic contractile recovery (PICR) to 39.0 ± 4.4 % of pre-I of P (vs. 24 ± 3 % in HypT-C, *p<0.05) at 45 min R in HypT, and to 28 ± 3 (vs. 12 ± 5 % in EuT-C, *p<0.05) at 15 min R in EuT, without changing muscle economy (P/Ht). In both cases Nbv increased the diastolic contracture during R (LVEDP: 16 ± 4 and 20 ± 6 mmHg respectively, *p <0.05 vs. 0). To evaluate whether the nitric oxide (NO) participates in the cardioprotection of Nbv, the NOS were blocked by L-NAME before I. The effects of Nbv on PICR and P/Ht were significantly reversed by the pre-treatment with L-NAME without changing LVEDP in HypT and EuT. Other groups of rats were treated with 20 mg/kg Nbv daily administered in drinking water during 7 days. In EuT, Nbv significantly improved PICR to 78.8 ± 10.6% of pre-I (*p<0.05 vs. EuT-C) and P/Ht to 5.4 ± 0.9 $mmHg.g.mW^{-1}$ (*p<0.05 vs. 1.0 ± 0.4, EuT-C), without changing LVEDP. In HypoT, oral Nbv significantly improved PICR and P/Ht only at the end of R, but increased LVEDP during R. Results suggest that: a) Nbv was directly cardioprotective in HypT and EuT hearts exposed to sI/R and this effect was mediated by NO production from NOS; b) Hypothyroidism reduced the prevention of cardiac stunning induced by oral Nbv in EuT hearts.

This work was supported by UNLP-X-795 grant.

0204 - GENETIC DELETION OF GALECTIN 3 REDUCED SURVIVAL AND FAVORED MYOCARDIAL HYPERTROPHY IN AGED MICE

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Galectin 3 (Gal-3), a ß-galactosidase binding lectin widely expressed in the immune system, has proinflammatory and profibrotic effects. Gal-3 participates in ventricular remodeling (VR), hypertension, acute myocardial infarction and heart failure. However, the role of Gal-3 on mortality and cardiac remodeling associated with aging has not been previously studied. We aimed to study if genetic deletion of Gal-3 modifies survival rate and VR associated with aging. Wild type (WT, n= 54) and Gal-3 knockout (KO, n= 55) mice were in-housed and maintained on a 12 h light/dark cycle during 24 months. Animals had access to water and food ad libitum. Water and food consumption, body weight and survival rate were quantified. After 24 months of follow up, systolic blood pressure was measured by plethysmography. Heart (HW), lungs (LW), kidneys (KW) and spleen (SW) were weight and tibia length (TL) measured. Student's T tes t was used for comparison among groups and log rank test for survival curves (Kaplan meier). Results (Mean±SEM): Survival rate was significantly reduced in Gal-3KO mice as compared with WT (p= 0.02). Water and food consumption was higher in Gal-3 KO mice: 5.5 ± 0.1 ml/animal/week vs 3.3 ± 0.1 ml/animal/week (p<0.0001) and 2.7 ± 0.04 gr/animal/week vs. 2.2 ± 0.03 gr/animal/week (p<0.0001). Body weight and systolic blood pressure was similar between groups: 34.4 ± 0.5 vs. 35.3 ± 0.5 and 111.6 ± 2.9 vs. 114.3 ± 2.8 in WT and Gal-3KO respectively (p= NS). HW/TL and KW/TL were 8.0 ± 0.3 vs. 9.0 ± 0.3 (p<0.05) and 25.0 ± 0.9 vs. 34.0 ± 1.3 (p<0.05) in WT and Gal-3KO respectively. In summary, genetic deletion of Gal-3 during aging reduced survival associated with an increase in myocardial hypertrophy despite showing no effects in blood pressure. Further studies are neccesary to understand the mechanisms that links Gal-3 with myocardial hypertrophy and ventricular function during aging.

0218 - ASSOCIATION BETWEEN CLINICAL AND GENETIC DIAGNOSIS IN PATIENTS WITH LQT SYNDROME: IMPORTANCE OF GENETIC TESTING

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Long QT syndrome (LQTS) is a congenital genetic disorder that cause cardiac arrhythmia and sudden death. The genes more frequently implicated are those encoding for the K^+ channels KCNQ1 (40 - 45 %) and HERG (40 – 45 %), and the Na^+ channel Nav1.5 (5 – 8 %). Dysfunction in these channels leads to QT interval lengthening in ECG. Molecular identification of the causes of this disease contributes to better diagnosis, risk stratification and pharmacological treatment improvement. Our aim is to correlate clinical diagnosis with genetic variants of LQTS. We examined the LQT-associated genes KCNQ1, KCNH2 and SCN5A using gDNA extracted from 6 subjects. Five of them showed a prolonged QT interval on the ECG (>460 ms) while 1 first-degree relative presented a normal QT interval (<450 ms). The group (3 males and 3 females, 0-62 years old) included 1 with sudden cardiac death history under 40 years old and 1 with presyncope and documented polymorphic ventricular tachycardia. We amplified all exons from the 3 genes by PCR followed by sequencing. For KCNQ1 we found the uncommon variant c.1638G>A (p.Ser546=) in 2 subjects. In 1 patient we could not amplify exon 16, suggesting exon deletion. For KCNH2 we found the following variants: c.1692A>G (p.Leu564=) in 1 patient, c.1956T>C (p.Tyr652=) in 5 out of 6 cases and c.2690A>C (p.Lys897Thr) in 1 patient. Finally, we found the likely-pathogenic variant c.982C>T (p.Arg328Cys). For SCN5A no variants were detected at the tested exons. We found benign and pathological genetic variants in either KCNQ1 or KCNH2 genes of our population. No information about the exon 16 deletion for KCNQ1 as a pathological variant has been reported. To our knowledge, this is the first genetic test of LQTS performed in Argentina. Genetic characterization will impact on patient habits, avoiding risk situations such as sports, acute stress or cardiotoxic drugs therapies. Moreover, these studies will enable to set patient-oriented pharmacological treatments.

0396 - TRYPANOSOMA CRUZI INFECTION INDUCES SLUG EXPRESSION IN HEART DURING CARDIAC REMODELING

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Chagas disease is caused by Trypanosoma cruzi infection. Chronic cardiac manifestation (CCC) is consequence of a cardiovascular remodeling (CR) process that elicit a dilated cardiomyopathy that can trigger heart failure. This process starts in heart and vessels during the acute phase of the infection, but the molecular mechanisms are poorly understood. We reported that inhibition of Wnt proteins reduces CCC severity n BALB/c mice. Despite smooth muscle cells (SMC) dedifferentiation and fibroblast (Fb) differentiation into myofibroblasts play an important role in CR, little is known about their contribution to this progression. Slug is a transcription factor crucial during development and pathogenesis. Our group demonstrated that Slug is associated with vascular remodeling and promotes SMC dedifferentiation. We also recently observed that in vitro TGF-ß; treatment of SMC and Fb promotes Slug downregulation. In this work, we tested the hypothesis that Slug is involved in CR process during T. cruzi infection. Consequently, we aimed to determine Slug expression in heart during acute and chronic T. cruzi infection in absence and presence of Wnt proteins secretion inhibition. We also evaluated the presence of myofibroblasts in heart. BALB/c mice were infected with 1,000 tripomastigotes and Slug was determined in hearts by q-PCR at different days post-infection (dpi). During acute phase of infection, a gradual upregulation of Slug that became statistically significant after 23 dpi respect to non-infected mice was observed ($p= 0.0277$). The expression of Slug remained upregulated in heart ($p= 0.0330$) during chronic phase of infection (180 dpi). Interestingly mice treated with a Wnt proteins secretion inhibitor (IWP-L6) increased TGF-ß; levels, partially blocked Slug upregulation and increased the number of myofibroblast in heart. Our results indicate that Slug could be involved in the regulation of CR during T. cruzi infection, probably by modulating SMC and Fb phenotype.

0471 - SUB-ANOREXIGENIC DOSES OF LEPTIN ACTIVATES THE LEPTIN-TRH CARDIAC HYPERTROPHIC PATHWAY.

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Cardiac TRH (cTRH) induce left ventricular hypertrophy (LVH) and fibrosis. Additionally Leptine (Lep) induces TRH, until now only described in CNS. Our previous results on obese mouse models suggest that high Lep levels may impact cardiac tissue inducing cTRH expression and consequently stimulate LVH mediated by cTRH. So far this novel Lep-cTRH pathway has not been described in lean mice. We used C57 adult male mice to evaluate if sub-anorexigenic Lep doses could stimulate cTRH system hypothesizing that low doses of Lep could activate the Lep -cTRH pathway. For 3 weeks mice received subcutaneous leptin (10 ug/kg/day) or saline, with or without cTRH blockage by intracardiac injection of siRNA-TRH or siRNA-control (n= 7). As expected, Lep did not modify body weight, food intake or blood pressure during all the experiment, as there were no differences between those treated with Lep vs saline confirming the sub-anorexigenic Lep dose. As hypothesized we found that Lep significantly increased ($p<0.05$) cTRH mRNA expression (rtPCR) and precursor protein (WB). This increase was only observed in the group with the native TRH system compared to the group blocked by siRNA-TRH, confirming that the TRH blockade was successful. The Lep-induced TRH rise ($p<0.05$) brought the expression of hypertrophy and fibrosis markers (TGF-β, BNP and Collagens) as these results were only observed in the group with the active TRH system. We confirm on heart cells (H9C2, 3T3, primary myocytes) the direct induction of TRH (WB and RT-PCR) ($p<0.05$) by stimulating with different Lep doses (10 and 100 ng/ml) at different times. Finally we show for the first time that sub-anorexigenic Lep dose impact the heart and provoke an increase in hypertrophic and fibrosis markers mediated by cTRH. These results open the possibility that in obesity, cardiac alterations could begin prior to overweight, due to the initial slight increase in leptin levels that impact the heart inducing the cardiac TRH system.

0539 - THE LACK OF PROTECTION OF ISCHEMIC POSTCONDITIONING IN DYSLIPIDEMIC MICE IS RESTORED WITH N-ACETYLCYSTEINE TREATMENT

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Dyslipidemia per se exacerbated ischemia/reperfusion (I/R) injury, mainly caused by an increased of reactive oxygen species increases infarct size (MI). Ischemic postconditioning (IP) decreases MI, but we proved that in mice fed a high-fat diet (HFD, 12 weeks) with atherosclerosis in the early stages, the protective effect of IP is abolished. Therefore, the objective of our work is to evaluate the effect of treatment (T) with n-acetyl cysteine (NAC) restores the cardioprotective effect of IP in HFD mice. Male C57/BL6 mice (20 g) were divided into a control diet group (CD) and HFD group. In the last 3 weeks of feeding, a subgroup was treated with NAC (10 mM). Hearts were subjected to 30 min of I and 120 min R (Langendorff Technique). In the IP group, after I, 6 cycles of R/I were performed. The biochemical profile, histological alterations, ventricular function and MI were evaluated. Data are expressed as mean ± SEM and $p < 0.05$ was considered statistically significant (n= 6 per group). There was a slight decrease in weight in the NAC group without variation in daily caloric intake, cholesterol levels and triglycerides. NAC reduced the moderate hepatic steatosis in HFD group. As we expected, IP reduced MI in CD group (I/R: 54.3 ± 2.3 vs. IP: 36.5 ± 1.8; $p<0.05$), but this protection was abolished in the HFD-IP group (I/R: 66.4 ± 3.8 vs. IP: 62.1 ± 3.6). NAC T reduced MI in CD-IP+NAC with respect to the CD-I/R group and the CD-I/R+NAC group (I/R: 54.3 ± 2.3; I/R+NAC: 36.3 ± 4.6 vs. PI+NAC: 19.2 ± 3.3; $p<0.05$). On the other hand, in both NAC groups we observed a significant decrease in MI (HFD-I/R+NAC: 39.7 ± 4.5; HFD-IP+NAC: 26.4 ± 2.0; $p<0.05$ vs. HFD-IP and HFD-I/R). Regarding left ventricular function, we did not observe a significant difference between the groups after 30 min of I evaluated at 60 min after R. This study showed that alterations in the redox state in an early stage of atherosclerosis were enough to abolish the protective effect of IP, however T with NAC restored the cardioprotective effect of IP.

0643 - MECHANISMS OF THE FIRST AND SECOND WINDOW OF PROTECTION OF PREISCHAEMIC VAGAL STIMULATION ON MYOCARDIAL INFARCTION IN MICE

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We recently proved that pVS mimics ischaemic preconditioning in having two windows of protection. Our current objective was to further study the mechanisms of the protective effect of VS during this so-called first and second window. Mice were randomly assigned to different protocols (n= 5 - 7 per group). The control group had 30 min of regional myocardial ischaemia and 2 h of reperfusion (I/R). The other groups underwent 10 min of pVS and

either 5 min, 3 – 6 – 12 – 2 4 – 48 - 72 or 96 h of recovery between VS and I/R. The 5 min, 3 and 72 h groups were redone to receive a muscarinic receptor inhibitor (atropine). The 5 min and 72 h groups were performed yet again to administer a mitochondrial K^+ ATP channel blocker (5-HD) and a nitric oxide synthase inhibitor (L-NAME). All groups were catheterised to assess ventricular function. Septum (S) and area at risk (AR) samples from the I/R, 5 min and 72 h groups were taken to assess mitochondrial active respiration (MAR), passive respiration (MPR), and the respiratory control ratio (RCR). Hearts were dyed with Evans Blue and incubated in TTC to measure IS. pVS - 5 min had smaller IS when compared to I/R (44 ± 3 % and 57 ± 2 %, respectively; p<0.05). The 3 and 6 h groups showed further IS reduction (34 ± 3 % and 34 ± 3 %, respectively; p<0.05 vs. 5 min). Cardioprotection was lost at 12, 24 and 48 h post-VS (56 ± 4, 53 ± 2 and 56 ± 2 %, respectively; p= NS vs. I/R), but reappeared at 72 h post-VS (42 ± 4 %; p<0.05 vs. pVS – 48 h) and was lost again at 96 h post-VS (56 ± 3 %; p= NS vs. I/R). The IS-reducing effect of pVS - 5 min, 3 and 72 h was abolished by atropine (56 ± 2, 56 ± 3 and 56 ± 3 %; p= NS vs. I/R), 5-HD in pVS – 5 min and 72 h (55 ± 2 and 62 ± 5 %, p= NS vs. I/R) and L-NAME in pVS – 5 min and – 72 h (56 ± 3 and 57 ± 2%, p= NS vs. I/R). There were no significant differences between S and AR in any of the groups in MPR. MAR, however, was decreased in the AR of the I/R group when compared to the S (131 ± 13 vs. 83 ± 8, p<0.05) and so was the RCR (5 ± 0.2 vs. 3 ± 0.2, p<0.01). This difference was reversed in the 5 min group both in the MAR (128 ± 12 vs. 126 ± 11, p= ns) and in the RCR (4 ± 0.3 vs. 4 ± 0.2, p= ns). However, in the 72 h group, the difference persisted both in MAR (150 ± 5 vs. 109 ± 6, p<0.01) and in RCR (4 ± 0.1 vs. 3 ± 0.1, p<0.01). In conclusion, VS has two protection windows as does classic preconditioning. The first window lasts 6 h and the second window appears at 72 h and disappears at 96 h. The mechanisms underlying the cardioprotective effect include muscarinic receptors, nitric oxide synthase and mitochondrial K^+ ATP channels and functional changes in mitochondrial oxygen consumption.

0719 - CARDIOPROTECTION CARRIED OUT BY ORAL ADMINISTRATION OF STEVIOSIDE; AN INSIGHT INTO MITOCHONDRIA STATUS AND ITS RELATIONSHIP WITH PROTEIN KINASE B (AKT).

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In previous studies carried out in our laboratory, we demonstrated that oral administration of stevioside (S), a mayor component of Stevia rebaudiana Bertoni, improved the recovery of contractile activity in hearts subjected to ischemia-reperfusion (I-R), decreased the infarct size and increased Akt and GSK3ß phosphorylation. These effects were reverted, at least in part, by the administration of Wortmannin (W), an upstream inhibitor of Akt. Since mitochondrial dysfunction is essential for the pathogenesis of I-R injury, we aimed to investigate the effects of oral administration of S (168 mg/kg for 15 days) in several mitochondrial parameters from Langendorff perfused rat hearts subjected to I-R. Hearts from female Wistar rats (200 – 250 g) fed ad libitum were used. W (100 nM) was added 15 min before I. Mitochondrial ultrastructure was analyzed by electron microscopy, the measurement of mitochondrial ATP synthesis was made by the luciferin-luciferase method and calcium-triggered mitochondrial swelling was determined as % of light scattering decrease at 540 nm (% LSD). We also studied the calcium retention capacity (CRC) exposing mitochondria to calcium pulses using the fluorescent dye: Calcium Green-5N. ANOVA, n= 8/group. At the end of reperfusion, results showed an increase in mitochondrial ATP synthesis rate of hearts treated with S that was partly canceled by the administration of W (C:66.3 ± 6.5, W: 59.5 ± 6.1, S: 87.3 ± 3.7*, S+W: 64.6 ± 6.9 nmol/min/mg mitochondrial protein; *p<0.05 vs. all groups). Likewise, electron micrographs showed better mitochondrial conservation in the group treated with S. Furthermore, % LSD produced by overload of calcium (300 µM) and CRC were significantly lower with S treatment compared to the other groups (% LSD= C: 2.6 ± 0.3, W: 2.5 ± 0.4, S: 1.3 ± 0.3*, S+W: 2.2 ± 0.3 %; CRC= C: 31.0 ± 3.1, W: 30.7 ± 3.7, S: 46.6 ± 5.4*, S+W: 36.8 ± 5.4 nmol calcium/mg protein; *p<0.05 vs. all groups). These findings suggest that oral administration of S presents cardioprotective effects due to better mitochondrial preservation and could be partly mediated by Akt activation.

0753 - LONG TERM EFFECTS OF PRE-ISCHEMIC VS ON LEFT VENTRICULAR FUNCTION AFTER MYOCARDIAL ISCHEMIA AND ISCHEMIA/REPERFUSION IN MICE

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The aim of this study was to analyze the effects of brief vagal stimulation (VS) applied before ischemia on acute myocardial infarction and its long-term benefits on an experimental ischemia/reperfusion and non-reperfusion model. Male FVB mice were randomly assigned to different groups and a regional myocardial ischemia of the left descendant coronary artery during 45 min, followed by either 2 hours, 28 days of reperfusion or no reperfusion at all, with or without 10 min of pre-ischemic vagal stimulation (VS) was performed. In order to evaluate de participation of muscarinic receptors, atropine was administrated during VS. Left ventricular function (LVF) was assessed by echocardiography and catheterization of the left ventricle via the left carotid artery. Morphometric parameters were also analyzed by a comparison of both ventricles weight (VW) and lungs weight (LW) with the length of the tale (TL) and the tibia (TiL). Finally, infarct size (IS) on the 2 h reperfused hearts was measured with TTC. VS+IR-2h presented smaller IS compared to IR-2h and administration of atropine reverted the protection. On the other hand, while the IR-28 d group showed a significantly higher LVEDP with a lower EF % and SF % compared to the Sham-28 d group, and VS reverted this findings, the administration of atropine did not reduced echocardiographic parameters. In addition to this, a reduction of the FE % and SF % was observed in the non-reperfused group that could be reverted with VS and did not changed with atropine during stimulation. Finally, morphometric results showed in the IR-28d group an increment in the VW compared to TiL and TL which did not descend with VS and similar behavior was found in non-reperfused groups. In conclusion, brief VS applied before ischemia confers cardioprotection, reducing the acute infarct size and improving long-term LVF independently of the action of muscarinic receptor and infarct size.

0821 - THE ARM AND HINDLIMB ISCHEMIA TRIGGER DIFFERENT COMMUNICATION PATHWAYS IN REMOTE ISCHEMIC PRECONDITIONING

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Remote ischemic preconditioning (rIPC) is a cardioprotective phenomenon by which transient non-lethal ischemia and reperfusion of one organ or tissue confers resistance to a later episode of lethal ischemia reperfusion injury in a remote organ or tissue. The aim was to determine the most appropriate anatomical site to stimulate rIPC and achieve protection. A second objective was to evaluate some of the possible pathways (neural and

humoral) involved in the rIPC mechanism. Isolated rat hearts (n= 6 per group) were subjected to 30 min of global ischemia followed by 60 min of reperfusion (I/R), in a Langendorff system. In additional groups, a rIPC protocol of 3 cycles of I/R (5 min) were performed on both in leg and the arm. We studied the involvement of a vagal neural pathway by performing a bilateral cervical vagotomy (BCV) prior to the rIPC protocol. The humoral pathway was evaluated by administering DPCPX (adenosine A1 receptor blocker) before rIPC. The opening of mitochondrial permeability transition pore (MPTP) was assessed in fresh isolated left ventricle mitochondria by the occurrence of cyclosporin A sensitive mitochondrial membrane potential disruption followed by swelling as determined by the changes in optical density of the mitochondrial suspension. The ischemia/reperfusion protocol induced an infarct size of 47.1 ± 0.8 %. The hindlimb and arm rIPC reduced infarct size to 35.6 ± 1.2 ($p<0.05$) and 27.1 ± 4.4 ($p<0.05$), respectively. Bilateral vagotomy completely abolished cardioprotection induced by hindlimb rIPC but not by arm rIPC. The administration of DPCPX (A1 adenosine receptor blocker) abolished the beneficial effect of arm rIPC but nor hindlimb rIPC. After I/R, mitochondria showed an increased rate of MPTP opening (18 %). A protection against MPTP opening was evidenced in mitochondria from rIPC rats with values only 10 % and 7 % higher than in mitochondria from sham animals for hindlimb rIPC and arm rIPC, respectively. These results indicate that hindlimb rIPC is critically dependent on parasympathetic efferent innervation (vagal nerve), while arm rIPC appears to rely on a different signaling pathway(s) that involved the A1 adenosine receptor activation. However, both cardioprotective mechanisms seem to converge in the preservation of mitochondrial integrity as evidenced through the inhibition of MPTP opening.

Oncología / Oncology II

Chairs: Gabriel Fiszman | Cinthia Masillo

0106 - GALECTIN-1 PROMOTES KSHV-MEDIATED PDGFRA ACTIVATION ON MESENCHYMAL STEM CELLS

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Galectin-1 (Gal1) contributes to tumor immune-scape by inducing apoptosis of T cells and promoting angiogenesis via recognition of complex N-glycans on VEGFR2. Kaposi Sarcoma (KS) is characterized by the proliferation of spindle cells and deregulated angiogenesis. Recent reports have shown that Kaposi-associated herpes virus (KSHV) usurps PDGFRA signaling to drive KS. This study aims to investigate the influence of Gal1-N-glycan interactions in KSHV-mediated sarcomagenic signaling of PDGFRA. First, we differentiated mesenchymal stem cells (mMSCs) from C57BL/6 mice and explored their glycophenotype by using a panel of biotinylated lectins. mMSC exhibited high levels of complex branched N-glycans with poly-LacNAc elongations, indicating permissive glycoepitopes for Gal-1 ($p<0.05$). Accordingly, Gal-1 binds to mMSC in a dose and carbohydrate-dependent manner ($p<0.05$). Moreover, Gal-1 interacted directly with PDGFRA and pPDGFRA suggesting that Gal1 activate PDGFRA signaling in MSCs. To better define the role of Gal1 in PDGFRA activation, we performed WB analysis of mMSC incubated with rGal-1. Results showed that Gal-1 effectively activates PDGFRA signaling inducing pAkt, pErk, and pSTAT-3 ($p<0.05$). Next, in a bioinformatic approach, we performed coordination analysis of Gal1-PDGFRA showing that Gal-1 and PDGFRA associated in a stable complex that affected lipidic membrane composition, suggesting an interaction mediated by lipid rafts. Finally, in order to determine whether KSHV could increase Gal-1-mediated PDGFRA signaling, we analyzed the glycan profile of mMSCs transfected with vGCPR (one of the KSHV genes activated in late infection). We observed increased exposure of glycosylated structures and binding of Gal1 ($p<0.05$). Taken together, these data suggest that KSHV infection of MSCs may increase oncogenic signaling of PDGFRA trough a glycosylation-dependent and Gal-1-mediated mechanism, thereby promoting virus-induced sarcomatogenesis.

0126 - RHBDD2 BINDS TO GRP78/BIP AND PROMOTES CHEMORESISTANT AND INVASIVE PHENOTYPES TO RECTAL CANCER TUMORS VIA MODULATING UPR AND ADHESION GENES

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The current standard of care for locally advanced rectal cancer (RC) is neoadjuvant radio-chemotherapy (NRC) with 5-fluorouracil (5Fu) as the main drug, followed by surgery and adjuvant chemotherapy. A group of patients will achieve a pathological complete response, while a significant percentage will not respond to the treatment. The unfolding protein response (UPR) is generally activated in tumors and results in resistance to radio-chemotherapy. We previously showed that RHBDD2 gene is overexpressed in the advanced stages of colorectal cancer (CRC) and would be involved in the UPR. Moreover, its expression is induced by 5Fu. We hypothesized that the overexpression of RHBDD2 in the advanced stages of CRC could have an impact on the regulation of the UPR pathway providing tumor cells with a stress-resistant phenotype. We stably overexpressed and silenced RHBDD2 expression in the Caco2 and HCT116 cell lines, respectively. Results indicated that RHBDD2 overexpression conferred to Caco2 cells resistance to 5Fu, favored cell migration, adhesion and proliferation and had a profound impact on the expression of the UPR genes BiP, PERK and CHOP ($p<0.01$), and on the adhesion genes FAK and PXN ($p<0.01$). Moreover, by immunoprecipitation, we determined that RHBDD2 binds to GRP78. GRP78, also called BiP, is a master regulator of the UPR, reducing ER stress levels and apoptosis. In paired tumor samples (before and after NRC treatment) of patient with RC, we found that tumors that maintained a high expression of RHBDD2 even after treatment were associated with patients who had developed local or distant metastases. The collected evidence positions RHBDD2 as a promising predictor of response to neoadjuvant therapy in patients with RC.

0129 - THE IAP FAMILY MEMBER BIRC5/SURVIVIN CONFERS RESISTANCE TO PANCREAS CANCER CELLS BY AUTOPHAGY PATHWAY MODULATION

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Pancreatic ductal adenocarcinoma (PDAC) is a deadly and highly aggressive cancer. Gemcitabine (standard chemotherapy for PDAC) induces low levels of apoptosis as consequence that it is partially blocked by the autophagy induced during the treatment. Autophagy inhibition allows apoptosis triggered by inhibitors of MAPKs or NFkB pathway. In this context, a relationship between the Baculoviral IAP Repeat-Containing Protein 5 (BIRC5/Survivin), from IAPs family, and autophagy is observed. The aim of this work

was to evaluate the modulation apoptosis-autophagy mediated by Survivin. By Western blot, an increase of Survivin is observed in PDAC cell lines in response to the MAPKs inhibitor UO126. This increase is lessened by the autophagy inhibitor 3MA. Using a specific shRNA against Survivin, it is possible to observe, by TUNEL assay, the Survivin dependence of apoptosis induced by UO126. Moreover, this Survivin-dependent cell death resistance is mediated by autophagy. Morphologically, Survivin have a moderated partial colocalization with the autophagy marker LC3 at basal condition which is increased by starvation. Interestingly, under the blockade of autophagy finalization steps, by chloroquine, Survivin accumulates in a nuclear proximity area where it colocalize with LC3 in close relationship to omegasome. In conclusion, Survivin is a key factor in PDAC cells resistance to treatment within an intricate mechanism with the autophagy pathway.

0145 - SURVIVIN: ¿A LINK BETWEEN AUTOPHAGY AND SENESCENCE?

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Pancreatic cancer is highly resistant to chemotherapy. Gemcitabine (Gem) raises the levels of survivin expression accompanied by increases in autophagy levels. It has been described a link between autophagy and senescence. Hence, we proposed to evaluate if survivin would be mediating these processes in our cell lines and whether the SASPs produced by senescent cells could have an impact on the surrounding cells. Accordingly, we treated MIAPaCa-2 wt and KDSurv cells with Gem (10, 100 and 1,000 µg/ml) alone or in combination with 3MA for 48 h and evaluated senescence by SAß-Gal assay. Then, we determined apoptosis induction by Gem alone or in combination with 3MA by tunel assay. Besides that, we study the effect of SASPs on cell death and senescence. Supernatant of the cells treated with Gem were collected and added on fresh cells. We could determine in both lines that Gem increases the levels of senescence in a dose-dependent manner reaching values of 30 % in the wt line and 60 % in the KDSurv line ($p<0.0001$); that decrease when autophagy is inhibited. No differences were found in the percentage of TUNEL + cells with or without 3-MA in wt cells, while in the KDSurv line, inhibition of autophagy sensitizes cells to the pro-apoptotic action of Gem ($p<0.001$). Additionally, we could observe that the supernatants with Gem vs. basal induces senescence obtaining values of 20 % in the both cell lines ($p<0.0001$), whilst they induce cell death in a time-dependent manner, catching up values of 80 % at 72 h, in both cell lines ($p<0.0001$). From our results, we postulate a potential role of survivin as a mediator of the autophagy and senescence processes in pancreatic tumor cells by the treatment with Gem. We also propose that SASPs release by senescent cells provoke similar effects about inducing senescence or cell death, regardless of the presence or absence of survivin.

0147 - MAST CELLS INFILTRATION IN PREMALIGNANT TONGUE LESIONS UPON TTP ABLATION IN CONDITIONAL TRANSGENIC MICE.

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Oral squamous cell carcinoma (SCC) is the sixth most common cancer worldwide and tongue SCC is the most common oral malignancy. RNA-binding proteins (RNA-BP) that regulate transcript stability play a key role in disease pathogenesis. Tristetraprolin (TTP) is a RNA-BP that regulates proinflammatory mediators which promote tumorigenesis. We have previously developed TTP knockout mice for oral cavity (TTP-KO:K14-CreERtam/TTP-/-). TTP-KO mice developed dysplastic lesions in the tongue along with inflammatory cells infiltration in the connective tissue. TTP-KO mice were bred with a K-rasG12D line, obtaining compound mice (K14-CreERtam/TTP-/-/K-rasG12D+/-). They developed a complete oral phenotype with more aggressive histologic features and persistent inflammatory infiltrates. Compound mice heterozygous (het) for TTP showed a different phenotype. Here, to study the inflammatory infiltrate in these premalignant lesions we characterize their cell composition. We performed immunohistochemistry for S100A (monocytes, neutrophils, macrophages) and 2 different staining for mast cells: alcian blue-safranin (AB-S) and toluidine blue (TB). We found S100A positive cells in the tongues of TTP-KO, compound and het-compound mice. These cells were distributed equally between groups. AB-S positive cells were found in the connective tissue of the tongues in all experimental groups. However, the distribution and the amount of these cells were not homogenous. We performed TB staining and quantified positive cells (cell/area). We found a statistically significant increase in TB positive cells in TTP-KO (19.8 ± 1.8) compared with compound (11.04 ±2.01), het compound (6.26 ± 1.9) and wild type tongues (10.68 ± 4.27), ANOVA $p<0.05$. We conclude that TTP ablation is sufficient to recruit mast cells infiltrate to the connective tissue underlying epithelial dysplastic changes in the TTP-KO tongues. S100A positive infiltrate deserve further analysis to elucidate the types of cells that constitute it.

0150 - METRONOMIC THERAPY FOCUSED ON MUSCARINIC RECEPTORS COULD BE USEFUL ON TRIPLE NEGATIVE BREAST TUMORS, MDA-MB231 AND MDA-MB468

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The treatment of triple negative (TN) breast tumors is a problem not yet resolved in oncology, due to the lack of specific therapeutic targets. We had previously demonstrated that in vivo treatment with low doses of paclitaxel (PX) combined with the muscarinic agonist carbachol (Carb) reduced the growth of MDA-MB231 tumors in NUDE mice. The participation of muscarinic acetylcholine receptors (mAChRs) was demonstrated by reducing the effect observed by the administration of the muscarinic antagonist atropine (AT). In this work we analyzed the effect of PX or doxorubicin (DOXO), both drugs often used in the treatment of breast cancer combined with two different agonists: Carb or arecaidine propargyl ester (APE) all in suboptimal concentrations, on the viability of MDA-MB231 and MDA-MB468 tumors measured with by the MTT reagent. We observed that the combination of PX (10^{-8}/10^{-9} M) + Carb (10^{-12}/10^{-10} M) reduces the viability of MDA-MB231 and MDA-MB468 cells (36.8 ± 6.2; $p<0.001$ and 33.4 ± 2.5; $p<0.001$ respectively). When Carb was replaced by the selective M2 agonist APE (10^{-5}/10^{-7} M), there was also a significant decrement in cell viability (MDA-MB231: 35.8 ± 3.17; $p<0.001$; MDA-MB468: 26.9 ± 3.6; $p<0.001$). The effects produced by the combination containing Carb or APE were blocked in the presence of AT (10^{-7} M) or methoctramine (MET; 10^{-5} M) (non-selective or M2 selective antagonists respectively). Similar results were obtained when DOXO was employed instead of PX in the combination (DOXO (10^{-8} M) + Carb: MDA-MB231: 35.3 ± 0.8; $p<0.001$ and MDA-MB468: 21.1 ± 0.7; $p<0.01$. DOXO (10^{-8} M) + APE: MDA-MB231: 33.3 ± 2.1; $p<0.001$ and MDA-MB468: 31.2 ± 0.9; $p<0.01$). The observed effects were inhibited in the presence of AT or MET respectively. We conclude that the combination of a conventional cytotoxic drug with a muscarinic agonist is useful to reduce the viability of triple negative tumor cells, which could be a new form of treatment focused on mAChRs for these tumors.

0151 - AUTOPHAGY MODULATES THE IMMUNE RESPONSE OF PANCREATIC TUMOR CELLS BY CONDITIONING THE EXOSOME COMPOSITION.

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by inducing immunotolerance, where exosomes act as intercellular messengers, carrying molecules from the tumor cells to the immune cells. In this work we investigated the role of autophagy in the composition of tumor-derived exosomes and their impact on the activity of Dendritic (DC) and Natural Killer cells (NK). For the experiments we used two PDAC cell lines, MIAPaCa-2 and PANC-1, and two inhibitors of autophagy, 3-Methyladenine (3-MA) and Spautin-1 (SP-1). First, we demonstrated the presence of exosomes in culture cells supernatants with or without 3-MA or SP-1 by electron microscopy. Interestingly, both treatments also increased the exosomal marker CD63 observed by WB. Afterward, monocyte-derived-dendritic-cells (MDDC) were treated with the different populations of exosomes and after 1 h LPS. Cytokine production by ELISA was evaluated in 48 h supernatant. MDDCs incubated with exosomes from cell culture without SP-1 secreted TGF-ß, meanwhile the exosomes from cells with SP-1 induced the secretion of IL-12, and increment in HLA-DR expression on MDDC membrane (observed by flow Cytometry) ($p<0.01$). No differences were observed in IL-10 profile. NK cytotoxic activity was evaluated in K562 cell line. We incubated NK cells with exosomes from supernatant of MIAPaCa-2 and PANC-1 cells treated or not with SP-1, for 2-6 h. After CFSE staining of K562 cells, co-cultures of NK:K562 (ratio 5:1) were performed for 4h. Cytotoxicity of NK was evaluated by CFSE/PI stain. Exosomes from SP-1 treated cell supernatant stimulated cytotoxic activity of NK cells ($p<0.05$). Moreover, this treatment increased the IFNg production by NK cells ($p<0.01$). Our results suggest that autophagy condition exosome-composition, activating NK activity but inducing a tolerogenic profile in DC. Furthermore, we speculate that autophagy pathway status in cancer cell may modulate the immune tumor microenvironment through the exosome profile composition.

0153 - THE TREATMENT OF MCF-7 CELLS WITH CARBACHOL AND PACLITAXEL IS EFFECTIVE TO REDUCE TUMOR CELL GROWTH IN VITRO AND ANGIOGENESIS IN VIVO.

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Previously we demonstrated that muscarinic receptors (MR) are expressed in different types of human and murine breast tumors. Their activation with the synthetic agonist carbachol (Carb) promotes cell death and improves the effect of paclitaxel (PX), a cytotoxic drug commonly used in breast cancer treatment. In this work, we analyzed the ability of a combination of low concentrations of Carb+PX, simulating a metronomic schedule, to reduce cell growth in bi (2D) and tridimensional (3D) MCF-7 cell cultures (by MTT assay and by microscopy respectively). We also studied the effect of this combination in HMEC-1 cells´ tubulogenesis and the in vivo effect on the neovascular response (Nº vessels/mm^2) in mice tumor bearers. We observed that the treatment of MCF-7 cell spheroids with Carb (10^{-11} M) + PX (10^{-9} M) significantly reduced their 3D growth compared to control spheroids by 33 ± 3 % at day 6 of culture ($p<0.01$). In addition, Carb+PX significantly decreased HMEC-1 cells tubulogenesis (55 ± 7 %; $p<0.01$). The administration of two cycles of subtherapeutic doses of Carb+PX to tumor bearer mice, diminished the neovascular response produced by MCF-7 cells (MCF-7: 3.9 ± 0.3; MCF-7+Carb+PX: 3.1 ± 0.3; $p<0.0001$). The previous treatment with the antagonist atropine reverted the effect produced by the combination (4.2 ± 0.2 %).Interestingly, the administration of PX at therapeutical doses increased the neovascular response produced by MCF-7 cells (4.4 ± 0.4; $p<0.001$). Our results demonstrate that the combination of Carb+PX has more specificity than conventional chemotherapy, since it targets MR and it has an anti-angiogenic effect not seen with the cytotoxic drug at therapuetical doses.

0154 - DIFFERENTIAL ROLE OF AHCYL1 GENE IN TUMOR PLASTICITY

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Malignant reprogramming of cells is responsible for tumor development. During this process stem-like tumor cells that acquired self-renew capacity produce heterogeneity, tumor dissemination, and relapse after cancer therapy. We have previously identified AHCYL1 as a potential regulation target of core transcription factors OCT-4, SOX-2, and NANOG responsible for cell reprogramming. We studied AHCYL1 by analyzing its cellular location and expression levels during cell plasticity events of tumor cells. We used the glioblastoma (GBM) cell line U87 and lung carcinoma (LC) cell line H1299 as in vitro models since brain and lung have the highest Ahcyl1 expression. We cultured these cell lines in a 3D format in DMEM/F12 medium supplied with FGF, EGF, and B27 and compared with 2D format cultured cells with DMEM serum complemented medium. Ahcy1 localization was determined by immunofluorescence assay and cell fractioning followed by Western blotting. To generate U87 and H1299 Ahcyl1 knockdown stable lines, three different shRNAs were tested and the expression levels of Ahcyl1 and the core factors were determined by Western blot and RT-qPCR. Stemness potency was evaluated by ELDA assay (extreme limiting dilution analysis). We found that AHCYL1 localizes both in nuclei and cytosol, in addition to a putative processed isoform in nuclei. In 3D cultures, Ahcyl1 expression is differently regulated compared to 2D cultures. Also, in GBM, AHCYL1 expression was significantly increased, in contrast in LC was decreased ($p<0.0001$ and $p<0.05$ respectively). In Ahcyl1-depleted LC cells, the core factors expression levels and the stem potency were increased ($p<0.05$). Altogether, we conclude Ahcyl1 has a key role as a regulator of stem potency and would be dependent on tumor type.

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0186 - INHIBITION OF BREAST TUMOR GROWTH BY N(G)-NITRO-L-ARGININE METHYL ESTER (L-NAME) IS ACCOMPANIED BY ACTIVATION OF FIBROBLASTS

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Nitric Oxide (NO) is generated by a family of NO synthases (NOS), being the inducible isoform (iNOS) which produces higher NO levels. This, often acts as a survival factor, hence inhibition of iNOS has been proposed as a targeted therapy. Fibroblasts, the main cell type in tumor microenvironment, have been described as a heterogenic population and their role in breast cancer associated to NO inhibition has not been yet elucidated. In this work we use

murine and human breast cancer cell lines to evaluate the impact of NO inhibition in tumor progression. LM3 and its more aggressive variant LMM3 cell line expressed iNOS, as well as the human MDA-MB-231 cells (qPCR $p<0.05$; $p<0.001$). On the other hand, LM2 and human MCF10DCIS.com, line did not express iNOS. Inhibition of NO production by L-NAME abrogates viability and treatments with the NO-donor, DETA/NO, induced cell viability only in iNOS positive cancer cells (MTS assay $p<0.05$; $p<0.01$; $p<0.001$). L-NAME abrogates ERK activated signalling pathways only in iNOS positive human and murine cancer cell lines (Wenstern blot $p<0.05$; $p<0.01$). In vivo, L-NAME inhibited tumor growth in iNOS positive cells ($p<0.001$ vs. CRL). In pararallel, collagen deposition and α-SMA positive stromal cells was observed. In iNOS negative cells, no effect on viability, ERK activation and tumor size reduction was observed with L-NAME. On the other hand, L-NAME induces an opposite effect on fibroblast, showing an increase in viability and differentiation. In contrast, DETA-NO reduced their viability (MTS assay $p<0.05$). Our results reveal that NO inhibition contributes to stimulate proliferation and activation of fibroblasts in parallel with tumor reduction only in iNOS positive breast cancer. Hence, iNOS inhibition could be considered as new therapeutic targets to be added to conventional therapies.

0502 - CHANGES IN APOPTOSIS LEVELS ARE ASSOCIATED WITH HYPOXIA-MEDIATED TRASTUZUMAB AND T-DM1 RESISTANCE IN HER2+ BREAST CANCER

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Trastuzumab and trastuzumab emtansine (T-DM1) immunotherapies are the treatment of choice for HER2+ breast cancer patients. However, although their success, de novo or acquired resistance is still the main obstacle in clinical practice. Our previous studies demonstrated that hypoxic microenvironment promotes trastuzumab and T-DM1 resistance in HER2+ breast cancer cells. In this work we further analyzed mechanisms determining hypoxia-induced resistance. First, we confirmed in concentration-response curves the significant differences between normoxic and hypoxic conditions with (1 - 50) µg/mL trastuzumab and (0.1 - 10) µg/mL T-DM1 three-day treatments on BT-474 cell line ($p<0.001$). In order to determine whether it was due to a reduction of cell viability or to a modulation of cell cycle, we performed flow cytometry analyses. Interestingly, we observed that hypoxic conditions reduced trastuzumab and T-DM1-mediated apoptosis ($p<0.05$). In contrast, there were no significant differences between drug effects on the cell cycle either under normoxic or hypoxic conditions. Since modulation in the HER2 expression is associated not only with trastuzumab mechanisms of action but also with drug resistance, we asked if hypoxia regulated BT-474 HER2 levels in response to drug treatment. Further flow cytometry analyses showed that trastuzumab and T-DM1 decreased HER2 expression on cell surface ($p<0.05$) regardless of hypoxic conditions. In summary, our results show that lower levels of apoptosis under hypoxia mediate trastuzumab and T-DM1 resistance. However, the question of the mechanism underlying this effect is still open. In our laboratory, by mammosphere assay, we observed that hypoxic BT-474 cells developed a higher proportion of breast cancer stem cells than normoxic cells. These results highlight an increase in the breast cancer stem population as a potential mechanism of hypoxia-mediated trastuzumab and T-DM1 resistance, which deserves to be studied.

0711 - RANITIDINE HINDERS RADIATION INDUCED MESENCHYMAL TRAITS IN EXPERIMENTAL PANCREATIC ADENOCARCINOMA

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We have previously set that the antihistamine ranitidine (R) hindered growth of human pancreatic PANC-1 and BxPC3 grafts and the development of PANC-1 lung metastasis in nude mice. Two Gy irradiation increased PANC-1 tumor growth while slowered BxPC3, though increased lung metastasis in both. R reduced irradiated tumor growth rate and lung metastases. The aim of this work was to evaluate the effect of irradiation and R on epithelial to mesenchymal transition (EMT), a process associated with invasion and metastasis, in pancreatic tumors. Dedifferentiated PANC-1 and more differentiated BxPC3 tumors were irradiated (I) or not (C) with 2 Gy of gamma radiation, transplanted to non-irradiated mice, and treated with R 150 mg/kg.day, p.o. (I+R; C+R) or not (I; C). Immunohistochemistry was performed to evaluate the expression of EMT molecular markers (E-cadherin, vimentin, Slug) and of TGF-ß1 (a major promoter of EMT). In PANC-1 tumors epithelial marker E-cadherin was not detected in any group, while transcription factor Slug nuclear expression was similar in all of them. In C-grafts we observed a big number of vimentin (mesenchymal marker) and TGF-ß1 positive cells that was even bigger in I-tumors ($p<0.05$), but not in R and I+R. In BxPC3 only I-tumors did not show E-cadherin at cell membrane in the inner areas of slices. Very few cells expressed vimentin and TGF-ß1 in C-tumors; this expression was enhanced in I-group ($p<0.01$) but not changed in I+R or R. An increase in nuclear Slug and TGF-ß1 was detected only in I-grafts ($p<0.05$). TGF-ß1 correlated positively with vimentin in both tumor types ($p<0.01$). Nuclear Slug positively correlated with vimentin and TGF-ß1 only in BxPC3 ($p<0.01$). In vitro studies showed an increase in vimentin expression and cell migration in both irradiated cell lines that was blocked by R. In conclusion, R could reduce radio-induced gain of mesenchymal features, pointing out the relevance of research on drugs that control both growth and metastasis.

Genética / Genetic

Chairs: Florencia Giliberto/ Ariel López

0109 - DIVING THE OCEAN OF VARIANTS IN PURSUIT OF A MUSCULAR DYSTROPHIES DIFFERENTIAL DIAGNOSIS

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Muscular dystrophies (MD) are a group of rare inherited diseases that cause weakness and progressive degeneration of skeletal muscle. They are caused by mutations in genes encoding structural skeletal muscle proteins or proteins necessary for the stability and proper functioning of muscle fibers. However, the clinical symptoms of these pathologies overlap, hindering differential diagnosis, which is of paramount importance to establish the standard of care. Therefore, it is important to carry out molecular studies to be able to differentiate between each type of MD. Here, we focus on the case of Limb-Girdle MD, which are frequently misdiagnosed as Dystrophinopathies, the most frequent type of MD and caused by mutations in the DMD gene. The present work aims to detect molecular alterations in MD genes in patients with a presumptive dystrophinopathy clinical diagnosis but no DMD mutation identified. A cohort of 106 Dystrophinopathy suspected males, with no alteration detected in DMD by MLPA, was referred to our laboratory for WES analysis. In a subset of 21, no small mutation in the DMD gene was detected. Therefore, we deepened the screening to all the MD genes included in the Gene Table of Neuromuscular Disorders. For recessive MD disorders, when only

one mutation was identified, MLPA (SGCA, SGCB, SGCD, SGCG and FKRP) was implemented for deletion/duplication screening. Dystrophinopathy mutations were detected in the DMD gene in 81.1 % of patients. Further analysis of the WES results of the remaining individuals, allowed us to identify possibly pathogenic molecular alterations, in other MD associated genes, in 11 of them (10.4 %). Thus, reaching a WES detection rate of 91.5 %. We found 2 large deletions in SGCA and SGCD by MLPA. Finally, our work highlights the importance of extending the mutation screening to all the MD associated genes in patients without alterations in DMD, given that a misdiagnosis could lead to an error in the selection of the standard of care.

0110 - HOW TO SURPASE THE WES TSUNAMI OF VARIANTS: THE IMPORTANCE OF THE HUMAN FACTOR

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Dystrophinopathies are neuromuscular X-linked recessive diseases caused by mutations in the DMD gene. Molecular alterations in this gene are large deletions/duplications in 80 % of cases, identified by MLPA, and small mutations in the remaining 20 %, detected by whole exome sequencing (WES). The use of next generation sequencing (NGS) techniques generates a large quantity of data that is analyzed by a bioinformatics pipeline. However, this analysis can lead to errors in the variant calling. The present work aims to emphasize the importance of the human factor in order to detect these errors. A cohort of 106 patients with presumptive clinical diagnosis of dystrophinopathy and negative MLPA results was analyzed by WES. Raw data was evaluated using the Integrative Genomics Viewer (IGV) software. Sanger sequencing was used to corroborate the identified variants. Two cases have been selected as an example to illustrate variant calling errors. Even though the WES technique and its bioinformatic pipeline proved to be fruitful, allowing us to identified pathogenic variants in muscular dystrophy genes in 91.5 % of patients, we detected 2 variant calling errors among the studied individuals. In other words, the VCF results did not resemble the alteration observed in the raw data analysis. These discordances were due to the presence of deletions in the DMD gene, which caused problems in the alignment process. In both cases, alignment and annotation had to be manually re performed. While one of the patients carries a small delins, the other one has a complex rearrangement, a deletion and a 20 pb insertion in the same allele. Specific primers were design to corroborate these findings. Finally, this work highlight the importance of analyzing the NGS raw data, corroborating the identified mutations by an alternative technique and the expertise of the scientist in charge of the study, so as to detect the occurrence of variant calling errors and provide reliable results to the patient.

0250 - A NOVEL HUMAN HETEROZYGOUS STAT5B VARIANT LEADS TO GROWTH AND DEVELOPMENTAL DEFECTS IN ZEBRAFISH EMBRYOS

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Signal transducer and activator of transcription 5B (STAT5B) has been identified as a key downstream mediator of Growth Hormone (GH) signaling in somatic growth. Autosomic recessive human mutations in STAT5B lead to severe growth retardation associated to immune dysregulation. On the other hand, some heterozygous STAT5B mutations have been associated to a milder form of the disease. The aim of our study was to evaluate the functional consequences of a novel heterozygous human STAT5B variant (K632N), described in a child presenting short stature with mild immunological dysfunction, during zebrafish embryo development to determine its pathogenicity. To do this, we microinjected 100 and 200 pg of wildtype (WT) and mutant mRNA into zebrafish embryos and measured the embryo length at 72 hours post fertilization (hpf). In addition, we characterized the morphological phenotypes observed in these embryos. Zebrafish embryos microinyected with 100 and 200 pg of mutant mRNA show a dose dependent significant reduction of body length at 72 hpf compared to those microinyected with the same dose of WT mRNA ($p<0.001$) for both 100 and 200 pg. Moreover, the body length is significantly shorter in those embryos injected with 200 pg ($p<0.001$) compared with 100 pg of mutant mRNA. In addition, a significant number of embryos injected with mutant mRNA show developmental defects including pericardial edema, bent spine, and cyclopia compared to those injected with WT mRNA ($p<0.01$). These morphological phenotypes also increase with the mutant mRNA dose. In conclusion, our study was able to evidence the pathogenic nature of the STAT5B K632N variant since it leads to growth and developmental defects in zebrafish embryos. The zebrafish, and its conserved GH-IGF-I axis, constitutes an ideal in vivo model for characterizing the functional effect of genetic variants in ortholog human genes.

0560 - PORPHYRINOGENICITY OF DRUGS IN ACUTE INTERMITTENT PORPHYRIA: ARE THE CYP-450 POLYMORPHISMS THE ANSWER?

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CENTRO DE INVESTIGACIONES SOBRE PORFIRINAS Y PORFIRIAS (CIPYP)

Acute Intermittent Porphyria (AIP) is a hepatic pathology characterized by the accumulation of porphyrin precursors. It has sudden neurovisceral manifestations, commonly known as acute attacks or crises, which are triggered by exposure to different factors (fasting, stress, hormones and commonly used medications). Many hypotheses try to explain the variability in the prediction of the porphyrinogenicity of drugs, among which are the genetic polymorphisms of the CYP-450 enzymes. In this study, we wanted to verify if the difference in the triggering of an acute crisis was given by some of the most frequent and clinical significant polymorphisms of the enzymes of the CYP3A family, or even by a difference in their expression, given by the AKR1D1, an enzyme that regulates the expression of various CYPs and whose polymorphisms would augment CYPs expression. Peripheral blood samples of AIP patients (n= 50) and no AIP patients were analyzed (n= 74) by PCR-RFLP and PCR-sequencing. The variants analyzed were CYP3A4*22, CYP3A5*3, and AKR1D1 rs1872929 and rs1872930. Both allelic and genotypic frequencies found in the AIP group are very similar to those of the non-AIP group. So, when they were statistically analyzed with the X^2 test ($p < 0.05$), it was not a surprise to find out that there is no trend of the AIP population to any SNP. Therefore, the genetic polymorphisms analyzed do not explain the interindividual variability that exists in these patients when an acute attack arises. Another interesting conclusion obtained from this study is that there is a significant amount of extensive drug metabolizers compared to poor drug metabolizers in both groups. This is important for adjusting the dose of numerous drugs that are metabolized by this path. If these results are maintained with a higher number of patient in the Argentinian population, it will mean that doses of the drugs affected by these CYPs need to be adjusted, contributing to the safety and efficacy of therapeutics.

0572 - . GENOMIC DIAGNOSIS IMPLEMENTATION IN A PEDIATRIC HOSPITAL. PRELIMINARY RESULTS

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The access to new technologies, like Next Generation Sequencing (NGS) and microarray, has allowed the development of effective high-performance diagnosis algorithms for genetic pediatric diseases. The aim of this work was to establish standardized procedures for genomic diagnosis of genetic pediatric diseases in a pediatric hospital. Patients with presumptive diagnoses of genetic diseases (intellectual disability, metabolic, hematological or immune diseases or delay of growth and puberty) were included. DNA from peripheral blood was obtained from the patients and their parents. Genomic diagnosis procedures were performed by NGS (Clinical exome, TruSight One, NextSeq 500 Illumina) and microarray studies (8x60K Platform, Agilent). NGS results were analyzed by own designed bioinformatic pipelines, and B platform (Bitgenia) was used to prioritize variants. All variants found (sequence changes or Copy Number Variations) were classified according to American College of Medical Genetics and Genomics recommendations. This study was approved by the hospital ethical review board. Diagnostic flowchart was implemented according to designed operative protocols. Patients referred by specialized pediatricians were evaluated by the interdisciplinary team to agree on the best diagnostic pathway. From March 2018 to August 2019, 200 probands were included (86 with delay of growth and puberty, 12 hematologic, 4 immunologic and 55 metabolic disorders and 43 with intellectual disability). Among the 36 cases studied by microarray, 5 pathogenic variants (13.9 %), and 3 variants of uncertain significance were found. In 24 of the 60 patients (40 %) studied by NGS, genic variants related to patient's phenotype were found. Conclusion: Interdisciplinary team work has enabled the successful implementation of these new genomic diagnosis techniques in the hospital. Diagnosis efficiency achieved agrees with international standards.

0588 - ASSESSMENT OF F9 GENOTYPE SPECIFIC INHIBITOR RISKS ASSOCIATED WITH A LARGE SERIES OF ARGENTINE PATIENTS WITH HEMOPHILIA B

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Hemophilia B (HB) is an X-linked disorder caused by pathogenic variations in the coagulation factor IX gene (F9). Currently, HB is successfully treated by substitution of the deficient FIX. Development of inhibitory antibodies (INH) against the therapeutic FIX represents a major complication affecting patients and the public health economy. INH development in HB is typically associated with allergic and/or anaphylactic reactions. The objective was to estimate the global and partial F9 genotype risks of INH in a large series of Argentine HB patients (about 1/3 of all HB patients registered in Argentina (WFH Global Survey 2018)). We characterized the HB causative variation in 98 out of 104 studied patients (94 % of efficiency) by application of an in-house developed algorithm including 12 PCR-amplifications allowing gross deletion detection in hemizygous probands, small-mutation screening by CSGE (conformation sensitive gel electrophoresis), and Sanger DNA-sequencing of the suspected region. The case (INH+)/control(INH-) study included 10 cases and 94 controls (n= 104) assessing a global absolute INH-prevalence (GAIP) of 9.6 %. Absolute and relative risks of each F9-genotype are presented as INH-prevalence, AIP and odds ratios, OR (CI95%), respectively. Large F9-deletions showed increased risks, AIP of 50 % (6/12) and an OR of 22 (4.8 - 99.7) p=0.0001; and considering entire F9-deletions, an AIP of 71 % (5/7) and OR of 46 (7.1 - 298) p<0.0001. F9-nonsense variations showed non-significant INH risks 3/17 (18 %) OR of 2.4 (0.5 - 10) p= 0.2 as well as F9-splicing defects, 1/7 (14 %) OR of 1.6 (0.2 - 15) p= 0.5. On the other hand F9-missense showed the lowest risks, 0/49 of AIP and an OR of 0.043 (0.002 - 0.7) p= 0.001. Our GAIP is placed on the upper limit as compared with other international series (9.6 % vs. range 3 – 11 %). Our findings about F9-genotype associated INH risks in Argentina may help hemophilia therapists in designing a case-specific treatment and properly fitted follow-up regimes.

0589 - GENOTYPING PARTIAL F8 DELETIONS CAUSING SEVERE HAEMOPHILIA A IN HEMIZYGOUS AND HETEROZYGOUS STATE: NEW APPLICATIONS OF INVERSE-PCR

Karen WAISMAN (1) | Betiana Michelle ZIEGLER(1) | Vanina MARCHIONE(1) | Pamela RADIC(1) | Carlos DE BRASI(2) | Martín ABELLEYRO(1) | Liliana Carmen ROSSETTI(1)

INSTITUTO DE MEDICINA EXPERIMENTAL (IMEX), CONICET- ACADEMIA NACIONAL DE MEDICINA (ANM) (1); INSTITUTO DE MEDICINA EXPERIMENTAL (IMEX), CONICET- IIHEMA - ACADEMIA NACIONAL DE MEDICINA (ANM) (2)

Large F8 deletions are responsible for approximately 8 – 15 % cases with severe haemophilia A (sHA) and predispose to the development of FVIII therapeutic inhibitors. This work presents two practical approaches for genotyping large deletions both based on inverse-PCR that permitted resolving the cases of two unrelated families with sHA. Family 1 includes an affected patient with a deletion of F8-exon 24–26 whilst family 2 is composed by a family proband with a F8-exon 5-6 deletion and two female relatives (his mother and sister). The objective was to develop cost-effective approaches to diagnose large F8 deletions in hemizygous patients and their potential heterozygous female carriers. We designed and developed a protocol of inverse-PCR (iPCR) combined with long distance-PCR (LD-PCR) to analyse and characterise the breakpoint junctions in family 1, and the approach of inverse shifting-PCR (IS-PCR) to detect the presence/absence of the specific F8-deletion of family 2 in the proband and their female relatives. Based on the BclI restriction map, a LD-iPCR amplification system was designed to discriminate the normal variant (3.5 kb) and the deletion variant associated with 4.2 kb using primers on F8-intron 22 (IVS22-lo) and on F8-intron 23 (24A). Standard-size IS-PCR discrimination system was designed to detect and recognize the 1 kb normal allele (primers Bup1B and IVS6M-up) and the deleted variant (product size of 1.3 kb) obtained from primers IVS65-lo and IVS6M-up. As it was expected, in both families, deletion-specific LD-iPCR or IS-PCR amplification products were obtained in familiar probands and not-observed in normal control samples. Carrier diagnosis in family 2 indicated that both the mother and the sister resulted heterozygous for the deletion. Our findings points the utility to apply cost-effective and reliable approaches such as LD-iPCR and IS-PCR to allow detection and diagnosis of large deletions on X-linked

genes, like the F8, to provide valuable information for carrier detection and prenatal diagnosis in families with X-linked disorders like HA.

0615 - GENETIC STUDY OF 367 PATIENTS WITH MULTIPLE CONGENITAL ANOMALIES (MCA) AND ISOLATED CONGENITAL HEART DISEASE (CHD)

Marisol DELEA (1) | Soledad MASSARA(2) | Lucia ESPECHE(1) | Ma. Paz BIDONDO(1) | Jaen OLIVERI(2) | Paloma BRUN(2) | Pablo BARBERO(1) | Celeste MARTINOLI(3) | Verónica CAZAYOUS(2) | Lilian FURFORO(1) | Viviana COSENTINO(4) | Mónica RITTLER(5) | Sandra ROZENTAL(1) | Liliana DAIN(1)

CENTRO NACIONAL DE GENÉTICA MÉDICA- ANLIS (1); HOSPITAL DE ALTA COMPLEJIDAD EN RED EL CRUCE – SAMIC (2); HOSPITAL SOR MARIA LUDOVICA (3); HOSPITAL INTERZONAL GENERAL DE AGUDOS LUISA CRAVENNA DE GANDULFO (4); HOSPITAL MATERNO INFANTIL RAMÓN SARDÁ (5)

Congenital anomalies (CA) are morphological and/or functional disorders that originate before birth. Affecting 3 to 5 % of newborns, they represent the second leading cause of infant mortality in Argentina, after perinatal conditions. In approximately 50 % of the patients, the underlying causes are unknown. Cases with MCA are those with 2 or more unrelated birth defects. MCA are present in 2.26 / 1000 births. CHD are the most frequent CA, with a prevalence of 4.06 / 1000 births. The goal of this work was to identify the genetic causes of MCA and isolated CHD cases from our population. We studied 367 patients (169 MCA and 198 isolated CHD) born between June 2015 and August 2017 in 13 public hospitals participating in the National Network of Congenital Anomalies of Argentina (RENAC). Peripheral blood and DNA was obtained from all patients and a karyotype was performed in MCA patients. Patients with conotruncal CHD or DiGeorge phenotype (n= 126) were studied by MLPA. Array-CGH was performed in 77 MCA selected patients. A total of 15 CHD patients were analyzed by a Next Generation Sequencing (NGS) gene panel or by Exome Sequencing. one hundred and seventeen MCA patients displayed a normal karyotype, 12/129 presented cytogenetic anomalies: a trisomy 13, 5 trisomy 18, a 47,XXX/47,XX,+14, 2 translocations a (t(1;2)(q25;q21)) and t(11;17)(p10;p10), a del(15)(q11.2q13) and 2 supernumerary marker chromosome. The karyotype could not be performed in 40 patients due to culture failure. Among 126 cCHD patients, 21 presented a typical 22q11 deletion, three 22q11 short deletion, one 22q11 duplication, and one TBX1 gene deletion. We found that 13/77 MCA patients had a causal or potentially causal CNVs. After NGS analysis, five patients presented 5 different nucleotide variants with possible impact on protein function. Using this algorithm that combines a technical and clinical strategy, 20 % of the patients analyzed were diagnosed.

0626 - MOLECULAR GENETIC STUDIES IN A LARGE ARGENTINEAN COHORT (1152) OF 21-HYDROXYLASE DEFICIENT PATIENTS AND RELATED INDIVIDUALS

Melisa TABOAS (1) | Cecilia FERNANDEZ(1) | Carlos David BRUQUE(1) | Belen BENAVIDES-MORI(1) | Aldana CLAPS(1) | Susana BELLI(2) | Mirta STIVEL(2) | Titania PASQUALINI(3) | Liliana ALBA(1) | Marisol DELEA(1) | Lucia ESPECHE(1) | Eduardo CHARREAU(4) | Noemi BUZZALINO(1) | Liliana DAIN(1)

CENTRO NACIONAL DE GENÉTICA MÉDICA- ANLIS (1); HOSPITAL DURAND (2); HOSPITAL ITALIANO DE BUENOS AIRES (3); IBYME-CONICET (4)

Congenital Adrenal Hyperplasia (CAH) is an autosomal recessive disease produced in 95 % of cases due to 21-hydroxylase deficiency. CAH is presented in 3 clinical forms, 2 severe or classical: salt wasting (SW) or simple virilizing (SV) and a mild or non-classical form (NC). The gene encoding 21-hydroxylase, CYP21A2, shares 98 % sequence identity with the pseudogen CYP21A1P. Our objective was the molecular characterization of 21-hydroxylase deficiency in patients of our population. In this work, we analyzed 1,152 individuals from our populations: 628 21-hydroxylase deficient patients (78SW, 90SV, and 460NC), 398 relatives and 126 partners. All were recruited between 1996 and 2018. Until 2011, the 10 most frequent derived-pseudogene point mutations in the CYP21A2 gene were screened by allele-specific PCR or PCR-RFLP. For those samples with at least one non-determined allele, as well as for those recruited from 2011 to 2018 (n= 343), direct sequencing was performed. Deletions/duplications were analyzed by MLPA SALSA P050-C1 CAH MLPA kit. The most frequently mutated allele in NC patients was the p.V282L. In classical patients were c.293-13C>G and p.I173N. Patients presenting c.293-13C>G or p.I173N showed more than one possible phenotype. In NC patient, 86.9 % of the alleles presented mutations. A total of 60 alleles disclosed novel or rare mutations. From these, 11 mutations were found for the first time and published by our group in recent years. In addition, 3 novel mutations, p.(S166F); p.(P189R) and p.(R436L) are being described in this study. From the 330 parents analyzed, all but one were carriers. One of the probands disclosed a de novo mutation. Interestingly, 5 fathers, 3 brothers, 1 sister and 2 mothers presented both alleles with a mutation but without clinical signs. By last, 105 of the 126 partners were non-carriers. Thus, several techniques and molecular approaches need to be applied for a comprehensive characterization of the diseased alleles. In that sense, our work represents one of the larger and complete genetic characterization of 21-hydroxylase deficient patients from our region.

0628 - ATYPICAL MOLECULAR CAUSE OF FRAGILE X SYNDROME: A CASE REPORT

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CENTRO NACIONAL DE GENÉTICA MÉDICA- ANLIS (1); HOSPITAL SOR MARIA LUDOVICA (2)

Fragile X Syndrome (FXS), the most common heritable form of intellectual disability (ID), is usually due to a CGG expansion, named full mutation (> 200 CGG repeats) in the FMR1 gene, located in Xq27.3. This mutation leads to hypermethylation of the promoter silencing it and lowering the expression levels of the FMRP protein, involved in synaptic plasticity and maturation. The mothers of children with FXS generally have an X chromosome with a premutation (55-200 repeats) or, less frequently, a full mutation. In 2 % of the cases, the absence of FMRP is due to single nucleotide variants or deletions. We present the case of a 3 years-old male child (who presented ID, language development delay, autism, hyperactivity, hyperlaxity, prominent ears, high palate, surgically resolved hypospadias and left testicle in elevation), and his mother (with ID without dysmorphisms), who were tested for FXS. Fluorescent PCR, TP-PCR with the AmplideX FMR1 PCR kit (Asuragen) and MS-MLPA (ME029-B3 FMR1/AFF2 kit, MRC Holland) were performed for both of them. Fluorescent PCR did not show amplification product for the child's sample and capillary electrophoresis of the fluorescent PCR from the mother's sample showed a single peak of 33 triplets. Unexpectedly, the TP-PCR also showed no amplification for the child and a normal pattern of 33 repetitions for the mother. Finally, the MLPA showed a deletion of at least 1.05 Mb in the Xq27.3-q28 region, involving entire FMR1 and exons 1 to 14 of AFF2 (NM_002025.3), both in the child and his mother. No mosaicism was observed. So, the mother presented one allele with 33 repeats and another with deletion of FMR1. Her son inherited the allele with the deletion, probably resulting in no FMRP protein levels. Although the MS-MLPA for FMR1 is usually used to assess the methylation state of its promoter, in this case, this technique helped to evidence the presence of a deletion, a very rare molecular cause of FXS, reaching to an accurate diagnosis.

0700 - APPLICATION OF LDL GENETIC RISK SCORE IN PATIENTS WITH HYPERCHOLESTEROLEMIA TO EVALUATE A POLYGENIC ORIGIN CAUSES

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ANLIS ¨DR. C. MALBRÁN¨, CENTRO NACIONAL DE GENÉTICA MÉDICA (1); INSTITUTO NACIONAL DE SAÚDE DOUTOR RICARDO JORGE, GRUPO DE INVESTIGAÇÃO CARDIOVASCULAR (2); UNIVERSIDAD FASTA, FACULTAD DE MEDICINA (3); UNIVERSIDAD DE BUENOS AIRES, FACULTAD DE FARMACIA Y BIOQUÍMICA, LAB DE LÍPIDOS Y ATEROSCLEROSIS (4)

High LDL-cholesterol (LDL-c) values may be due to monogenic variants in the LDLR, APOB, PCSK9 or LDLRAP1 genes, leading to Familial Hypercholesterolemia (FH); or to a polygenic origin arising from polymorphisms in different genes related to cholesterol metabolism. Two genetic risk scores (GRS) are the most widely used, one in Canada (SNPs: 11) and the other in European population (SNPs: 6). Here we present the application of the European GRS in a sample of hypercholesterolemic patients in the province of Buenos Aires from the FH DaVinci study. The 6 SNPs of the GRS were obtained from the VCF files or by Sanger sequences, from 116 index cases with clinical evaluation (DCLN score) and genetic studies of FH. Twentyu eight % showed a positive GRS. The mean value was: 0.68 ± 1.72, median=0.704, mode=0.760. The quartiles values were: Q1= 0.581, Q2= 0.704 and Q3= 0.798. The SNP with the strongest effect on the score was the rs6511720 G allele on the LDLR gene (frequency 0.95). Four groups were considered: with monogenic origin (22); non-monogenic and GRS- (62); non-monogenic and GRS+ (26); and both monogenic and GRS+ (6). DLCN score was: 9.14 ± 2.98; 6.47 ± 2.53; 6.72 ± 1.90 and 10.71 ± 3.15 respectively, (ANOVA $p<0.001$), no differences were observed for: GRS+ vs. GRS- and for monogenic vs. both monogenic and GRS+. The mean LDL-c was: 269.36 ± 100.06; 188.17 ± 78.80; 218.30 ± 56.70 and 278.43 ± 93.14 respectively (ANOVA $p<0.001$), no differences were observed for GRS+ vs. GRS- .The GRS showed a sensitivity of 39 % and specificity of 81 % using a cut-off value of > 0.76. We conclude that hypercholesterolemic individuals from this sample of our population, with positive polygenic GRS showed lower values of DCLN clinical score compared to those with a monogenic origin, levels of LDL-c are higher for those with monogenic origin, and that it would be preferable to apply the GRS in a healthy group to assess their ability to discriminate polygenic causes in our population.

0709 - COPY NUMBER VARIANTS IN CHILDREN WITH INTELLECTUAL DISABILITY/GLOBAL DEVELOPMENTAL DELAY, DYSMORPHIC FEATURES AND/OR CONGENITAL ANOMALIES.

María Eugenia FONCUBERTA | Gabriela ZELAYA | Angélica MORESCO | Mara BONETTO | Edgardo BAIALARDO | María Gabriela OBREGON | Cristina Noemí ALONSO

HOSPITAL DE PEDIATRIA JUAN P. GARRAHAN

Chromosomal microarray analysis (CMA) has emerged as a major tool to identify clinically relevant copy number variants (CNV) in children with intellectual disability/developmental delay (ID/DD), autism spectrum disorders (ASD) and multiple congenital anomalies (MCA).The aim of this study is to present the spectrum of anomalies detected by CMA in patients with ID/DD, dimorphism and/or MCA in whom standard karyotyping analysis had shown normal results. CMA analysis was performed in 56 patients using two microarray platforms: Sure Print G3 ISCA v2 8x60K or Baylor CGH 8x60K (Agilent). Pathogenic or likely pathogenic CNVs were identified in 12 cases (21.4 %), a variant of uncertain significance (VOUS) was detected in 1 patient (1.8 %) and 3 patients (5.3 %) showed likely benign CNVs. Microdeletions were observed in 11 cases and only in one patient a microduplication syndrome was identified. Regarding deletions, 7 cases were associated with known syndromes, 2 patients showed intragenic deletions involving DYRK1A and SCL9A6 genes respectively, one patient presented an uncommon chromosome 19q13.12-q13-2 deletion, and a mosaic deletion of 18.3 Mb on chromosome 20 was observed in one case. CNVs detected in our cohort ranged from 8 Kb to 6.6 Mb regardless the mosaicism case. The resolution of 8x60K microarrays format used in this study demonstrated to be useful and offered an excellent diagnostic yield in patients with ID/DD, dysmorphic features and/or MCA. In addition, it provided a low rate of VOUS detection which may be difficult to interpret and may represent a counseling challenge. In conclusion, this study emphasizes the usefulness of CMA in detecting genomic imbalances in this group of patients with normal standard karyotype.

0902 - IMPLICATION OF GLUTATHIONE S-TRANSFERASES GENE VARIANTS ON ACUTE INTERMITTENT PORPHYRIA ONSET.

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CIPYP-UBA-CONICET (1); CIPYP - UBA-CONICET Y FCEN, UBA (2)

Acute intermittent porphyria (AIP) is a result of a partial and primary deficiency in Porphobilinogen deaminase (PBG-D), the third enzyme in the heme pathway. The presence of the mutation is not enough for the manifestation of AIP which can be triggered by therapeutic drugs, so genetic variants in cell detoxification system could be involved in AIP onset. Glutathione-S-transferases (GST) are Phase II enzymes involved in detoxification of reactive oxygen species, environmental carcinogens, metabolism of steroid hormones and chemotherapeutic agents. Some polymorphisms in this gene, GSTT1 null, GSTM1 null and GSTP1 (rs1695, c.313 A>G), alter GST activity affecting hormones and xenobiotics levels. The aim was to analyze these variants in relation with AIP manifestation. The study was performed in control individuals (non porphyric) and in AIP patients carrying PBG-D mutation who at the moment of the diagnosis were symptomatics (S-AIP) or without clinical/biochemical alterations (latent group, L-AIP). GSTT1 and GSTM1 were amplified by multiplex PCR; GSTP1 variant by PCR-RFLP. The deletion frequencies in homozygosis for GSTT1 null were: 8.3 (control), 20.5 (S-AIP) and 6.1 % (L-AIP). Frequencies for GSTM1 null were: 41.7 (control), 51.3 (S-AIP) and 45.5 % (L-AIP). In S-AIP, null GSTT1 frequency was significantly high respect to control ($p<0.05$) and L-AIP ($p<0.01$); GSTM1 gene frequency were higher but no significant than the other cohorts. GSTs null variants are considered of risk. GSTP1 allelic frequencies for non-wild type (G) variant were: 0.42 (control), 0.47 (S-AIP), 0.35 (L-AIP). GG genotype frequency in GSTP1 was significantly high in S-AIP respect to others groups ($p<0.01$). When the combination of GSTM1/GSTT1/GSTP1 were calculated, a high frequency for the presence of 2 risk variants was observed for the S-AIP group respect to L-AIP and Control. In conclusion, results here presented would suggest a possible implication of GSTs in AIP onset.

0918 - ATM KINASE ACTIVITY PROMOTES THE REMOVAL OF ETOPOSIDE-INDUCED TOP2A CLEAVAGE COMPLEXES THROUGH TDP1 FUNCTION

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The Top2 poison Etoposide (ETO) stabilizes a covalent intermediate Top2-DNA, which represent a protein blockage of DNA ends. Tyrosil-DNA-Phosphodiesterase 1 (TDP1) can remove phosphodiester bonds between proteins and the phosphate group

of DNA to allow the repair of blocked DNA ends. We determined the role of TDP1 in the removal of abortive Top2A-DNA complexes (ccTop2A) during different metabolic processes of DNA. A possible regulatory effect of ATM on the TDP1-dependent ccTop2A removal was also analyzed. The study was performed in a HeLa TDP1-knock down cell line (TDP1kd) by assessing ETO-induced ccTop2A by flow cytometry. We showed that TDP1kd cells accumulated higher amounts of abortive ccTop2A than control (NS) cells after 1 h exposure to ETO ($p<0.05$, t-test). In addition, pre-incubation of TDP1kd cells with the proteasome inhibitor Bortezomib (Btz, 2.5 μM) gave rise to similar levels of ETO-induced ccTop2A than those found in NS cells treated with Btz+ETO and ETO-treated TDP1kd cells. This suggests a proteasome-dependent activity of TDP1 in the removal of ccTop2A. TDP1kd cells showed higher levels of ETO-induced ccTop2A in the S-phase compared to NS ($p<0.05$), which correlated with increased DNA damage signals (γH2AX) by 2 h. No significant differences were found in ETO-induced ccTop2A levels in NS and TDP1kd cells after pre-treatment with the transcriptional inhibitor DRB (300 μM)+ETO compared with ETO alone. On the other hand, pre-treatment with an ATM kinase inhibitor (KU55933, 10 μM) resulted in higher accumulation of ETO-induced ccTop2A in both NS and TDP1kd cells ($p<0.05$), without evidence of synergistic or additive effect; thus suggesting both enzymes are involved in the same pathway. Together, our results demonstrate that TDP1-mediated removal of ETO-induced ccTop2A occurs during DNA replication and is dependent on proteasome activity. Similarly, we showed the kinase activity of ATM promotes the removal of abortive ccTop2A by the same pathway that TDP1 does.

0940 - VARIATION OF THE ABSOLUTE TELOMERE LENGTH AFTER DIFFERENT TREATMENT IN PATIENTS WITH OBESITY

Andrea Liliana MILLÁN (1) | María Constanza PAUTASSO(1) | Andrea Elena IGLESIAS MOLLI(1) | Martina CERRATO GARCÍA(1) | Mailén ROJO(1) | Susana Rutt GUTT(2) | Gustavo Daniel FRECHTEL(1) | Gloria Edith CERRONE(3)

UNIVERSIDAD DE BUENOS AIRES – CONICET. INSTITUTO DE INMUNOLOGÍA, GENÉTICA Y METABOLISMO (INIGEM) (1); HOSPITAL ITALIANO DE BUENOS AIRES (2); UNIVERSIDAD DE BUENOS AIRES, FACULTAD DE FARMACIA Y BIOQUÍMICA, CÁTEDRA DE GENÉTICA (3)

Overweight and obesity are one important risk factors for mortality in the world. Telomeric length is considered a marker of cell aging and is closely related to biological situations like oxidative stress and inflammation. Our objective was to analyze different treatments on absolute telomere length (aTL) in obese patients. We studied a group of 21 patients with obesity treated with diet, physical exercise and pharmacological treatment and a group of 31 obese patients with indication of bariatric surgery. The biochemical-clinical characteristics were measured and we evaluated the difference in their absolute telomere length after 6 months of intervention. aTL was determined in genomic DNA extracted from peripheral blood leukocytes by the method of absolute quantification by quantitative real-time PCR. Statistical analysis was carried out by SPSS with a significance level of 0.05. We observed by linear regression a negative association between the variation of aTL between basal time and after 6 months and age in all patients with obesity ($p= 0.03$). A change in telomere length significantly correlates with weight loss ($p= 0.01$) and decrease in BMI ($p<0.01$), and depended on the type of treatment ($p<0.01$) and with the incidence of T2DM ($p= 0.02$). The significant increase in aTL in patients with an indication of bariatric surgery ($p<0.01$), was attributed to a significant correlation found with the greatest decreases in the levels of inflammation measured by PCR-us ($p<0.01$). In patients undergoing pharmacological treatment we only found a significant positive association between the variation of aTL after treatment and the dose of metformin ($p= 0.01$). The treatments had different effects on the absolute length of the telomere in obese patients. We show the impact of inflammatory status and high doses of metformin on telomere length.

Metabolismo y Nutrición / Metabolism and Nutrition I

Chairs: Marcelo Choi | Miriam Wald

0060 - CHRONIC ADMINISTRATION OF HIGH FRUCTOSE-HIGH FAT DIETS INCREASES ABDOMINAL ADIPOSE TISSUE AND LIVER WEIGHT BUT NOT BODY MASS IN RATS

Laura Mercedes LINARES | **Pablo Antonio SCACCHI BERNASCONI** | Graciela Clelia BORTOLAZZO | Verónica Soledad ZUCCARELLA | Pilar BALCARCE | María Laura REYES TOSO | Osvaldo Juan PONZO | Carlos Felipe REYES TOSO

DEPARTAMENTO DE CIENCIAS FISIOLÓGICAS, UAII, FACULTAD DE MEDICINA, UNIVERSIDAD DE BUENOS AIRES

Diets in industrialized nations have shifted to increased consumption of fructose and saturated fat. These diets are linked with metabolic complications such as metabolic syndrome (MS). Fructose is a lipogenic substrate which can induce metabolic alterations in the liver and has increasingly been used as a sweetener since the introduction of high-fructose corn Syrup in soft drinks and other carbohydrate-sweetened beverages. Similar effects are not observed with the administration of other simple sugars such as glucose. In animal models, the administration of high fructose -10 % fructose in drinking water- and fat -20 %- (known as Western diet –WD-), induce features of MS including weight gain, insulin resistance, hypertriglyceridemia, hypertension, abdominal obesity and non-alcoholic fatty liver disease (NAFLD) among other pathological alterations. The objetive of this work was designed to evaluate body composition modification in long term administration of WD to male Wistar rats. Animals were exposed from 6 to 24 weeks of age to a standard diet –SD- (n= 8) or WD (n= 8). Every 6 weeks rats were fasted overnight and baseline blood samples were collected from the tail vein. Blood glucose, insulin and fasting triglyceride and cholesterol levels were assayed. Homeostatic model assessment (HOMA) was calculated using a software from Oxford University. Body mass was measured weekly, and Systolic blood pressure (SBP) was measured monthly by using a manometer and employing an inflatable tail-cuff pressure transducer connected to an amplifier and a data acquisition system. An average value from three SBP readings (that differed by no more than 2 mmHg) was determined for each animal. Weight gain was calculated by subtracting the final weight from the initial weight. After euthanasia by decapitation, abdominal white adipose tissue (WAT) and liver were extracted, and weights were expressed as a percentage of body weight. Lipid peroxidation of hepatic tissue was estimated by Thiobarbituric acid reactive substances (TBARS). All data are shown as the mean ± SEM. Data were analyzed by two-way ANOVA or ANOVA for repeated measures, adjusted by Bonferroni correction. WD induced a metabolic syndrome. Diet-induced weight gain in WD-treated group was not found. However, significant differences were detected between SD- and WD-treated group in SBP ($p<0.01$), HOMA ($p<0.01$), fasting serum triglycerides levels ($p<0.01$), fasting serum cholesterol levels ($p<0.05$), insulin ($p<0.01$), WAT ($p<0.01$), TBARS in liver homogenates ($p<0.001$) and liver weight ($p<0.05$). WD in Wistar rats induces MS with increased WAT and liver weight, and altered metabolic parameters but not body mass. The augmentation in liver weight observed during WD supplementation is interpreted as a first sign of fatty liver. Increased HOMA shows that WD rapidly induced insulin resistance. Furthermore, greater TBARS levels in hepatic tissue suggest that these rats have an imbalance between reactive oxygen species generation and removal by the antioxidant defense systems. Longer term effects of such a diet are still left to future analysis.

0081 - PERINATAL TAURINE EXERTS A HYPOTENSIVE EFFECT IN MALE

SPONTANEOUSLY HYPERTENSIVE RATS AND DOWN-REGULATES ENDOTHELIAL OXIDE NITRIC SYNTHASE IN THE AORTIC ARCH

Adriana Laura BURGUEÑO (1) | Melisa Florencia MENSEGUE(2) | Noelia Luz GONZALEZ MANSILLA(2) | Mariana L TELLECHEA(2)

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Essential hypertension is considered to be a result of the interaction between genetic and environmental factors, including perinatal factors. Different advantageous perinatal factors proved to have beneficial long-lasting effects against an abnormal genetic background. Taurine is a ubiquitous sulphur-containing amino acid present in food. The antihypertensive effects of taurine have been reported in experimental studies and in human hypertension. We aimed to investigate in spontaneously hypertensive rats (SHR), a known model of genetic hypertension, whether taurine administration during pregnancy and lactation would influence 1) systolic blood pressure (SBP), 2) aortic geometry, and 3) cardiac hypertrophy in adult offspring. Additionally; we aimed to determine whether perinatal taurine administration is associated with changes in relative telomere length (RTL) and gene expression of target genes. Female SHR were administered with taurine (3 g/l) during gestation and lactation (SHR-TAU). Untreated SHR and Wistar-Kyoto rats (WKY) were used as controls. Long lasting effects in offspring were investigated. Addition of taurine to the mother's drinking water improved SBP in adult offspring. No differences were observed in aortic morphometry or cardiac hypertrophy. We suggest that taurine programming albeit sex-specific, is associated with gene expression changes which ultimately may lead to improvement of aortic remodelling and enhanced endothelial function because of augmented nitric oxide production. Specifically, we found modifications in gene expression of Bcl-2 family members and upregulation of endothelial nitric oxide synthase in the aorta of 22-week-old male offspring. No differences were observed on RTL in different cardiovascular tissues between SHR and SHR-TAU. Although SHR have responded only moderately to perinatal treatment, our findings supports the possibility of improving the genetically hypertensive state by manipulating the early environment.

0111 - HYPOLIPIDEMIC EFFECTS OF N-ACETYLCISTEINE IN CF-1 MICE FED A HIGH-FAT DIET

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We showed that antioxidant N-acetylcysteine (NAC) inhibits cellular lipid accumulation during adipocyte differentiation in vitro (Soto et al, 2016 and Pieralisi et al, 2017). Here we evaluated effects of NAC on mice fed a high-fat diet and in vivo lipid parameters. FCEyN – UBA CICUAL approved this protocol. Thirty-six CF-1 male mice weighting from 39 to 42 g were randomly assigned to 4 treatment groups, fed a normal-fat diet with 2.9 Kcal/g without (group C, n= 12) or with NAC (group CN, n= 12) and a high-fat diet with 5.7 Kcal/g without (group O, n= 6) or with NAC (group ON, n= 6) for 45 days. NAC supplementation in drinking water was 1.2 g/L, animals fed and drank ad libitum. We did not observe toxic effect of NAC and, all mice consumed similar amount of food and water during treatments. At day 45, we determined mice body weight (BW) and sacrificed them. We evaluated: a) serum cholesterol (Chol) and triglyceride (Tg) levels; b) liver and omental adipose tissue histology; c) omental fat weight (OFW). At day 45 CN had significantly lower BW than C (46.8 ± 1.2 [C] vs. 42.0 ± 0.7 g [CN], $p<0.01$); but ON had similar BW as O (43.9 ± 1.1 g [O] vs. 44.7 ± 3.1 g [ON], $p= 0.5$). Nevertheless, O showed significantly higher Chol and Tg levels than C (Chol: 3.32 ± 0.05 mmol/L [O] vs. 1.92 ± 0.05 mmol/L [C], $p<0.01$; Tg: 2.94 ± 0.07 mmol/L [O] vs. 2.58 ± 0.11 mmol/L [C], $p<0.01$). Chol and Tg levels significantly decreased in ON compared to O (Chol: 2.80 ± 0.02 mmol/L [ON], O vs. ON $p<0.01$; Tg: 2.81 ± 0.03 mmol/L [ON], O vs. ON $p<0.01$). Hematoxylin-eosin stained samples did not present significant difference in hepatic cells between the groups; NAC reduced almost 30 % adipocyte size in ON compared to O. We observed 40 % reduction in OFW in mice treated with NAC compared to untreated mice. Our results suggested that NAC administration through drinking water could decrease serum Chol and Tg in CF-1 mice fed a high-fat diet, NAC also could decrease omental fat weight.

0134 - FISH OIL SUPPLEMENTATION OF A DIET PROVIDED OMEGA 9 FATTY ACIDS. STUDY ON SERUM, THYMUS AND BRAIN OF GROWING RAT.

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Dietary lipids have an important role in nutrition. This work analyzed the effect of diet containing olive oil, with and without the supplementation with omega 3, on serum, thymus and brain's fatty acid profiles of growing rats. Weanling Wistar rats fed during 10 days a diet containing olive oil as fat (O group). Other group received the same diet supplemented with 24 mg/day of fish oil (OS group). Control group (C) received diet according AIN´93. Serum, thymus and brain's fatty acids profiles were determined by gas chromatography. Statistical analysis used ANOVA. Results (%Area) were: SERUM: OLEIC O:23.44 ± 3.68a; OS: 18.31 ± 2.22b; C: 10.60 ± 2.01a. LINOLEIC (LA) O: 12.44 ± 1.65b; OS: 12.98 ± 4.31b; C: 18.27 ± 2.81a; LINOLENIC (ALA) O: 0.30 ± 0.09b; OS: 0.32 ± 0.08b; C: 0.92 ± 0.34a; EPA O: 0.65 ± 0.17a; OS: 1.63 ± 0.49b; C: 0.80 ± 0.23a; DHA: O: 1.57 ± 0.58a; OS: 4.00 ± 1.70b; C: 1.33 ± 0.19a. THYMUS: OLEIC O: 21.54 ± 5.92; OS: 24.40 ± 5.04; C: 18.22 ± 3.23. LINOLEIC O: 5.90 ± 0.56b; OS: 6.5 ± 0.61b; C: 10.89 ± 2.18a; ALA O: 0.27 ± 0.02b; OS: 0.30 ± 0.07b; C: 0.49 ± 0.19a; EPA O: 0.49 ± 0.28; OS: 0.50 ± 0.13; C: 0.50 ± 0.12; DHA O: 0.47 ± 0.10a; OS: 0.70 ± 0.12b; C: 0.52 ± 0.16a. BRAIN: OLEIC O: 13.11 ± 2.64; OS: 12.94 ± 1.07; C: 13.14 ± 1.56. LA O: 1.17 ± 0.46; OS: 1.05 ± 0.33; C: 1.26 ± 0.19; ALA O: 0.15 ± 0.03; OS: 0.12 ± 0.04; C: 0.16 ± 0.06; EPA O: 0.46 ± 0,18; OS: 0.38 ± 0.09; C: 0.33 ± 0.07; DHA: O: 11.39 ± 2.04; OS: 11.32 ± 1.69; C: 11.66 ± 1.63. Data with one letter (a,b) in common, were different ($p<0.05$). In sera, O and OS showed lower ALA and LA and higher oleic levels, compared to C. OS presented high levels of EPA and DHA. In thymus, O and OS groups showed lower levels of ALA and LA than C. The OS group only increased DHA. No changes were presented in brain. The results suggest that olive oil exacerbated omega-9 family with diminution of essential fatty acids while organism tries to compensate brain essential fatty acids. Fish oil supplementation increased serum and thymus DHA levels, not modifying low levels of essential fatty acid. Other source of supplementation may be convenient.

0140 - EFFECTS OF PRENATAL STRESS AND POSTNATAL HIGH FAT DIET FEEDING ON BALB/C MICE METABOLISM.

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In-utero exposure to maternal stress increases short and long term risk of suffering metabolic diseases. Exposure to stressful events leads to an increase in glucocorticoids release by activation of the HPA axis, therefore early programming of the HPA axis has emerged as a key underlying mechanism of stress-related disorders. Evidence suggests that a stressful prenatal environment seems to favour adverse metabolic conditions. To test this hypothesis in BALB/c mice, a strain susceptible to stress but resistant to metabolic effects of a high fat diet (HFD), we exposed female pregnant mice to restraint stress during the last week of pregnancy (2 h/day). Offspring were fed with HFD between weeks 4 and 28 of age. Prenatally stressed (PS) females and males fed with HFD showed higher body weight (females: $p<0.001$, n= 8; males: $p<0.01$, n= 8) and adipose tissue content (adipose tissue weight/body weight, both sexes: $p<0.001$, n= 8). Females were hyperinsulinemic ($p<0.001$, n= 5), with decreased expression of Foxo1 (Forkhead box protein O1) a transcription factor that plays important roles in regulation of gluconeogenesis and glycogenolysis by insulin signaling ($p<0.05$, n= 5) and Adiponectin ($p<0.05$, n= 5) in adipose tissue. On the other hand, PS males (fed with standard or HFD) had hypertriglyceridemia ($p<0.001$, n= 8) and hypercholesterolemia ($p<0.001$, n= 8). PS per se, in males, decreased the expression of Adiponectin ($p<0.01$, n= 5). PS animals showed a great susceptibility to develop obesity. We conclude that PS may give rise to some adverse effects, and abnormal phenotype may be provoked by or exacerbated in a later life nutritional challenge. We intend to continue our research by evaluating whether epigenetic alterations are responsible for the observed gene expression alterations.

0144 - MECHANISMS UNDERLYING SKELETAL MUSCLE LIPOTOXICITY IN DYSLIPEMIC INSULIN-RESISTANT RATS: EFFECTS OF DIETARY CHIA (SALVIA HISPANICA L.) SEED.

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Lipid accretion in skeletal muscle (SM) is related to the development of lipotoxicity and insulin resistance (IR), however, the mechanisms involved are not fully clarified. We previously shown that rats fed a sucrose-rich diet (SRD) develops IR, dyslipidemia, and SM lipid accretion. Moreover, we demonstrated that all of them were reversed when chia seed (Salvia hispanica L.)-rich in alpha-linolenic acid (ALA, 18:3 n-3)- was administered as a dietary source of fat in SRD-fed rats. The aims of this study were: (i) to explore the mechanisms underlying SM lipotoxicity in SRD-fed rats (ii) to investigate the effects of chia seed on these mechanisms. Male Wistar rats were fed a SRD for 3 months. Half of the animals continued with the SRD until month 6, the other half was fed a SRD in which the fat source, corn oil, was replaced by chia seed from month 3 to 6 (SRD+chia). Another group consumed a reference diet all the time. In SM we analyzed: a. muscle-type carnitine palmitoyltransferase (M-CPT), fatty acid synthase (FAS), and glucose-6-phosphate dehydrogenase (G-6-PDH) enzyme activities, b. protein mass levels of PPARalpha, PPARgamma, total AMPK, pAMPK, precursor and mature forms of SREBP-1 and sarcolemmal FAT/CD36, c. fatty acid composition of SM phospholipids. SM of SRD-fed rats showed a significant reduction ($p<0.05$) of M-CPT 1 enzyme activity, PPARs and pAMPK protein levels. FAS, G-6-PDH enzyme activities, the mature form of SREBP-1 and FAT/CD 36 were increased ($p<0.05$). In SRD+chia-fed rats M-CPT 1 enzyme activity, PPARs and pAMPK protein levels were normalized ($p<0.05$). The precursor and mature forms of SREBP-1 and lipogenic enzyme activities were decreased ($p<0.05$). FAT/CD36 and n-3/n-6 fatty acids ratio of membrane phospholipids were increased ($p<0.05$). In summary, this study shows some mechanisms involved in SM lipotoxicity of insulin-resistant rats fed a SRD and provides novel information on the beneficial effects of chia seed on these mechanisms.

0207 - ALPHA-LINOLENIC ACID-RICH CHIA (SALVIA HISPANICA L.) SEED AMELIORATES ADIPOSE TISSUE DYSFUNTION IN AN EXPERIMENTAL MODEL OF VISCERAL ADIPOSITY AND INSULIN RESISTANCE BY MODULATING LIPID METABOLISM.

Victoria AIASSA | Michelle Berenice VEGA JOUBERT | María Eugenia OLIVA | Maria Del Rosario FERREIRA CORDONEDA | María Eugenia Guadalupe D'ALESSANDRO

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Salvia hispanica L. (chia) seed is one of the richest botanical sources of alpha;-linolenic acid (ALA, 18:3 n-3)- and it has generated considerable research interest in recent years. We previously shown in dyslipemic insulin resistant rats fed a sucrose-rich diet (SRD), which have visceral adiposity, that the replacement of corn oil by chia seed in the SRD reduces epididimal adipocyte hypertrophy, triglyceride content, lipogenic enzyme activities and lipolysis. This study aimed to further explore if changes in adipocyte lipid metabolism could be involved in the beneficial effect of chia seed on reducing visceral adiposity. Male Wistar rats were fed a SRD for 3 months. Half of the animals continued with the SRD until month 6, the other half was fed a SRD in which the fat source, corn oil, was replaced by chia seed from month 3 to 6 (SRD+chia). Another group consumed a reference diet all the time. We analyzed: a. morphometrical parameters -body weight (BW), body length, thoracic (TC) and abdominal circunference (AC), body mass index (BMI)- and energy intake; b. Carcass composition; c. visceral adiposity index (VAI) and d. in epididymal adipose tissue (EAT): carnitine palmitoyltransferase (CPT 1, CPT 2 and total CPT) enzyme activities, total AMPK, pAMPK and plasma membrane FAT/CD 36 protein levels. Besides, glucose, insulin, triglyceride, free fatty acids serum levels and insulin sensitivity (IS) were determined. Compared with SRD-fed rats, SRD+chia group shown: a- a decrease ($p<0.05$) in TC, AC, BMI and VAI. BW an energy intake not change, b- a reduction ($p<0.05$) in carcass fat content and weight, c-in EAT: a normalization ($p<0.05$) of both the reduced protein levels of pAMPK and the increased levels of FAT/CD 36. No changes were observed in CPT enzyme activities. Besides chia seed normalizes hyperglicemia, dislypemia and IS. This work shows new possible mechanisms involved in the beneficial effect of chia seed on adipose tissue dysfunction and visceral adiposity.

0243 - EFFECT OF DEHYDROEPIANDROSTERONE SUPPLEMENTATION IN A RAT MODEL OF HIPOESTROGENISM AND OBESITY

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Obesity is a major worldwide health concern that predisposes individuals to a higher risk of cardiovascular disease (CVD), diabetes and metabolic syndrome. The menopausal period is followed by an increased risk of CVD and is associated with higher rates of overweight and obesity. According to intracrinology, intracellular enzymes may convert dehydroepiandrosterone (DHEA) into active steroids in peripheral tissues without systemic exposure to high estrogens levels, presenting DHEA supplementation as a low risk treatment for menopause. The aim of this study was to analyze the effects of DHEA supplementation in a model of hipoestrogenism and obesity. Four groups (n= 5) of ovariectomized Wistar rats were feed with standard diet (ND) (4 % fat w/w) or high fat (HF) diet (27 % fat w/w) for 8 weeks. Hormonal treatment of rats consisted in daily injectections of vehicle (Cont) or DHEA (1 mg/kg day of DHEA). Daily food intake was 165.3 ± 6.8; 201.1 ± 9.1 Kcal/kg (ND; HF

p<0.01) with an increase of body weight of 12.5-52.5; 43.9-82.1 (95%CI, ND; HF) and an adiposity index of 5.7 ± 0.41; 6.7 ± 0.46 (Mean±sd ND vs. HF, p<0.01). Using commercial kits, serum glucose, total cholesterol, HDL-cholesterol and triglycerides where evaluated, and no differences between Cont and DHEA were observed. In HF group, serum ROS (H2-DCFDA) was diminished in DHEA group 12.2-42.3 % (95 % CI VH-DHEA). Nitric oxide production (2,3-DAN) in rat aortic rings was enhanced by DHEA in both ND and HF 4.0 ± 0.6; 5.2 ± 0.4; 4.2 ± 0.4; 5.8 ± 0.5 nmol NO/mg protein (Mean±sd ND-Cont; ND-DHEA; HF-Cont; HF-DHEA, p<0.01). DHEA efect on NO showed to be dependent on its convertion to more active steroids since presence of trilostane completely abolished the effect. The results show that DHEA supplementation may be benefitial in terms of ROS and NO leveles sugesting that the therapy may be beneficial for the treatment of hipoestrogenism.

0294 - EFFECT OF MATERNAL FRUCTOSE-RICH DIET (FRD) INTAKE ON THE ADULT OFFSPRING BROWNING POTENTIAL OF RETROPERITONEAL WHITE ADIPOSE TISSUE (RPWAT)

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IMBICE (1); CENEXA (2)

Beige adipocytes dissipate energy as heat through uncoupling protein-1 (UCP1) activity. Cold exposure or ß3-adrenergic agonist treatment stimulates white to beige adipocyte conversion. Although not completely matched, UCP1 expression levels are largely related to beige adipocyte thermogenesis. Our aim was to assess whether maternal FRD intake throughout pregnancy affects the development of browning capacity in RPWAT from the adult male offspring. On pregnancy day 1, dams were provided with either tap water (control) or FRD (10 % w/v; in tap water) and fed ad libitum with chow up to delivery. Weaned pups received water and chow ad libitum up to 60 days of age (experimental day). C and F indicate pups born to control and FRD dams, respectively. On day 53 of age, C and F pups were submitted to cold (4 ºC) for one week. Body weight and food intake was daily registered. On experimental day, trunk blood was collected and RPWAT pads were dissected and weighted. RPWAT mRNA expression levels of beige adipogenic markers were measured, and additional tissue was used for H&E staining, IHC and Western blot analysis. Data indicate that basal Pgc1a; and UCP1 expression were lower in F rats (p<0.05 for UCP1). Nevertheless, when submitted to cold F animals increased Pgc1a; and UCP1 expression above C cold animals (p<0.05). And the later increase well correlated with RPWAT H&E stain showing beige areas and UCP1 positive cells (by IHC). Increased UCP1 protein (WB) also correlated with enhanced UCP1 gene expression (p<0.05). In accordance, cold exposure induced in both groups an increase in brown AT (BAT) mass, while a decrease in body weight and in RPWAT mass (p<0.05) was noticed. Our data strongly suggest that decreased RPWAT browning capability could be involved in metabolic-endocrine dysfunction characterizing F adult male animals in basal conditions. However, cold exposure provokes exacerbated browning in F animals, an overreaction probably due to a protective compensatory mechanism.

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0552 - EFFECTS OF HIGH FAT DIET ON RENAL DOPAMINERGIC SYSTEM IN THE RAT: ITS IMPACT ON SODIUM EXCRETION AND BLOOD PRESSURE

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Renal dopaminergic system (RDS) promotes sodium excretion and anti-inflammatory actions. Kidneys are one of the main targets of metabolic syndrome, leading to intracellular lipid accumulation, tubular atrophy, interstitial fibrosis and glomerulosclerosis. However, little is known about the effects of a high fat diet (HFD) on RDS and its impact on hypertension development. Male Sprague-Dawley rats (body weight of 180-200 g) were randomized on two groups: control (C) and HFD during 4, 8 and 12 weeks; C: standard diet and tap water to drink, HFD: 50 % w/w fat added to control diet. From week 4, HFD vs. C (H&E stain) showed intracytoplasmatic inclusions in the tubular cortex cells; increased interstitial fibrosis (Picrus Sirius red); and foot processes effacement (transmission electron microscopy). FENa, UNa.V and diuresis/min decreased significantly comparing HFD vs. C (p<0.01 for each one), GFR showed no variation during the studied periods. Systolic blood pressure (SBP) (mmHg): HFD4: 137 ± 3 vs. C4: 120 ± 2, HFD8: 138 ± 2 vs. C8: 120 ± 2, HFD12: 147 ± 2 vs. C12 115 ± 3, (p<0.01). Metabolic results: triglyceridemia (mg/dl): HFD4: 132 ± 9 vs. C4: 60 ± 8, HFD8: 170 ± 9 vs. C8: 60 ± 6, HFD12: 168 ± 14 vs. C12: 58 ± 8, (p<0.01); insulinemia (ng/ml): HFD8: 4.2 ± 0.5 vs. C8: 1.2 ± 0.1, HFD12: 5.3 ± 0.3 vs. C12: 1.1±0.1, (p<0.01); glycemia increased from week 8 (p<0.05) and body weight from week 12 (p<0.01). Regarding RDS: urinary L-dopa (ng/day/kg): HFD4: 209.6 ± 62.5 vs. C4: 22.0 ± 0.9, HFD8: 519.8 ± 33.6 vs. C8: 27.2 ± 0.4, (p<0.01); urinary dopamine (ng/day/kg): HFD4: 353.0 ± 32.8 vs. C4: 7.8 ± 0.6, HFD8: 315.5 ± 60.4 vs. C8: 5.3 ± 1.6, HFD12: 207.7 ± 37.7 vs. C12: 48.7 ± 6.7, (p<0.05). In conclusion, HFD reduced urinary dopamine excretion while increased L-dopa excretion in association with intracytoplasmatic inclusions in tubular cells and modifications in glomerular ultrastructure. Structural alterations might lead to RDS dysfunction and subsequent development of hypertension induced by sodium retention in this experimental model.

0580 - SUPPLEMENTATION WITH DOCOSAHEXAENOIC ACID PLUS HYDROXYTYROSOL PREVENTS WHITE ADIPOSE TISSUE IMPAIRMENT BY IMPROVING MITOCHONDRIAL ACTIVITY AND THE EXPRESSION OF NRF2, NF-Kß, SREBP-1C AND PPAR-GAMMA IN MICE FED A HIGH-FAT DIET

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Overnutrition, lead to white adipose tissue (WAT) expansion, adipocyte hypertrophy, antioxidant defense depletion, inflammation, insulin resistance and dysregulated lipolysis. Docosahexaenoic acid (C22:6n-3, DHA) have beneficial effects on metabolic disorders linked with obesity. The hydroxytyrosol (HT), a polyphenol that provides antioxidant and anti-inflammatory protection, also impact positively on metabolism. Both bioactive compounds can modulate the activation of transcription factors and gene expression involved in lipid, antioxidant and inflammatory responses. Previously we found that HT improved the antioxidant system and inflammation of altered WAT in mice. Thus, the aim of our work was test if DHA+HT co-administration could avoid adiposity increase and WAT deterioration in a mouse model of high-fat diet (HFD)-induced obesity and the possible action mechanisms related. Male C57BL/6J mice received: control diet

(CD) (10 % fat), CD+DHA, CD+HT, CD+DHA+HT, high fat diet (HFD) (60 % fat), HFD+DHA, HFD+HT or HFD+DHA+HT for 12 weeks constituting 8 experimental groups (Doses: DHA, 50 mg/kg/day; HT, 5 mg/kg/day). In WAT we evaluate: oxidative stress damage; mRNA levels and antioxidant enzymes activities; citrate synthase, mitochondrial Complex I and II activities; binding activity and gene expression of transcription factors: nuclear factor erythroid 2-related factor 2 (Nrf2), nuclear factor-kß (NF-kß), sterol regulatory element-binding protein 1c (SREBP-1c) and peroxisome proliferator-activated receptor gamma (PPAR-gamma). The treatment with DHA+HT avoid the increase of adipose mass; maintained the levels of TBARS, F-2 isoprostanes and protein carbonyl compared to CD group; increased enzymatic activity of superoxide dismutase and catalase; preserved mitochondrial activity; up-regulated Nrf2 and PPAR-gamma; and down-regulated the NF-kß and SREBP-1c. Co-administration of DHA+HT prevent the development of obesity conserving mitochondrial function and preventing the dysregulation of WAT.

0641 - PROBIOTIC ADMINISTRATION TO DOG PETS WITH GASTROENTERITIC SYMPTOMS IN A CONTROLLED, RANDOMIZED, DOUBLE BLIND TRIAL

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There is a renewed interest in the design of formulas containing probiotic or beneficial microorganisms for the prevention of infections and animal welfare, exerting a physiological effect (ISAPP). The objective of this work was to determine the effect of the oral administration of four strains previously selected by their beneficial properties: Lactobacillus johnsonii CRL1693, L murinus CRL1695, L mucosae CRL1696 and L salivarius CRL1702 to dog pets (from 1-4 months old) with gastrointestinal symptoms (diarrhea, fever, vomiting). The randomized, double blind placebo-controlled trial was conducted at a veterinary center in Tucumán (June 2018-July 2019) in a Treated TG (n= 60) and Placebo PG (n= 60) groups. The probiotic was administered as freeze-dried bacteria (10^8 CFU) or excipients for 7 days by oral route. Race, weight, sex, type of feeding and previous sickness were registered, while clinical protocols were applied for diagnostic and treatment. Fecal stools were collected for microbiological and parasitological evaluation at days 0 and 8. No significant differences were observed between the two groups at the beginning of the trial. Pets' recovery was significantly different between the groups, having an excellent score (less than 3 days) in TG, while not as good in the PG (more than 7) (Fisher, $p<0.0001$). The stools consistency (by Bristol-adapted stools test) was harder in most of the TG after 7 days, but softer in PG (Fisher, $p= 0.024$). No significant differences were obtained in the number of cultivable mesophylls, lactic acid bacteria, enterobacteria and enterococci, or parasitological evaluation after the treatments. The dead animals were 3 and 4 in the TG and PG, respectively. The pets died after 6 days in the TG, but at days 2-4 in the PG. The results indicate that probiotic administration exerts a positive effect on the recovery of pets, and can be used as adjuncts for diarrhea treatment, supported by the stopped symptoms and high recovery levels.

0685 - ARTERIAL HIPERTENSION INDUCED BY SALINE OVERLOAD: ROLE OF CHLORIDE ANION

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A chronic saline (as sodium chloride) overload (SO) in the diet induces a renal inflammatory response and oxidative stress, which lead to the development of hypertension. The aim of this work was to demonstrate the hypothesis that chloride anion (Cl^-), besides sodium cation (Na^+), is involved in these inflammatory and oxidative responses. These alterations might be diminished if Cl^- is replaced by other anion (like citrate), or if Na^+ is replaced by other cations. Male Wistar rats were randomly divided into four experimental groups (n= 8): control (C); SO (NaCl 8% W/W); high Na^+ without Cl^- (Na: $Na_3C_6H_5O_7$ 11,8%); high Cl^- without Na^+ (Cl: $CaCl_2$ 3.80 %; KCl 3.06 % and $MgCl_2$ 1.30 %). After three weeks, systolic blood pressure (SBP) was measured, and rats were housed in metabolic cages in order to collect 24-hour urine to assess renal function. Oxidative stress parameters were measured in renal cortex: TBARS production andantioxidant enzymes activities and expression: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). In all the experimental groups we observed a significant increase of diuresis (*$p<0.05$ vs. C) while SBP was increased only in those rats fed with Cl^- (mmHg, C: 125 ± 9; NaCl: 164 ± 8*; Na: 133±4; Cl: 152±7*). These changes were accompanied by an increase in TBARS production in renal cortex (mol TBARS/mg protein) ($x10^{12}$): C: 1.30 ± 0.10; NaCl: 1.82 ± 0.18*; Na: 2.01 ± 0.32*; Cl: 1.91 ± 0.34*. No changes were observed on the activity or expression of SOD and CAT. Despite the fact that GPx expression was unaltered, the enzyme activity was increased in those groups with Cl^- (µmol GSSG/mg protein .min, C: 1.34 ± 0.14; NaCl: 2.31 ± 0.37*; Na: 1.30 ± 0.14; Cl: 2.77 ± 0.52*). These results suggest a relevant role of Cl^- in the development of hypertension, independently on Na^+. Saline overload, and both ions separately, induced lipid oxidative damage. Nevertheless, only Cl^- salt diets produced an increase in GPx activity, resulting in a high prooxidant state in kidney.

0818 - PARTICIPATION OF GUT MICROBIOTA ON THE METABOLIC CHANGES INDUCED BY HIGH FAT DIET

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Environmental factors, such as a fat-enriched diet are among the causes of the great prevalence of obesity and type 2 diabetes in the population. Recent studies have shown that diet-induced alterations the gut microbiota composition play a pivotal role in the development of obesity. Changes in the predominant gut bacterial phylums: Bacteroidetes and Firmicutes (B-F) characterizes different metabolic phenotypes. Our objective is to study in a high fat diet (HFD) feeding model of obesity if the treatment with probiotics induces changes in glycidic metabolism and B-F DNA. Four week-old male C57B6/6J mice were fed with a normal chow diet (fat content: 7.5 g/100 g) or an HFD diet (fat content: 31 g/100 g, butter and lard). When HFD mice reached a 5 % weight gain with respect to the controls ($p= 0.07$, n= 12) and a cumulative increase in food intake of 19 % kcal (week 16), probiotic treatment was started. We used two type of probiotics (P1 and P2) in two concentrations: 10^7 CFU and 10^8 CFU, supplied in drinking water. Genomic DNA of gut microbiota was isolated from feces samples and were analyzed by real time PCR reactions using selective primers. HFD induced an increment in basal (Gb) and after 2 hours of glucose administration (G2h) glycemia. Lower dose probiotic treatment did not produce changes in those parameters, however the higher dose induced an improvement in Gb and G2h (ANOVA Gb $p= 0.0204$, G2h $p= 0.022$, n= 4) being P2 most effective. No changes were observed in body weight and food intake. Concerning gut microbiota, we observed a non-significant increase in the Bacteroidetes DNA under treatment

with HFD and P1 (interaction diet probiotic p= 0.06, n= 4). We conclude that probiotic treatment improved metabolic parameters that were altered during HFD treatment. These data suggest the importance of gut microbiota as a therapeutic target in the treatment of obesity complications.

Bioinformática, genoma, proteoma y nuevas tecnologías / Bioinformatic II

Chairs: David Brudke/ Alberto Penas Steinhardt

0117 - CYTOTOXICITY OF METHYL VANILLATE AND METHYL DIVANILLATE IN BREAST CANCER CELLS

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UNIVERSITY OF SÃO PAULO, BAURU SCHOOL OF DENTISTRY (1); SÃO PAULO STATE UNIVERSITY "JULIO DE MESQUITA FILHO" - BAURU (2)

Breast cancer is the most common cancer among women worldwide, with over 1.3 million new cases per year resulting in about half a million deaths. Current treatment strategies are based on surgical removal of the tumor and/or radiotherapy followed by chemotherapy, which are usually associated to harmful side effects. Regarding this, there is a constant search for new selective and low toxicity drugs. Phytochemicals and their chemically modified derivatives are potential candidates in this scenario. Recently, some studies have evaluated the antioxidant properties of vanillic acid and its esters in which methyl vanillate has been found to have higher antioxidant activity than vanillic acid itself and vanillin. This effect was related to their higher lipophilicity and self-dimerization that occurs when they react with free radicals, as vanillin. Considering previous studies with vanillin and vanillic acid and the fact that there are no reports in the literature about the effects of methyl vanillate and its dimer methyl divanillate on human breast cancer cells, the aim of this work was to study the cytotoxic and antitumor effects of these compounds on MCF-7 and MDA-MB-231 cancer cell line, estrogen dependent and triple negative, respectively. For cytotoxicity assays, MTT reduction viability assay, flow cytometry cell apoptosis, and Hematoxylin/Eosin and DAPI/Phalloidin stains were performed. The MTT reduction assay showed that divanillate was 15-fold more cytotoxic than vanillate for MCF-7 and 9-fold higher for MDA-MB-231 cell lines ($p<0.05$). The cells incubated with the average of IC50 and IC25 values were stained and showed lower cell damage for IC25. This concentration was chosen for the apoptosis assay, which showed higher cytotoxicity for the MCF-7 than MDA-MB-231. In conclusion, divanillate presents higher cytotoxicity than vanillate and the MCF-7 strain is more sensitive to both compounds.

Financed by The São Paulo Research Foundation-FAPESP, n. 2017/26309-9.

0318 - GENETIC DIAGNOSIS OF CONGENITAL HYPOPITUITARISM BY MOLECULAR INVERSION PROBES SEQUENCING: NOVEL PATHOGENIC VARIANTS

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Congenital hypopituitarism (CH) is a life-long and threatening disease, associated with an abnormal pituitary development. CH is highly variable comprising a spectrum of disorders that range from isolated growth hormone deficiency (IGHD) to combined pituitary hormone deficiency (CPHD). Mutations in at least 30 genes have been implicated in CH, but at present, precise diagnosis remains a challenge. In the present study, we report variants found in pediatric patients with CPHD (n= 116) or IGHD (n= 55) from Argentina using the molecular inversion probes sequencing (MIPS) method and our own custom designed gene panel. We identified pathogenic, likely pathogenic or variants with uncertain significance but predicated to be damaging for at least 3 independent software in about 23 % of the cases. We have identified a number of phenotypes associated with mutations in known genes that cause hypopituitarism (HESX1, LHX3, LHX4, GLI2); in less frequently reported genes (BMP4, FGFR1, GLI3, TGIF1, FOXA2) and in genes that require additional evidence about causality (ARNT2, ZSWIM6, GPR161, PNPLA6, CDH2). We have identified de novo heterozygous variants in LHX3 and LHX4, transcription factors involved in the development of the pituitary. Two variants on LHX3 (p.L220S and p.P187S) were found in a patient with IGHD and a patient with CPHD, micrognathia, chiasm hypoplasia and bilateral cryptorchidism. LHX4 variants (p.Q100H, p.W204L and p.R84H) were found in a child with septo optic dysplasia, a child with CPHD and a third patient with GH and TSH deficiency, respectively. Transient transfection of HEK293T cells with human wild-type or mutant hLHX3/ hLHX4 showed an impairment in transcriptional reporter activity by the mutant variants, except for variant LHX4 p.R84H. Collectively, using the first screening panel for known genes and candidate genes for CH, we identified a significant number of variants in a large cohort of patients associated with the complex phenotype. Our studies will facilitate early diagnosis and prognosis, assessing the risk of future affected individuals. Furthermore, understanding the mechanisms behind new genes involved in CH would lead us to develop new tailor-made therapies that could benefit the patients.

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0591 - EXPRESSION OF RECOMBINANT FATTY ACID DESATURASE IN A BOVINE MAMMARY GLAND CELL LINE INDUCES CHANGES IN LIPID PROFILES

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INBIAS/CONICET. DEPARTAMENTO DE BIOLOGÍA MOLECULAR-UNIVERSIDAD NACIONAL DE RIO CUARTO (1); FRIEDRICH-LOEFFLER-INSTITUT, INSTITUT FÜR NUTZTIERGENETIK (2)

Vertebrates are unable to synthesize a subset of polyunsaturated fatty acids (PUFA) known as omega 3 and omega 6. The nematode C. elegans is able to synthesize them thanks to a family of lipid desaturases (delta desaturases, i.e., FAT2). Our hypothesis proposes that heterologous expression of a FAT2 in a bovine mammary gland cell line (MAC-T) will induce synthesis of PUFA. The aim of the present work was to transpose the C. elegans fat2 gene into the genome MAC-T and to study the resulting PUFA profile. Cotransfections of MAC-T with the Sleeping Beauty (SB) transposon system were performed. Two transposons, one carrying a cassette for the expression of a GFP, and a second one for expression of the FAT2 enzyme and neomycin resistance were used. For transfection

essays, 2:0.5:0.5 molar ratios of FAT2, GFP transposons and SB-helper plasmid were used. After cotransfection, MAC-T cells were subjected to antibiotic selection (G418). After 15 days, fluorescent and resistant colonies were isolated and expanded. PCR confirmed presence of the FAT2 sequence in five clonal cell lines. Two transgenic cell lines and one unmodified cell clone were grown to confluence in order to analyze the profile of the cellular phospholipid. Gas chromatography analysis of phospholipids confirmed the presence of linoleic acid (C18:2) in both transgenic cell lines, and the absence of linoleic acid in the unmodified cell line. The results indicate that recombinant FAT2 is functional, since it catalyzed the synthesis of linoleic acid, an omega-3 FA. Experiments are ongoing in order to confirm FAT2-mediated production of longer omega-3 and omega-6 lipids (C20 and C22), which are derived from C18:2. In conclusion, we successfully use the SB transposon system to generate stable transgenic bovine cell lines that express functional recombinant FAT2 enzyme. These results pave the way for the production of genetically engineered animals with improved PUFA profiles in tissues or milk for human consumption.

0622 - SCANNING ELECTRON MICROSCOPY ANALYSIS OF HAIR TREATED WITH PYLORIC CECAE EXTRACT FROM PACU (PIARACTUS MESOPOTAMICUS)

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Hair is composed mainly of keratin (90 %) is a fibrous and insoluble protein with high content of amino acid cysteine, responsible of disulfide bonds presence, which gives it high resistance to degradation. Hair structure consists of a medulla, cortex, and cuticle. This last cover the cortex, made of long filaments packed together (microfibrils). Currently, the industrial processing of animal sources generates significant volumes of highly polluting waste. The meat packaging and tannery industry, hairdressing salon, generate waste of keratinous nature. On the other hand, aquaculture discards considerable amounts of viscera, but these are considered an alternative source of enzymes with potentials industrial applications. Treatment of these protein-rich wastes is an attractive option that results in products with high added value. In the present study we evaluated the degradative capacity on hairs of a pyloric cecae extract from pacu (Piaractus mesopotamicus) under reducing and non-reducing conditions. The extract was prepared by mechanical digestion of pacu viscera in buffer pH 7.8, 1:5 g tissue/ml and proteolytic activity were assayed over Nα-Benzoyl-dl-arginine-p-nitroanilide as substrate. Hairs were pre-treated with buffer 7.8, 1 % 2-mercaptoethanol (2-ME), for 20 min at 100°C, then 5.0 U/ml pacu extract was added (1:5) and incubated for 1h, 3h, 24h and 7 days at 37°C. Hairs were observed at scanning electron microscopy (SEM). Other samples of the same hair were exposed in parallel under different treatments, such as the absence of reducing agent, heat or fish extract. SEM analysis showed that viscera extract, only in presence of 2-ME and heat, was able of degrading the hair cuticle, exposing the cortex microfibrils just at the first day. Results demonstrate that pyloric cecae pacu extract is able to degrade hair pre-treated with heat and reducing agent, so this treatment could be used in the recovery of hair keratins.

0663 - PROTEOMIC STUDY OF BREAST CANCER CELL LINE AFTER HEMEOXYGENASE-1 MODULATION BY HEMIN TREATMENT

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LABORATORIO DE BIOLOGÍA DEL CÁNCER - INIBIBB - UNS-CONICET. DPTO. DE BIOLOGÍA BIOQUÍMICA Y FARMACIA. (1); LABORATORIO DE BIOQUÍMICA DE LÍPIDOS POLIINSATURADOS – INIBIBB -UNS-CONICET DPTO. DE BBYF (2); DEPARTMENT OF MOLECULAR MEDICINE MAX PLANCK INSTITUTE OF BIOCHEMESTRY (MPI) (3)

Hemoxygenase-1 (HO-1) is a microsomal enzyme that catalyzes the degradation of the heme group and it can be translocated to multiple subcellular compartments. Our laboratory, among others, has shown that HO-1 regulates several processes related to cancer progression such as: proliferation, invasion migration, metastasis and the epithelial-mesenchymal transition. The aim of this work is to investigate the role of HO-1 in the proteome modulation in a breast cancer cell line. Protein extracts of LM3 cell line treated with hemin, a pharmacological HO-1 inducer (80 µM, 24 h), were obtained and studied by Western blot and Mass Spectrometry (MS). MS-data analysis showed 1,033 from 7,292 proteins were modulated after hemin treatment (ANOVA, $p < 0.05$). We observed that 595 proteins were increased, including HO-1 and 353 proteins were decreased in the group treated with hemin respect to their controls. Hemin treatment in LM3 cells induce lipid metabolism, heme and iron related protein expression. By thin layer cromatography, we observed an increase fraction of phosphatidyl serine, phosphatidylinositol, phosphatidylethanolamine and triglycerides after hemin treatment in LM3 cells, confirming the role of HO-1 in lipids metabolism. In addition, hemin treatment decreases the ribosomal RNA biogenesis and cytoskeleton and microtubules related proteins. These results show the multiple physiological effects of HO-1 in a breast cancer cell line.

0699 - METAGENOMIC ANALYSIS OF THE HUMAN GUT MICROBIOME IN INFLAMMATORY BOWEL DISEASE

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Gut microbiota is implicated in many human disorders. Ulcerative colitis (UC) is a chronic inflammatory bowel disease. Although the specific cause is unknown, some genetic and environmental factors have been defined. The purpose of this study was to investigate whether there were differences in gut microbiota in Argentine UC patients vs. non-UC controls. In this sense, 23 UC patients and 27 non-UC healthy controls matched by sex, age and BMI were included. DNA extraction was performed from 200 mg of feces using Power Fecal DNA Isolation Kit (Qiagen).The hypervariable regions V3-V4 of the bacterial 16S gene was sequenced using MiSeq-Illumina system. Sequences generated were analyzed using quantitative insights into microbial ecology (QIIME) version 1.9.1 software package. To compare taxa relative abundance between groups, we performed linear discriminant analysis (LDA) effect implemented in LEfSe. Alfa diversity did not differ between CU patients and non-CU controls. However, we found that CU patients differ from non-CU controls in the observed community structure. In CU patients, the dominant phyla were Bacteroidetes (43.49 ± 20.18 %), Firmicutes (48.94 ± 18.61 %), Proteobacteria (4.13 ± 7.12 %), Actinobacteria (2.12 ± 1.97 %) and Verrucomicrobia (0.38 ± 1.01 %) while the principal phyla found in Controls were Bacteroidetes (60.06 ± 13.54 %), Firmicutes (32.82 ± 13.51 %), Proteobacteria (4.27 ± 3.32 %), Verrucomicrobia (1.45 ± 3.18 %) and Actinobacteria (0.81 ± 1.46 %).The linear discriminant analysis (LDA) effect size (LEfSe) method revealed that the genus Bacteroides and Akkermansia were higher in non-CU control and Bifidobacterium, Eubacterium, Lactobacillus, Collinsella, Peptostreptococcus,

Actinomyces, Streptococcus, Slackia and Dialister in CU patient ($p<0.05$, LDA score >2).Our results demonstrated that there were differences in gut microbiota in UC patients. These findings could provide the bases for deeply understand cause-effect relationship between microbial communities and CU in Argentine population.

0760 - TRANSCRIPTOME ANALYSIS REVEALED DIFFERENTIAL EXPRESSION OF SPECIFIC LONG NON-CODING RNAS (LNCRNAS) IN PROSTATE CANCER.

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IQUIBICEN (UBA-CONICET). FACULTAD DE CIENCIAS EXACTAS Y NATURALES UBA.

The human genome contains about 20,000 protein-coding genes, representing 2% of the genome. Historically, the other 98% of the genome was referred as junk DNA. However, there is now robust evidence that 85% of the genome can be transcribed and that approximately half of disease- and trait-associated genomic regions are intergenic. These non-protein-coding regions are transcribed to non-coding RNAs (ncRNA), which are known to be key regulators of the transcriptional and translational repertoire of the cell. Moreover, there are solid proofs that alterations of these ncRNAs are highly associated with tumor development and progression. Prostate cancer (PCa) is the second most incident type of cancer in men and the fifth leading cause of cancer-related deaths worldwide. However, the biology of PCa is still mostly unknown. Therefore, we aimed to identify long non-coding RNAs (lncRNAs) differentially expressed after androgen deprivation therapy (ADT). We downloaded raw transcriptome data from public repositories. The datasets comprise paired samples from PCa patients before and after ADT. We analyzed differential lncRNA expression using the algorithms edgeR and DESeq2 from R/Bioconductor. The lncRNAs were considered differentially expressed when there was an agreement between both algorithms and FDR-adjusted p-value <0.05. We found 456 differentially expressed lncRNAs after ADT. We next looked into lncRNAS with $Log_2FC>|3|$ to further reduce the list and we identified 46 lncRNAs that met all three criteria. All of them are annotated in different genomic databases and 11/46 have been validated in different tumor samples including PCa, e.g.: PCAT18 (p= 1.5e-28, $Log_2FC= -5$), PCA3 (p= 2.9e-10, $Log_2FC= -5$) and PCGEM1 (p= 1.2e-5, $Log_2FC= -7$). Interestingly, all 46 lncRNAs were downregulated after ADT. This study revealed the lncRNAs modulated after ADT. These results warrant further investigation to give new insights in the biology of PCa and to identify potential therapeutic targets.

0817 - ANALYSIS OF PUBLIC DATABASES FOR THE DETECTION OF PCA BIOMARKERS USING BIOINFORMATIC TOOLS

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INSTITUTO DE BIOLOGÍA Y MEDICINA EXPERIMENTAL (IBYME)

Prostate cancer (PCa) is the most common type of cancer and is the third cause of death by cancer among men in Argentina. Since the implementation of prostate specific antigen (PSA) analysis, early detection of PCa have improved; however, the lack of its specificity demands the discovery of new specific biomarkers for PCa. The Cancer Genome Atlas (TCGA) is a cancer genomics program from the National Cancer Institute in conjunction with the Human Research Institute that has characterized more than 20,000 primary tumors which are available for public use. Our aim was to identify miRNAs in prostate tumors from patients that could be used as biomarkers for early PCa diagnosis using bioinformatic tools. We extracted TCGA patient's clinical data and miRNAs expression values and downloaded both databases using R and Rstudio. We generated a code to merge both databases and we created a table to compare clinical data with PCa miRNA expression profiles. We filtered the new database to compare miRNA expression with other variable of interest, such as tumor grade (Gleason score) and patient´s age. We found 10 miRNAs with very high expression on the TCGAs data set: hsa-miR-143, -21, -375, -148a, -miR-22, -10b, -30a, -182, -99b and -200c. In addition, in our laboratory, we detected a set of miRNAs that were overexpressed in the plasma of PCa patients compared to healthy donors using miRNA microarrays (Affymetrix). We checked the expression of these miRNAs on the TCGAs data set, and found that all of them had low expression on tumors regardless of Gleason score. Moreover, we analyzed patient survival rates in correlation with the miRNA expression using the online tool PROGmiR. Surprisingly, we found that high expression of hsa-miR-3613 was significantly associated to low survival rates on patients with low Gleason score. Altogether, we identified a miRNA set with high expression in prostate tumors that might be useful as candidate biomarkers for this disease.

0945 - PRELIMINARY ANKYLOSING SPONDYLITIS SALIVA PROTEOMIC ANALYSIS

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Saliva could be an informative fluid useful for diagnosis, prognosis and treatment surveillance of patients with oral and systemic diseases. Proteomics is a novel approach in searching for protein biomarkers. Candidate biomarkers identified through high-performance proteomic platforms have potential applications in diagnosis, prognosis, and therapy. Ankylosing spondylitis (AS) is an inflammatory disorder that leads to the bony fusion of vertebral joints causing chronic back pain. The long delay in diagnosis and the insufficient response to currently available therapies advocate for greater knowledge of the disease. The objective of this study was to investigate AS patient salivary proteome (SP) to identify proteins that could be used as biomarker. Saliva samples were collected from 60 patients ldiagnosed with AS and 58 controls patients with health status. Samples were analyzed by nanoHPLC coupled to a mass spectrometer with Orbitrap technology and analyzed by the Proteome Discoverer program. And the proteins preliminary analysis were performed using Uniprot and String. A total of 234 proteins were identified in both samples. Differences and similarities between both protein profiles were observed. The overexpression of haptoglobin and annexin-2 were observed in the pathological samples. Conclusion: the results of the present study contribute to the knowledge of the salivary proteomics in AS. These preliminary results could be a useful tool for developing clinical applications for systemic disease.

Biología celular y molecular de procesos fisiológicos y patológicos / Biology II

Chairs: Alejandra Erlejman | Ayelén Toro

0083 - MECHANISMS FOR PTTG/SECURIN PROTEIN ABUNDANCE IN PITUITARY TUMORS

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Proto-oncogene pituitary tumor transforming gene (PTTG) is a cell cycle regulator whose overexpression was correlated with malignant status and poor prognosis in many tumors. Securin PTTG upregulation is a hallmark of pituitary adenomas. We described that high PTTG protein levels are induced by the RWD-containing SUMOylation enhancer (RWDD3 or RSUME), a protein originally identified in the same pituitary tumor cell line in which PTTG was also cloned. In this work, we explore the mechanism of stabilization of PTTG focusing in a tight control of protein levels by post-translational modifications. YAPA, a RSUME mutant with loss of activity as SUMOylation enhancer, showed by Western blot (WB) a decreased action on the stability of PTTG. Accordingly, we found diminished protein levels of PTTG in the presence of Gam1 (a viral enzyme that inhibits SUMOylation), even in the presence of RSUME. By inmunoprecipitation (IP) assays in COS-7 we observed that PTTG is conjugated to SUMO-1 and RSUME enhances this SUMOylation. We validated PTTG SUMOylation at endogenous level in pituitary lactosomatotroph tumoral GH4 cells and HeLa cells, and in vitro SUMOylation experiments. RSUME knockdown with a specific small hairpin RNA (shRNA), has not effect on PTTG protein levels when the proteasome degradation is inhibited. By IP of ubiquitinated proteins we observed that both RSUME and SUMO-1 (with less potency) decrease ubiquitin conjugation to PTTG. RSUME knockdown has the opposite effect on PTTG ubiquitination. Gam1 restored PTTG ubiquitination levels by preventing SUMOylation, even in the presence of RSUME. We conclude that PTTG tight regulation and stabilization by SUMOylation, which reduces its degradation by means of the ubiquitin proteasome system, accounts for its abundance in tumoral cells.

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0087 - ADH5 forms a novel pathway that sustains cellular redox balance in cancer cells

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Fundamental metabolism provides energy and precursor molecules required for cell growth and development. Some biological processes such as histone and nucleic acid demethylations or the one carbon cycle can also generate surplus genotoxic metabolites such as endogenous formaldehyde (FA). This simple aldehyde avidly reacts with electron-rich groups, adducting proteins, nucleic acids and thiol-rich components like glutathione (GSH) and thus threatening cell integrity. Here, we show that cancer cells harbour specific systems to prevent cellular redox imbalance caused by FA. These systems are centred on the enzyme alcohol dehydrogenase 5 (ADH5) that converts FA into the less reactive molecule formate. Inactivation of ADH5 by CRISPR/Cas9 renders colorectalcarcinoma cells severely sensitive to FA showing early induction of apoptosis, DNA damage and oxidative stress. Mechanistically, FA triggers a phosphorylation cascade that leads to cell cycle arrest and cell death, which is prevented by the inactivation of the tumour suppressor P53. Overall, this work characterizes the response of cancer cells to FA and the role of ADH5 in preventing FA cytotoxicity, which might have wide implications for Fanconi Anemia and Ruijs–Aalfs syndrome patients, and for cancer development in carriers of BRCA2 mutations, all diseases whose onset is associated to endogenous FA.

0090 - MODULATION OF PH BY EGFR IN CYSTIC FIBROSIS CELLS

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Cystic fibrosis (CF) is an autosomal recessive disease characterized by mutations in the CFTR (cystic fibrosis transmembrane conductance regulator) gene. The pulmonary parenchyma is affected severely by a vicious cycle of persistent inflammation and infections. Several hypotheses tried to explain the high susceptibility to lung infections in CF patients, including a reduction in the airway surface liquid (ASL) pH. A reduced bicarbonate transport through CFTR and an increased lactic acid secretion could explain the changes in extracellular pH. The aim of the present work was to study if the EGFR pathway is involved in the pH regulation in CF cells. We use two cellular models: IB3-1 cells (bronchial epithelial cells derived from a CF patient with a deltaF508/W1282X CFTR genotype) and C38 cells (IB3-1 cells transduced with an AAV vector to stably express a functional truncated CFTR version). The results obtained suggested that CFTR modulates significantly ($p<0.05$) the pH, lactate secretion and LDH expression and activity in bronchial epithelial cells. IB3-1 cells reported a decreased in pH in extracellular medium culture ($p<0.05$), and an increased in lactate secretion, LDH expression and activity ($p<0.05$) compared with C38 cells. Studying the possible mechanisms involved in this regulation, we observed that EGFR modulates significantly ($p<0.05$) the pH in the extracellular medium, the lactic acid secretion ($p<0.05$) and the LDH expression and activity ($p<0.05$). In conclusion, the CFTR channel activity regulates not only the pH, but also the lactic acid secretion and LDH expression and activity. The EGFR pathway is partially involved in this regulation. This acidic phenotype presented in CF cells could favor the establishment of infections characteristic of CF.

Acknowledgements: ANPCYT, UCA and CONICET.

0104 - RSUME AND VHL ASSOCIATION IN VHL RENAL CELL CARCINOMA TUMORS PHENOTYPE

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RSUME or RWDD3 is a hypoxia inducible protein isolated from highly tumorigenic and angiogenic cells, which targets Von Hippel-Lindau (VHL) protein. Renal Cell Carcinoma (RCC) tumors, are the main common cause of death in VHL patients. Survival analysis available at The Human Protein Atlas with a recent dataset of Cancer Genome Atlas Research Network (TCGA) shows that 20.07 % of the 528 RCC tumors samples studied express elevated levels of RSUME, which correlate with a 23 % decrease of patients' survival rate. Using the same bioinformatics' platform, we observed that patients carrying VHL mutations have increased levels of RSUME compared with those without VHL mutations ($p= 0.048$). RSUME expression levels were significantly higher in stage IV tumors than those in stages I-II and III ($p= 0.036$ and 0.021). Analyzing in more detail the levels of RSUME in stage IV, we observed increased levels of RSUME in patients carrying VHL mutations and specifically in those carrying VHL missense mutations compared with those patients without VHL mutations ($p= 0.012$ and $p= 0.027$, respectively). We validated these data in a RCC cell line in which we observed by a Luciferase assay of the RSUME promoter and by WB that, while VHL wild type transfection decreases RSUME expression levels, VHL missense mutants transfection do not. Moreover, in cells cultured under HPX (1 % O_2) and next exposed to NMX, we observed by WB that when VHL wild

type was transfected RSUME protein levels were lower at 0, 5 and 30 minutes compared with VHL missense mutants transfection. Data about RSUME expression were validated in paraffin samples of RCC patients by immunohistochemical staining. A first metabolomics analysis in the RCC cell line in which RSUME is silenced show a differential pattern in acetylcarnitine levels. These in silico and experimental results strongly support the association of VHL mutants and RSUME in the phenotype of RCC tumors.
Supported by ANPCyT, CONICET, UBA and FOCEM (COF 03/11).
Keywords: VHL, RSUME, RCC.

0114 - INTRACELLULAR ACTION OF IL-6 IN PITUITARY TUMOR SENESCENCE

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Pituitary adenomas are frequent, benign, non-metastatic and monoclonal tumors that have premature proliferative arrest. Senescence restrains proliferation, but allows the cell to remain viable and perform its physiological function. Oncogene induced senescence (OIS) represents a protective mechanism against potential oncogenic risks that can explain the benign nature of these tumors. It has been reported the role of interleukin 6 (IL-6) in the induction and maintenance of the OIS. In previous work we demonstrated that IL-6 contributes to maintain senescence by its autocrine action in a pituitary tumoral model. In order to determine the mechanisms that mediate the endogenous action of this cytokine we developed knock out (KO) clones with the CRISPR/Cas9 technology of IL-6 in the somatotrophic cell line MtT/S in which we proved the absence of protein expression through an ELISA assay in six clones. Two of these KO clones injected into NOD/SCID mice (four mice of each group) generated tumors 10 days after injection, unlike wild type (WT) cells that did not show tumor formation in vivo. We also observed that these KO clones had decreased senescence markers, p16INK4 and pRb. Contrary to WT cells in which inhibition of the secretion pathway with Brefeldin A (BFA) 100 ng/ml generated an increase in senescence markers and the activity of senescence-associated ß-galactosidase (SA-ß-Gal) ($p<0.05$), KO clones treated with BFA did not show any significant changes. From these results we can conclude that the intracellular synthetized IL-6 mediates the senescent actions in pituitary tumor cells.
Supported by ANPCyT, CONICET, UBA, FOCEM (COF 03/11) grants.

0116 - INCREASED RESISTANCE OF MC3T3-E1 CELLS DIFFERENTIATED OR NOT COMPARED TO UMR-106 IN RESPONSE TO CAFFEIC ACID AND CAFFEIC ACID PHENETHYL ESTER (CAPE)

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Osteosarcoma is the most common primary malignant bone tumor that represents the eighth most common cancer group in the world. In vitro studies use the UMR-106, murine osteosarcoma osteoblast cell, as a study model, however the used murine pre-osteoblast control cell line MC3T3-E1 is more sensitive than a completely differentiated one. Thus, to perform the assays, the MC3T3-E1 cell line was differentiated into osteoblast with osteogenic medium for 96 hours. Currently, the development of new therapeutic strategies for cancer are being sought. In this way, Caffeic acid and caffeic acid phenethyl ester (CAPE) are naturally found and reported for their antioxidant properties, protecting the cell membrane in various cancers. According to the effects of the osteogenic medium on MC3T3-E1 cells we assume that the differentiated cell is more resistant than the undifferentiated one and less sensitive to the effects of Caffeic Acid and CAPE when compared to the UMR-106. To investigate whether the osteogenic medium would increase MC3T3-E1 cell resistance compared to UMR-106 tumor cell, against the effect of the Caffeic acid and CAPE through its cytotoxic and apoptotic action. Cell differentiation of MC3T3-E1 strain with osteogenic medium was performed and then cell viability assays were performed through the MTT and apoptosis assayed by flow cytometry. As for the statistical analyzes the viability results showed that the resistance of MC3T3-E1 cells increased with their differentiation and were more resistant to the compounds than the UMR-106 cell. CAPE IC50 was 10 times lower than Caffeic acid IC50 for both cells ($p<0.05$). The results showed that once differentiated, the MC3T3-E1 cells are more resistant to the compounds than the undifferentiated ones, and that the compounds are more specific for UMR-106 cells, therefore promising for studies of cancer treatment.
Project financed by the São Paulo Research Foundation – FAPESP, n. 2018/10321-2 grant.

0118 - EVALUATION OF MYRCIA BELLA IN MURINE OSTEOSARCOMA CELLS: EFFECT OF THE CRUDE EXTRACT AND FRACTIONS OF ELLAGITANNINS AND FLAVONOIDS

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Medicinal plants have been widely used in traditional communities as home remedies, they are considered raw material for the manufacture of herbal and other medicines. Among the prominent medicinal species is Myrcia bella Cambess. Myrcia bella has shown promising potential against various pathologies, requiring an understanding of their action and optimal concentrations of use. This study aims to evaluate the cytotoxic and antitumor effects of crude extract (EB) and elagitanine fractions (ELT) and flavonoids (FV) on the UMR-106 osteosarcoma tumor and MC3T3-E1 control line. The evaluation of cell viability was assessed by mitochondrial activity analysis, by reducing MTT and cellular DNA damage by Crystal Violet colorimetric assay. In addition to the viability assays, UMR-106 cell migration inhibition capability was performed by the wound healing assay in cellular monolayer. The analysis of the MTT reduction showed that in the UMR-106 cell line there was a decrease in viability already in the 24 and 48 h periods for the higher concentrations of compounds when compared to the control. In the 72 h period there was an even more significant decrease in cell viability, when compared with the MC3T3-E1 control cell line, the decrease in viability is greater for the tumor strain, presenting statistical differences ($p<0.05$). To the Crystal Violet, both cell lines suffer increased DNA damage at higher concentrations of the compounds in the 48 h and 72 h periods, as well as a reduction in MTT. In the cell migration assay, EB and FV fraction showed higher efficacy, inhibiting the migratory process, which could be attributed to FV fraction. Until the present moment, both EB and fractions are more cytotoxic to UMR-106 tumor cell line than MC3T3-E1. In addition, the FV fraction was more efficient because it presented better results in lower concentrations.
Project financed by São Paulo Research Foundation, FAPESP – n. 2017/26261-6 grant.

0127 - SEASONAL DYNAMICS OF APIS MELLIFERA FILAMENTOUS VIRUS (AMFV),

BLACK QUEEN CELL VIRUS (BQCV), CHRONIC BEE PARALYSIS VIRUS (CBPV) AND DEFORMED WING VIRUS (DWV) IN APIS MELLIFERA COLONIES INFESTED BY VARROA DESTRUCTOR

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Current studies in Apis mellifera colonies of Argentina have reported the presence of the most relevant +ssRNA viruses of bees, such as DWV, BQCV and CBPV; and of the filamentous virus of dsDNA AmFV. Even so, studies that evaluate the role of the ectoparasitic mite Varroa destructor in the seasonal dynamics of these viruses in Argentina are scarce, hence the objective of the present study. Approximately 50 workers were sampled from 4 hives each, naturally infested by the mite, from the experimental apiary of Finca Santa Paula; and were stored at -80 °C. Sampling was done once for each of the seasons of the year, from autumn 2016 to summer 2016-2017. Total RNA and DNA were extracted from pools of 10 worker bees, randomly selected from each sample. DNA was isolated using Roche Diagnostics DNA extraction kit and then qPCR reactions were performed for AmFV detection. RNA was extracted using Trizol® reagent, digested with DNAse and then RT-qPCR reactions were performed for DWV, BQCV and CBPV detection. Bee's ß-actin qPCR was performed as an internal control to check the quality of the material from each sample, and to calculate the relative expression (ER) of each virus using the equation 2^(-DDCt). Statistical analyzes of the variables affecting the ER of each virus were carried out through the R statistical program using generalized linear mixed models (GLMM). Detections were positive in all samples except for CBPV from two samples of autumn. The seasonal dynamics of the 4 viruses analyzed coincided with the historical data records for these viruses in various parts of the world. The ER of the DWV was found to be significantly higher in autumn, consequently leading colonies to a greater vulnerability to Varroa and other pathogens, and to death in the short term. Future studies on a larger scale are necessary to elucidate the existing correlations between the seasonal dynamics of the A. mellifera/V. destructor parasitic system and these associated viruses.

0133 - IN VITRO ANTI-NEUROBLASTOMA ACTIVITY OF POLYPHENOLS FROM ANDEAN POTATO

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Andean potatoes (Solanum tuberosum L. ssp. andigena) are a good source of dietary polyphenols, such as chlorogenic acid and anthocyanins. We have previously demonstrated that polyphenol extracts from Andean potato tubers exerted a concentration-dependent cytotoxic effect in human neuroblastoma SH-SY5Y cells. The aim of this study was to investigate the mechanisms involved in the cytotoxic activity induced by flesh tuber´s polyphenols from Andean potato cultivar Santa María. In order to test the anti-neuroblastoma mechanisms, we assayed the CC50 (50% cytotoxic concentration) of total polyphenols extract on SH-SY5Y cells. First, we observed that polyphenols induced changes in the cell morphology, which acquired a rounded shape with a decrease in the neurites extent. After 24 h of treatment with polyphenols, apoptosis was found in 57.8 % of the cell population, being the 30 % of them positive for propidium iodide, which indicated impairment in the integrity of the plasmatic membrane. We also analyzed the effect of polyphenols on the cell cycle distribution using flow cytometry. We detected increase of cells in G2 phase after 24 h of polyphenols treatment, but a major change in the cell cycle was found at longer period of incubation. The results showed an increase of 14.5 % in both G2 and S phases, while decreased the number of cells in G1 phase. In addition, an increase of 4.08 % of cells in sub-G1 phase was detected after the polyphenols treatment, which is typical to happen when the apoptosis takes place. Finally, we performed the cell nuclei staining with DAPI in order to check the integrity of the genomic DNA, observing significant alterations such as bright nuclear condensation and in some cases fragmented nucleus. These findings demonstrated that polyphenols from flesh tuber´s Andean potato cultivar Santa María would be a good source of bioactive compounds with some impact in human health.

0136 - ROLE OF ANTIDEPRESSANTS ON FKBP51 SUMO-CONJUGATION AND ACTIVITY AS A GR CO-CHAPERONE

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FKBP51 is an Hsp90 co-chaperone that regulates the activity of the glucocorticoid receptor (GR) and is critical for the stress response. Abnormal FKBP51 function, and its impact on GR activity, has been widely associated to stress-related diseases and to the response to antidepressant (AD) treatment. Our group has demonstrated the key role of FKBP51 SUMO conjugation in the regulation of Hsp90-mediated inhibitory effect on GR activity. Taking this into consideration, we propose to study the role of antidepressants on FKBP51 SUMOylation and its impact on GR activity. We treated HEK293T cell with different antidepressants and performed Ni-NTA purification in order to enrich the cell extracts with SUMOylated proteins. We observed that ADs, and particularly Clomipramine (CLM), inhibit FKBP51 SUMOylation both in cells and in vitro assays (50.5 ± 2.9 %; $p<0.001$). Knowing that SUMOylation is key for FKBP51 interaction with the GR complex and its inhibitory effect on GR activity, we performed co-IPs and reporter assays. We observed that ADs inhibit FKBP51 interaction with Hp90 and GR both in HEK293T (66.7 ± 4.1 %; $p<0.0001$) and mice brain extracts (71.0 ± 3.2 %; $p<0.0001$) and decrease FKBP51 inhibitory effect on GR transcriptional activity (82.4 ± 2.5 %; $p<0.0001$). To deepen in the molecular mechanism of action we performed in vitro SUMOylation assays. We observed that CLM only decreased FKBP51 SUMOylation promoted by FKBP51 E3 ligase PIAS4 (71.2 ± 1.5 %; $p<0.0001$). Also, we studied if CLM could inhibit FKBP51 interaction with proteins that are key for its SUMOylation: the E2 Ubc9 and the E3 PIAS4 by co-IPs and in vitro pull-down assays. We observed that CLM inhibits FKBP51 interaction with PIAS4 but not with Ubc9 in both cellular context and in vitro assays (65.8 ± 3.2 %; $p<0.0001$). Our findings suggest that ADs inhibit FKBP51 SUMOylation. In particular CLM may be inhibiting PIAS4 E3 ligase activity on FKBP51 SUMO-conjugation. We also report that ADs inhibit FKBP51 SUMO-dependant interaction with Hsp90 and GR, providing a possible mechanism for restoring GR transcriptional activity.

Supported by ANPCyT, CONICET, UBA and FOCEM (COF 03/11) grants.

0148 - SNX10 AND PIKFYVE ARE REQUIRED FOR LYSOSOME FORMATION IN OSTEOCLASTS

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Osteoclast (OC) function require specialized intracellular trafficking for organelle homeostasis. Snx10, a PI3P binding protein, is a member of the sorting nexin family, which plays important roles in cargo sorting in the endosome pathway and localized in early endosomes. It was shown previously that the gene encoding sorting Snx10 is required for osteoclast morphogenesis and function as osteoclasts from humans and mice lacking Snx10 are dysfunctional. The present work aimed to study PIKfyve, another PI3P-binding kinase, which phosphorylates PI3P to PI(3,5)P2 to better understand the role by which Snx10 regulates vesicular transport. PI(3,5)P2 is required for endosome/lysosome maturation. Inhibition of PIKfyve causes endosome enlargement, while overexpression of Snx10 also induces accumulation of early endosomes. This suggests that both Snx10 and PIKfyve may be required for normal endosome/lysosome transition. Apilimod is a small molecule with specific nanomolar inhibitory activity on PIKfyve, but only if OC effector genes CLCN7, OSTM1 and Snx10 are expressed. This observation suggests that Apilimod's inhibitory effects may be mediated by endosome/lysosome disruption. Here we show that both Snx10 and PIKfyve co-localize to early endosomes in osteoclasts. Furthermore, we observed co-immunoprecipitation and co-localization of Snx10 and PIKfyve in osteoclasts and gastric zymogenic cells. Treatment with 10 nM Apilimod or genetic deletion of PIKfyve resulted in accumulation of early endosomes and the inhibition of osteoclast constitution, lysosome formation and secretion of TRAP from differentiated OC. Apilimod-specific inhibition of PIKfyve required Snx10 expression. These findings may provide novel therapeutic approaches to control bone loss by targeting osteoclast trafficking in pathologies that weaken bone structure.

0225 - EFFECT OF CALCIUM ON THE DISTRIBUTION OF TUBULIN AND SPECTRIN IN HUMAN ERYTHROCYTES

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INBIAS/CONICET. DEPARTAMENTO DE BIOLOGÍA MOLECULAR-UNIVERSIDAD NACIONAL DE RIO CUARTO

In previous work we demonstrated that membrane tubulin in erythrocytes of hypertensive patients is increased, PMCA is inhibited and associated with tubulin, which correlates with a decrease in erythrocyte deformability. On the other hand, tubulin associated with the membrane inhibits PMCA, which would cause an increase in intracellular calcium. Our goal is to determine the factors that cause the increase in tubulin in the erythrocyte membrane. At this time in our work we wonder what event occurs first in erythrocytes: 1- PMCA is inhibited by the increase in membrane tubulin, or 2- PMCA is inhibited by another factor and this causes the intracellular increase in calcium that causes the translocation of tubulin to the membrane. Given these hypotheses we decided to determine the effect of calcium (exogenous and endogenous) on the distribution of tubulin in erythrocytes. Our immunofluorescence and Western blot experiments reveal that tubulin translocates from the sedimentable structure to the membrane due to the increase in exogenous calcium or by inhibition of PMCA with caloxin. This effect was similar to that found by the addition of taxol (a microtubule stabilizer) in the absence of exogenous calcium, however, we now find that the simultaneous addition of exogenous calcium and taxol prevents translocation of the tubulin to the membrane observed with exogenous calcium only. This was expected because taxol activates PMCA which does not allow accumulation of intracellular calcium. On the other hand, in our laboratory we demonstrate, in vitro, that tubulin forms a complex with spectrin (major component of the erythrocyte cytoskeleton). If calcium affects the distribution of tubulin in erythrocytes, it may also affect the location of the spectrin. In this sense we determine the distribution of spectrin in erythrocytes treated with exogenous calcium. We found that exogenous calcium causes a less homogeneous redistribution of spectrin in 50 % of erythrocytes. This phenomenon does not change if taxol is added in addition to calcium. These data suggest that tubulin and spectrin, both components of the erythrocyte cytoskeleton, may change their cellular location by increasing intracellular calcium. The increase in calcium causes the translocation of tubulin to the membrane, while spectrin follows an independent redistribution of tubulin even though both proteins form an in vitro complex.

Endocrinología / Endocrinology I

Chairs: Susana Nowicki | Eleonora Sorianello

0046 - CURCUMIN INHIBITS LEYDIG CELL TUMOR GROWTH: IN VITRO AND IN VIVO STUDIES.

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Leydig cells (LC) comprise the steroidogenic population of the testes. Leydig cell tumors (LCT) are the most common non-germ cell gonadal tumor developing in the testicular interstitium. Symptoms include virilization in prepuberal boys and erectile disfunction, infertility and/or gynecomastia in adults. Herein, we investigated the in vitro and in vivo effect of curcumin, component of the traditional medicinal spice known as turmeric, on MA-10 tumoral LC. Given the vast evidence of this compound's therapeutic effects in a variety of pathological conditions, we hypothesized that curcumin reduces LCT growth. Cell proliferation, measured in vitro by the sulforhodamine B assay, was concentration-dependently inhibited by curcumin from 40 and 60 µM concentration after incubations of 24 and 48 h, respectively ($p<0.001$). Tripan blue assay showed a concomitant decrease in cell viability after a 24-h incubation period ($p<0.01$). For the in vivo assay, BALB/c x C57BL/6 mice were subcutaneously inoculated with MA-10 cells and, once the tumor reached a volume of 50 mm^3, intraperitoneal injections of 20 mg/kg curcumin or vehicle (10 % DMSO in corn oil) were administered every other day for 15 days. Results clearly indicate that tumoral volume is significantly reduced by a short-term treatment with curcumin ($p<0.01$). Curcumin-treated group elicited an increased doubling time of 5.37 days (CI: 4.303 to 7.150) compared to that of vehicle-treated control group (4.50 days, CI: 3.620 to 5.931). Tumoral weight also showed a tendency to decrease in curcumin-treated group. There were no significant differences between treatments in body, spleen, liver or testes weight. There were no changes in body weight between day 1 and 15 in neither of the groups. In conclusion, curcumin reduces LTC growth both in vitro and in vivo, being these the first evidences of its potential therapeutic action over LCT with no evidence of noxious effects on other organs.

0049 - VITAMIN D IN PREGNANT WOMEN

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Maternal vitamin D (25OHD) status is related to impaired glucose homeostasis, changes in lipid profile, increased maternal fetal morbidity and mortality, pregnancy hypertension, premature delivery, caesarean section and thyroid dysfunction. Our purpose was to study maternal blood vitamin D levels and its relationships with biomarkers and metabolic, inflammatory and hormonal indices in the pregnancy of the first trimester. We conducted a cross-sectional study of pregnant women (n= 48) in public health centers, until the 14th week. Anthropometric parameters, 25OHD, Thyrotrophin (TSH), blood glucose and basal insulin (Ins-b), total cholesterol (col-T), triglycerides (TG), uricemia (Uri), HOMA-IR, glycosylated hemoglobin and C-reactive protein (CRP) were evaluated. The hormones were measured by chemiluminescence (Architect PLUS iSYSTEM, ABBOTT) and serum biochemical parameters in Wiener Lab autoanalyzers. Vitamin D Status: Sufficiency (ES)> 30 ng / mL, Insufficiency (EI) between 21-29 ng/mL and Deficiency (ED) <20 ng/mL The age distribution and body mass index (BMI) was similar in ED, EI and ES (BMI: 36.6 % healthy index, 76 % > 25.0 overweight and obesity and 14.6 % thin). The BMI correlated positively with Ins-b (Spearman r: 0.469, p <0.05). The 25OHD levels were less than 30 ng/mL in 83.3 % (62.5 ED and 20.8% EI). Mean level of this vitamin in ED and EI were 14.1 and 25.1 ng/mL, respectively. No correlation was found between Ins-b and 25OHD levels. Uri and Col-T average levels were higher in ED compared to EI and ES, without significant differences. Seric TG was increased in ED respect EI and ES. We observed inverse relationship between Vit D and TSH. Thyrotrophin levels surpassed 4.0 IU/L in 13 % of ED. The results show vitamin D deficiency and thyroid dysfunction in a high proportion in this pregnant group. Both clinical situations are underdiagnosed problems in pregnancy with complications for the mother and the newborn.

0098 - THERAPEUTIC POTENTIAL OF CALCITRIOL, THE ACTIVE METABOLITE OF VITAMIN D, FOR TREATMENT OF LEYDG CELL TUMORS

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Leydig cell tumors (LCT) are rare neoplasms associated with endocrine dysfunctions in boys and adult men. Aromatase (CYP19) overexpression and excessive estrogen production sustain tumorigenesis. Although mostly benign, LCT can become malignant and unresponsive to chemo/radiotherapy, highlighting the need for new treatment options. Vitamin D (VD) deficiency is epidemiologically linked to cancer. Indeed, the potential therapeutic role of calcitriol (CAL, active form of VD) has been described for several tumor types, but not yet for LCT. Earlier, we detected VD receptor (VDR) in pediatric LCT. Also, we reported that CAL can diminish the proliferation of R2C Leydig cells, the best-known in vitro model for LCT, in which CYP19 is overexpressed. Herein, we studied the capacity of CAL to modulate StAR levels, as well as CYP19 expression and activity in R2C. Also, we evaluated the anti-tumor potential of CAL in vivo in murine LCT models. In vitro, CAL negatively modulated StAR and CYP19 expression, although it had no effect on CYP19 activity. In vivo, CAL treatment decreased (61,7 %, p<0.05) LCT volume, but there was no variation in plasma steroid levels vs untreated mice. Thus, based on our former in vitro findings in R2C showing that: a) CAL induces expression of histamine H4 receptor (H4R), currently considered a promising target for cancer treatment, b) H4R agonist VUF8430 (VUF) displays anti-proliferative and anti-steroidogenic activities, c) combined treatment with CAL+VUF attenuates CYP19 activity, we speculated that CAL+VUF treatment would boost the effectiveness of CAL as single agent in vivo. However, although VUF alone was effective at inhibiting (48.6 %, p<0.05) LCT growth, CAL+VUF treatment did not exhibit a synergistic effect. We have yet to determine if a different dose combination could result in a better outcome, nevertheless it can be concluded that both CAL and VUF are promising therapeutic alternatives for LCT treatment and deserve further research.

0180 - STEROIDOGENIC ENZYMES IN THE UTERUS OF RATS WITH POLYCYSTIC OVARY SYNDROME

María Virginia ACOSTA | Gisela Soledad BRACHO | Gabriela A. ALTAMIRANO | Enrique H. LUQUE | Laura KASS | Verónica Lis BOSQUIAZZO

INSTITUTO DE SALUD Y AMBIENTE DEL LITORAL (ISAL, UNL-CONICET),

Women with polycystic ovary syndrome (PCOS) have a higher rate of recurrent abortions, hyperplasia and endometrial adenocarcinoma. These effects could be associated with alterations in the tissue metabolism of steroid hormones. The aim of this study was to evaluate the uterine expression of steroidogenic genes and to investigate if these genes were regulated through androgen receptor (AR) in a rat PCOS model. Wistar rats were injected sc with sesame oil (control), dehydroepiandrosterone (DHEA) 6 mg/100 g bw (PCOS) or DHEA 6 mg/100 g + Flutamide (AR antagonist) 2 mg/100 g (PCOS+F) from 21 to 40 days of age. 24 hours later, blood and the uterine horns were obtained. No estrous cyclicity, hyperandrogenemia and similar estradiol serum levels were observed in PCOS and PCOS+F groups. mRNA transcripts for steroidogenic acute regulatory protein (STAR), 3a-hydroxysteroid dehydrogenase (HSD), 3b-HSD (isoforms 1, 2, 3, 5 and 7), 17b-HSD (isoforms 1, 2, 3 and 4), 5a-reductase type 1 (SRD5A1), aromatase (P450arom) and, steroid sulfatase (STS), were detected in the uterus of all experimental groups. In PCOS rats, an increase of the mRNA levels of 17b-HSD2 (converts estradiol into estrone), P450arom (converts androgens into estrone and estradiol) and SRD5A1 (converts testosterone into dihydrotestosterone) and a decrease of STAR (transport cholesterol into the mitochondria) was found compared to control rats. In PCOS+F animals, the same changes described in PCOS rats regarding SRD5A1, 17b-HSD2 and STAR enzymes were found. However, the increase in P450arom expression was not observed in PCOS+F. Present results suggest that P450arom expression is regulated by AR. The induction of 17b-HSD2 and P450arom expression in the PCOS rats, allow to propose that an increase in uterine estrogenic effects may be associated with the development of endometrial hyperplasia and/or cancer.

0197 - CORRELATION BETWEEN LXRS EXPRESSIONS AND LIPID PARAMETERS IN RATS FED WITH A HIGH FAT DIET

Mariana REY(1) | María Sol KRUSE(1) | Gabriela Egly FERESIN(2) | **Hector COIRINI** (1)

INSTITUTO DE BIOLOGÍA Y MEDICINA EXPERIMENTAL (IBYME-CONICET) (1); INSTITUTO DE BIOTECNOLOGÍA, FAC. DE ING. UNIVERSIDAD NACIONAL DE CUYO (2)

The liver X receptors (LXRs) are key factors in cholesterol metabolism and also participate in the metabolism of fatty acids, triglycerides (TG) and glucose, among other functions. There are two subtypes, LXRa and LXRb, which are equally functional. Previously, we described a link between increased plasma TG and hypothalamic LXRs expressions in glucose-intolerant rats, revealing that these receptors are probably sensitive to peripheral changes of lipids. In this work, adult male rats received normal chow diet (C) or high fat diet (HF; C + 38 % bovine fat + 2 % cholesterol + 0.2 % cholic acid). Total cholesterol (TC) and TG levels were measured in blood samples by using commercial kits; hypothalamic and hepatic LXRs expressions levels were determined by WB. These quantifications were performed every 2 weeks through an 8 weeks period. The TC levels in HF group were higher than C at all the weeks evaluated (42.80-93.70 %; p<0.05), but TG decreased toward the 8

weeks (75.30 %; p<0.05). At all the times evaluated, the LXRs expressions were increased in liver (LXRa: 11.70-42.24 % and LXRb: 29.00-100.60 %; p<0.05) and hypothalamus (LXRa: 8.92-29.67 % and LXRb: 25.50-49.52 %; p<0.05), but 2 weeks of HF diet did not modify the hypothalamic LXRa expression. The correlational studies considering all the weeks together revealed that TC and/or TG levels significantly correlated with both LXRs expression in liver (negatively to LXRa and positively to LXRb) and hypothalamus (both negative). The obtained results add relevant information about the role of LXRs on lipid homeostasis. Also, the hypothalamic LXRs results reinforce the idea that these receptors are sensitive to peripheral changes caused by dietary habits Supported by CONICET-PIP860, CONICET-PIP00243 and PIO-CONICET-022 grants.

0206 - HISTIDINE DECARBOXILASE INHIBITORS IN COMBINATION WITH CARBOPLATIN AS A NEW THERAPEUTIC OPTION FOR THE TREATMENT OF LEYDIG CELL TUMORS

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IBYME-CONICET (1); HOSPITAL DE PEDIATRÍA JUAN P. GARRAHAN - SERVICIO DE ENDOCRINOLOGÍA (2)

Testicular Leydig cell tumors (LCT) are endocrine tumors which lead to various clinical complications in boys and adults. To date, carboplatin (CP) and orchiectomy are considered the gold standard for LCT management. However, treatment-related infertility highlights the need for new therapeutic options. We have previously shown that histamine (HA), a biogenic amine synthetized by Histidine Decarboxylase (HDC), plays a role as autocrine growth factor in R2C and MA-10 Leydig tumor cells, the former characterized by aromatase (CYP19) overexpression. Given that both cell lines express high HDC levels as human pediatric LCT, herein we evaluated the efficacy of HDC inhibitors (a-MHD and EGCG, synthetic and natural, respectively) alone or in combination with CP, as anti-LCT agents. In vitro: R2C and MA-10 cells were treated with HDC inhibitors for 48 h and then subjected to [3H]-Thymidine incorporation to assess cell proliferation. C57 x BALB/c offspring (F1) and Swiss Nu/Nu mice were injected with MA-10 cells and R2C cells to generate allograft and xenograft LCT models, respectively. When LCT volume reached 40 mm^3, mice were treated every other day with a-MHD or EGCG, alone or combined with CP. a-MHD and EGCG decreased R2C and MA-10 cell proliferation in vitro (p<0.01). In F1 mice, a-MHD + CP was more effective than CP alone at reducing tumor growth (56 vs. 38 %, p<0.05), whereas EGCG had no effect. In Swiss Nu/Nu mice, EGCG was even more effective than a-MHD at enhancing CP anti-tumor potential (p<0.0001), possibly because R2C tumors rely on CYP19 overexpression for sustained tumor growth and EGCG can inhibit CYP19 expression as well as HDC activity. Conclusion: HDC inhibitors increase the anti-tumor effect of CP in murine LCT models and could be a promising therapeutic option in patients. EGCG is known to protect spermatogenesis against irradiation in vivo, underscoring the importance of our results in terms of fertility preservation.

0292 - PROGESTERONE AND ESTRADIOL MODULATE GONADOTROPIN-RELEASING HORMONE EFFECT OVER LH SURGE IN THE SOUTH AMERICAN PLAINS VIZCACHA, LAGOSTOMUS MAXIMUS.

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CEBBAD, UNIVERSIDAD MAIMÓNIDES (1); IBYME-CONICET (2)

During estral cycle, massive release of pituitary luteinizing hormone (LH) is required for the ovulatory event to occur. This is regulated by gonadotropin-releasing hormone (GnRH) and the ovarian hormones estradiol (E2) and progesterone (P4). Ovarian P4 enhances the positive feedback of E2 on GnRH and finally in the LH surge, suggesting that this hormone is also involved in the release of LH. Vizcachas have shown reproductive axis activity during gestation with release of ovarian and hypothalamic hormones and an increased LH surge at mid-pregnancy. The aim of this work was to determine the effects of E2 and P4 on GnRH receptor (GnRHR) and LH in the vizcacha. Ex vivo and in vivo approaches were developed: 1- Pituitaries of non-pregnant vizcachas were cultured under different conditions: a) Buffer, b) E2, c) GnRH, d) GnRH+E2; n= 4/group. 2- Non-pregnant females were ovariectomized (OVX) and treated with E2 (OVX+E2, 5 µg/kg); n= 4/group. 3- Pituitaries of non-pregnant females were probed in a pulsatil assay under different conditions: a) Buffer, b) P4, c) P4+RU486 (PR antagonist). LH release was measured by RIA, whereas pituitary GnRHR, ERalpha and PR expression was studied by immunohistochemistry and Western blot. A significant induction of LH release was determined in the pituitary cultures supplemented with GnRH and E2 (p<0.05). In addition, an increase in GnRHR expression was determined (p<0.05). On the other hand, significant increase of LH release was induced by P4 (p<0.05). Significant increment in the number of cells expressing PR was observed in OVX+E2 related to OVX (p<0.05). These results suggest that both ovarian hormones would be involved in the modulation of GnRH effect over LH release.
Supported by Fundación Científica Felipe Fiorellino, PIP110/14 and PICT1281/2014 grants.

0349 - MAMMARY GLAND-SPECIFIC REGULATION OF GNRH AND GNRH-RECEPTOR GENE EXPRESSION IS LIKELY PART OF A LOCAL AUTOREGULATORY SYSTEM IN FEMALE VIZCACHAS (CHINCHILLIDAE: RODENTIA).

María Clara CORSO (1) | Sofia PROIETTO(1) | Santiago Andrés CORTASA(1) | Alejandro Raúl SCHMIDT(1) | Kevin FEEHAN(1) | Victoria FIDEL(1) | Ruth CWIRENBAUM(1) | Pablo Ignacio Felipe INSERRA(1) | Marina Olga FERNANDEZ(2) | Alfredo Daniel VITULLO(1) | Verónica Berta DORFMAN(1) | Julia HALPERIN(1)

CEBBAD, UNIVERSIDAD MAIMÓNIDES (1); IBYME-CONICET (2)

Our laboratory has recently reported gonadotropin-releasing hormone (GnRH) protein expression in mammary gland (MG) epithelial cells of vizcachas during pregnancy and lactation. In addition, we also shown that prolactin (PRL) modulates GnRH MG content. The present work aims to study GnRH gene expression in MG and also, to explore a possible GnRH-autocrine mechanism over this tissue. We amplified GnRH, GnRH-receptor (GnRH-R), early growth response factor 1 (Egr-1), PRL, PRL-receptor (PRL-R) and α-lactalbumin by RT-PCR using mRNA from MG at different stages of pregnancy and lactation. We established local transcription of GnRH at all the analyzed stages and the amplicon sequencing confirmed GnRH identity. Maximum transcription of GnRH occurred at early pregnancy and this coincides with maximum transcription of GnRH-R (p<0.05, n= 5). Egr-1 showed a similar pattern although slighted shifted, i.e., its maximum was recorded at mid-pregnancy (p<0.05). To asses GnRH effect on its own expression, on that of GnRH-R and that of Egr-1 as well as over PRL signaling pathway, MG explants supplemented with or without a GnRH analogue were cultured for 6 and 24 h. According to the RIA, the GnRH released to the culture medium by the GnRH-treated explants was higher than that of controls, both after 6 and 24 h incubation (p<0.05, n= 6). GnRH and Egr-1 mRNA expressions were significantly higher in GnRH-treated explants vs. control explants (p<0.05). No differences were found in transcription levels of, PRL-R or α-lactalbumin between explants groups. In summary, the expression pattern of GnRH, GnRH-R and its target gene, Egr1, throughout pregnancy suggest that these gene transcriptions

would be coupled. GnRH would not directly affect the PRL signaling pathway. Yet, GnRH is able to modulate its own signaling pathway and induce its own local release in a positive autocrine-paracrine regulation. From these results, we propose an active role for GnRH in MG remodeling.

Supported by Fundación Científica Felipe Fiorellino, PIP110/14 and PICT1281/2014 grants.

0366 - AROMATASE (ARO) TRANSCRIPT VARIANTS AND ERRγ1 EXPRESSION IN HUMAN PLACENTAL (PL) TISSUES FROM PRETERM (PT) AND TERM DELIVERIES OF LARGE FOR GESTATIONAL AGE (LGA) NEWBORNS

Marcos PALLIGAS (1) | Cristina Patricia NEMER(2) | Claudia CANNIZZARO(2) | Maria Sonia BAQUEDANO(1) | Alicia BELGOROSKY(1) | Nora Isabel SARACO(1)

CONICET- SERVICIO DE ENDOCRINOLOGIA - HOSPITAL DE PEDIATRIA GARRAHAN (1); PROGRAMA DE DIAGNOSTICO Y TRATAMIENTO FETAL, HOSPITAL DE PEDIATRÍA "JP GARRAHAN" (2)

Aro is the key enzyme for estrogen biosynthesis and in PL is expressed exclusively in syncytiotrophoblast. In small and large newborns as well as in patients with Aro deficiency the prevalence of metabolic syndrome in adulthood tends to increases. Estrogen-related receptor (ERR)γ1 is an orphan nuclear receptor expresses in high levels in PL. ERRγ was found to act as an oxygen-responsive transcription factor that regulates Aro gene expression in PL. Our aim was to analyze ERRγ1 mRNA and Aro mRNA variants expression in PL from term LGA and PT (<35 weeks) compared to term adequate for gestational age (AGA) newborns. We propose that ERRγ1 expression in PL is involved in aromatase activity regulation and hence intrauterine estrogen-androgen balance. Total RNA and proteins were isolated from PL of PT (GA: 30-35 weeks, n= 4), LGA (GA: 39-41 w, n= 8) and two subgroups of AGA: AGA1 (GA: 37-38 w, n= 8) and AGA2 (39-40 w, n= 10). ERRγ1 mRNA and Aro mRNA variants were analyzed by Real-time RT-PCR with primers for total (TotAro, Ex2-Ex3), and active (ActAro, Ex9-Ex10) Aro. Aro protein analyzed by Western blot (WB). TotAro mRNA was higher in PT vs. AGA1 (8.91 ± 3.35 vs. 1.74 ± 0.41 AU, mean ± SE), while was lower in LGA vs. AGA2 (0.81 ± 0.36 vs. 2.29 ± 0.68), p<0.05. Similar results were found in Aro protein. ActAro/TotAro ratio was higher in PT (2.26 ± 0.26 vs. AGA: 0.70 ± 0.23) and in LGA (2.42 ± 0.31 vs. AGA: 1.47 ± 0.28), p<0.05. ERRγ1 mRNA was higher in LGA vs. AGA2 (GA: 39-40w), 8.66 ± 2.57 vs. 2.84 ± 0.61 AU, mean ± SE, p<0.05. While not significant difference was found in ERRγ1 mRNA from PT compare to AGA1, the high Aro expression found in preterm placenta agrees with previous reports of increments in maternal estrogens in PT parturition, suggesting a role for placenta Aro in local estrogen production associated to prematurity. High expression of ERRγ1 as well as ActAro/TotAro ratio in PL from LGA, suggest that ERRγ1 is involved in the regulation of ActAro variant expression in PL from LGA newborns.

0400 - COLD-INDUCED INGUINAL AND EPIDIDYMAL ADIPOSE TISSUES RESPONSES IN THE HYPERCORTICOSTERINEMIC MALE RAT

Florencia Magalí MARTÍN | Eduardo SPINEDI | Andrés GIOVAMBATTISTA

IMBICE

It is known that glucocorticoids (GC) have different effects on white adipose tissue (WAT) and inhibit brown adipose tissue (BAT) thermogenesis; however, it remains unclear how GC affect the thermogenic process in different WAT depots. Our aim was to study, in a hypercorticosteronemic rat model, the thermogenic activity in subcutaneous inguinal and epididymal WAT pads (scWAT and eWAT, respectively). For this aim, male S-D rats neonatally-treated with monosodium L-glutamate (MSG) were used. A subgroup of prepubertal 30 day-old male CTR and MSG rats were exposed to cold (4 °C) during 7 days. MSG and litter-mate CTR scWAT and eWAT pads were dissected, weighed and processed for mRNA (qPCR) and protein (Western blot) analysis. Data were analyzed by Two-way ANOVA. Body weight gain was reduced by MSG treatment (p<0.05) and cold exposure (p<0.05). Food intake was decreased by MSG treatment (p<0.05) and enhanced by low temperature exposure (p<0.05). Regarding plasma metabolites levels, corticosterone (B) was augmented (p<0.05) and triglycerides were lowered (p<0.05) by cold. Despite the larger WAT mass in MSG, scWAT and eWAT masses were reduced after cold exposure (p<0.05). While normal mRNA levels of GC (GR) and mineralocorticoid (MR) receptors in eWAT from MSG rats were found, conversely, they diminished in scWAT (p<0.01). As expected, after cold exposure, expression of the thermogenic marker UCP1 was enhanced (p<0.05) at both mRNA and protein levels in eWAT and scWAT from CTR rats; however, at room temperature, MSG eWAT mRNA and protein UCP1 levels were lower (p<0.05) and the thermogenic response to cold was inhibited (p<0.05). Finally, high B plasma levels tended to enhance UCP1 expression in scWAT. Our results indicate a depot-dependent WAT thermogenic response in hypercorticosterinemic rats and that this characteristic could be due, at least in part, to differences in GR and MR local levels.

This work was supported by PICT 2015-2352 grant.

0594 - EFFECTS OF TESTOSTERONE ON NEUTROPHIL BEHAVIOR IN ATHEROGENIC CONDITIONS

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CENTRO DE MICROSCOPÍA ELECTRÓNICA. FACULTAD DE CIENCIAS MÉDICAS. UNIVERSIDAD NACIONAL DE CÓRDOBA. (1); INSTITUTO DE HEMATOLOGÍA Y HEMOTERAPIA. UNIVERSIDAD NACIONAL DE CÓRDOBA. (2); INICSA Y FACULTAD DE CIENCIAS MÉDICAS (CONICET-UNC) (3)

In addition to their well-studied role in acute infections, neutrophils have been pointed as contributors of several inflammation-induced injuries, particularly during the onset and progression of atherosclerosis. In spite of the diverse effects of androgens on the immune and cardiovascular systems, there are no reports about the influence of male hormones on neutrophil action in atherogenic conditions. Our aim was to examine the effects of testosterone on neutrophil behavior in basal and hyperlipidemia milieu. Human neutrophils were isolated from peripheral blood of healthy men using Polymorphprep™ and stimulated in vitro with testosterone (T, 10^{-7}M) or vehicle in the presence or absence of low-density lipoprotein (LDL, 100 µg/ml). The surface expresion of CD11b, CD16, CD11a, L-selectin and CXCR2 as well as the phagocytosis of FITC-labeled latex beads were evaluated by flow cytometry. Neutrophil adherence to endothelial adhesion molecules (P-selectin, ICAM1 and VCAM1) was assessed by functional static adhesion assay. Statistical analysis: Paired t test, p<0.05 (at least 3 independent protocols). Under basal conditions, T increased the adherence capacity (p<0.05) and the surface expression of molecules involved in leukocyte-endothelial cell interactions and neutrophil extravasation to sites of inflammation including CD11a (p<0.05) and CD11b (p<0.05) integrins, L-selectin (p<0.05) and CXCR2 chemokine receptor (p<0.05), indicating that T prepares neutrophils to migrate to injury site. LDL challenge increased CD11b expression (p<0.05), endothelial adhesion (p<0.01) and phagocytic capacity (p<0.01) and induced L-selectin shedding (p<0.05) and CXCR2 down-regulation (p<0.05), indicating neutrophil activation. However, T did not modify the neutrophil behavior induced by LDL. All together, although testosterone favored a more responsive neutrophil profile in basal conditions, it was not able to regulate the neutrophil response to LDL.

0655 - ROLE OF THE OLIGOSACCHARIDES OF FSH ON THE REGULATION OF AMH PROMOTOR IN PREPUBERTAL SERTOLI CELLS

Mariela URRUTIA (1) | Mariela URRUTIA(2) | Helena SCHTEINGART(1) | Stella CAMPO(3) | Rodolfo REY(1)

CENTRO DE INVESTIGACIONES ENDOCRINOLÓGICAS "DR. CÉSAR BERGADÁ" (CEDIE) - CONICET - FEI (1); CEDIE (2); 2HOSPITAL DE NIÑOS RICARDO GUTIÉRREZ, CEDIE - CONICET (3)

The relative proportion of different FSH glycosylation variants (FSH-GV) changes during sexual development. FSH regulates anti-Müllerian hormone (AMH) secretion by testicular Sertoli cells from fetal life until puberty. The objective of this work was to evaluate the role of FSH-GV in the regulation of the human AMH promoter in prepubertal Sertoli cells. To test this, human recombinant FSH (hrFSH) was separated by isoelectrofocusing to isolate FSH-GV with different degrees of sialylation: the acidic (AC) pH 3-4 and the basic (BA) pH 5-7 variants. Alternatively, hrFSH was separated by Concavalin-A chromatography to isolate FSH-GV with different degrees of oligosaccharide complexity: unbound (UB) and weakly bound (WB). The concentration of each FSH-GV was determined by electrochemiluminescence immunoassay. Reporter assays were performed in the prepubertal murine SMAT1 Sertoli cell line co-transfected with a hFSH receptor expression plasmid and a firefly luciferase-associated AMH promoter plasmid. Luciferase was measured after incubation with the whole hrFSH or the FSH-VG fractions (20 ng/ml) for 24 hours. Results, expressed as relative luciferase units (RLU, mean ± SEM), were compared using ANOVA followed by Friedman post-test. Basal AMH promoter activity (1.66 ± 0.16 RLU) increased after incubation with BA (3.15 ± 0.67 RLU, n= 3, p= 0,023) or WB (3.01 ± 0.38 RLU, n= 3, p= 0,044). The AC fraction, most sialylated, did not significantly modify the AMH promoter activity (2.35 ± 0.37 RLU, n= 3, p>0,05) in SMAT-1 cells. To conclude, our results suggest that the degree of sialylation and complexity of FSH oligosaccharides may be involved in the regulation of the AMH promoter, conditioning the AMH production, in Sertoli cell in the prepubertal stage.

Neurociencias / Neurosciences II

Chairs: Juan Beauquis | María Ángeles Vinuesa

0040 - IGF-1 GENE THERAPY REVERSES HIPPOCAMPAL ALTERATIONS AFTER SPINAL CORD INJURY

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INSTITUTO DE BIOLOGÍA Y MEDICINA EXPERIMENTAL (IBYME-CONICET) (1); INIBIOLP (2)

After spinal cord injury (SCI), patients exhibit cognitive deficits that could be related to hippocampal alterations. We have previously described hippocampal neuroinflammation, neurogenesis reduction and cognitive impairments in rats after chronic SCI. Since insulin-like growth factor 1 (IGF-1) enhances neurogenesis and IGF1 gene therapy modifies the inflammatory response and ameliorates cognitive impairments in other models, we decided to evaluate whether an adenoviral vector expressing IGF-1 could reverse hippocampal alterations and cognitive deficits observed in rats after SCI. Sixty days post-injury (dpi), rats were injected in the lateral ventricles with a recombinant adenoviral constructed harboring the cDNA of rat IGF-1 as a therapeutic virus 1 (RAd-IGF-1) or the cDNA of red fluorescent protein, as a control virus (RAd-DsRed) or saline solution. Neurogenesis and cognitive hippocampal dependent-tasks were evaluated 15 dpi. As expected, the number of neuroblasts (doublecortin + cells) decreased after chronic SCI (p<0.01, SCI vs. sham). After the treatment with RAd-IGF1, neurogenesis increased in lesioned rats (p<0.05, SCI+RAd-DsRed control vs. SCI+Rad-IGF-1). Regarding cognitive performance, recognition and spatial working memory were assayed 60 dpi using the novel object recognition and Y-maze test respectively. The discrimination index decreased 24 h after the familiarization phase in the lesioned group (p<0.001 SCI vs Sham), while IGF-1 treatment increased the mentioned index in lesioned rats (p<0.05, SCI+RAd-DsRed vs. SCI+RAd-IGF-1). The percentage of spontaneous alternations decreased in lesioned rats (p<0.01 SCI vs Sham) while injured rats treated with the adenoviral IGF-1 injection increased the percentage of spontaneous alternation (p<0.05, SCI+RAd-DsRed vs. SCI+RAd-IGF-1). These results support that therapies which enhance endogenous IGF-1 expression might be a possible treatment for the encephalopathy developed by SCI.

0100 - ADULT CELL PROLIFERATION IN THE DENTATE GYRUS: MATERNAL SEPARATION MATTERS? RESULTS FROM A RAT DEPRESSION MODEL

Franco MIR | Antonella POLLANO | Julieta AGUGGIA | María Angélica RIVAROLA | Marta Magdalena SUÁREZ

CÁTEDRA DE FISIOLOGÍA ANIMAL. FCEFYN - UNIVERSIDAD NACIONAL DE CÓRDOBA

Depression is one of the most common and debilitating mental disorders; however, its etiology remains unclear. Some possible pathophysiological mechanisms include altered neurotransmission, HPA axis abnormalities involved in chronic stress, reduced neuroplasticity, and network dysfunction. Neonatal maternal separation induces long-term alterations in the morphology of hippocampus and may increase vulnerability to depression when a stressful situation occurs in adulthood. Treatment with the antidepressant tianeptine may reverse the stress-induced alterations in neuroplasticity. The aim of our study was to investigate if the interaction between neonatal maternal separation and chronic unpredictable stress in adulthood can alter cell proliferation in the dentate gyrus. Also, the effect of the antidepressant tianeptine in the alterations induced by the present model was assessed. Wistar derived male rats were separated from their mother for 4.5 hr during the first 3 weeks of life. From day postnatal 50, were exposed to an unpredictable chronic stress paradigm (depression model) during 24 days and were daily treated with tianeptine (10 mg/kg, i.p.) or vehicle. Cell proliferation was evaluated by bromodeoxyuridine (BrdU) immunohistochemistry in the subgranular stratum of dentate gyrus. Maternally-separated rats showed a decrease in cell proliferation (p<0.05) compared to non-maternally separated rats. Neither chronic stress nor tianeptine administration had effect in cell proliferation. This work contributes to the literature regarding neuroplasticity programming as a consequence of an early emotional stress.

0115 - RESVERATROL DOWNREGULATES LIPOPOLYSACCHARIDE-INDUCED MICROGLIAL ACTIVATION

Anibal STERN | **Ana Julia TICCHI** | Ana Maria FRANCHI | Fernando CORREA

CENTRO DE ESTUDIOS FARMACOLÓGICOS Y BOTÁNICOS (CEFYBO-CONICET), FACULTAD DE MEDICINA, UBA

Microglial cells play an important role in the central nervous system (CNS) innate immune response. Excessive activation of these cells is considered to be detrimental and has been implicated in many neurodegenerative and neuropsychiatric disorders. Therefore, a lot of efforts are being made in searching for compounds with good safety profile that could modulate microglial function. Reveratrol, a naturally occurring stilbene with anti-inflammatory, antioxidant and epigenetic properties, showed neuroprotective effects in several models of CNS disorders. However, little is known regarding the molecular mechanisms involved in resveratrol neuroprotective effects. Therefore, our aimed was to explore whether the MAPK and NF-kB signaling

pathways were involved in the modulatory effects of resveratrol on microglial BV2 cell line response to lipopolysaccharide (LPS). Cell cultures of the murine microglial cell line BV2 were expose to LPS and/or resveratrol and at different time-points supernatants, total mRNA or proteins were collected. We first evaluated the potential cytotoxicity of resveratrol with an MTT assay. We observed that only at concentration of 50-100 µM showed cytotoxicity. Therefore, we used a concentration of 10 µM resveratrol in our subsequent experiments. Next, we evaluated the mRNA expression of the proinflammatory cytokines Il-1b and Il-6, and found that resveratrol negatively modulated the LPS-induced mRNA levels of these cytokines. When we studied the potential involvement of MAPK and NF-κB signalling pathways, we found that resveratrol inhibited the activation (phosphorylation) of ERK1/2, SAPK/JNK, p38 MAPK and the p65 subunit of NF-κB complex. Overall, these results suggest that resveratrol dampens the LPS-induced activation of signalling pathways. Since it has been shown that resveratrol can modulate the activity of SIRT1 (a member of the NAD+-dependent histone deacetylase family of enzymes targeting both histone and non-histone substrates), we decided to analyze whether resveratrol could prevent the changes in the acetylation pattern of histone-3 (H3) induced by LPS. We found that LPS increased the acetylation of H3 and resveratrol prevented this effect. During inflammatory process in the CNS, microglia play an important role by creating an environment that enhances the production of various pro-inflammatory factors, which consequently cause neuronal dysfunction and neurodegeneration. Since resveratrol is capable of exerting anti-inflammatory and antioxidant activities in several cell types, we studied the effects of resveratrol on microglial LPS-induced activation. Collectively, our results suggest resveratrol exerts neuroprotective effects by suppressing the LPS-enhanced expression of pro-inflammatory mediators such as pro-inflammatory cytokines as well the epigenetic changes in microglia cell cultures. Since resveratrol has a good safety profile, it could result in an ideal candidate for neuroprotection in situations of neuroinflammation.

0123 - PHARMACOLOGICAL MODELING OF SCHIZOPHRENIA: BEHAVIORAL ANALYSIS FOCUS ON THE AT1 RECEPTORS ROLE

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Schizophrenia is a chronic mental illness with a high incidence worldwide affecting directly the patient's life quality. This pathology is characterized by positive (hallucinations, delusions) and negative symptoms (social withdrawal), and cognitive deficit; that have been related to alterations in glutamatergic and dopaminergic neurotransmission. The administration of ketamine is a validated preclinical/animal model of schizophrenia in rodents, which reproduces the typical symptoms of this pathology. Brain Angiotensin II, through AT1 receptors (AT1-R), modulates dopaminergic and glutamatergic neurotransmission. Previously, we showed the AT1-R involvement in behavioral and neurochemical responses in an amphetamine model of schizophrenia. The present aim was to study AT1-R role in behavioral responses in a ketamine model of schizophrenia. Male Wistar rats (250-320 g) were administered with AT1-R antagonist Candesartan/vehicle (3 mg/kg p.o., days 1-6 or 1-10) and Ketamine (30 mg/kg i.p.), Acute: day 6 or subchronic: days 6-10. For Acute protocol (day 6), the locomotor activity, the social interaction and the novel object recognition were evaluated. For Subchronic protocol (after 14 days withdrawal) the same behavioral tests were evaluated at basal or challenge condition (Ketamine 15 mg/kg i.p.). Data were analyzed using two-way ANOVA, followed by Bonferroni test. Ketamine administration protocols resemble the described signs of schizophrenia since they induced augmented locomotor activity, social withdrawal and cognitive deficit. Interestingly, the AT1-R antagonist avoids the development and expression of these behavioral responses. Since the available therapeutic treatments for schizophrenia related disorders have low efficacy and high incidence of side-effects, new pharmacological approaches become necessary. However, further studies are needed to postulate the AT1-R as an alternative pharmacological target.

0200 - INFLUENCE OF TRACE ELEMENTS ADMINISTRATION ON THE NATURAL LATERALIZED EXPLORATION OF DIFFERENT GEOMETRICAL ENVIRONMENTS IN MATURING RATS.

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Previous evidence from our laboratory showed that intact rats showed a non-consistent lateralized exploration response due to different geometrical characteristics of the novel environment. Since, in previous experiments Tellurium (Te) and Selenium (Se) were able to influence the biased response in one determined environment (T-shaped rectangular environment), the objective of the present study was to evaluate if the trace elements can affect the lateralized exploratory response in other geometrical environments. After systemic administration K_2TeO_3, or Na_2SeO_3 in non-toxic doses (1.55 nM) to pregnant mother and its litter rats, at 30 days of age litter rats were tested in three geometrically different environments: square (s), rectangular (r) and t-shaped rectangular (t) environments, as previously shown. Animals receiving tap water were considered control (n= 14). Results showed that control animals presented a right-biased exploration response in the S and R field (24.5 ± 5 vs. 96.5 ± 12 counts/3 min, left vs. right, $p<0.05$, and 43.5 ± 11 vs. 83 ± 11 counts/3 min, left vs. right, $p<0.05$) and a left-biased exploration response in the T environment (49 ± 6 vs. 29 ± 4 counts/3 min, left vs. right, $p<0.001$). Group of rats receiving Te (n = 10) showed impaired lateralized response in the R and T environments, not affecting the biased exploration of the S field (83 ± 13 vs. 68.5 10.2 counts/3 min, p n.s., left vs. right, and 33 ± 3.5 vs. 32.5 ± 3.8 counts/3 min, p n.s., left vs. right). Animals receiving Se (n= 13) showed impaired lateralized response in the S and R, but not in the T environment. Conclusion: Trace elements can modify or block selectively the lateralized exploration response induced by different geometrical environments.

0208 - SPINAL CORD INJURY: SPINAL TRACTS RECONNECTION PROMOTES THE RECOVERY OF LOCOMOTOR SYSTEM FUNCTIONS.

Hector Ramiro QUINTÁ

CONICET - LABORATORIO DE MEDICINA EXPERIMENTAL - HA

In spinal cord injury (SCI) trauma, the shaft of long motor and sensory spinal tracts is interrupted. Therefore, the goal in this pathology is focused not only in promotes re-growth of spinal tracts but also in an effective re-connection whit the lower targets to achieve a recovery of locomotor functions. According to this background, I focus my research in the design of protein treatments to this traumatic pathology. Netrin-1, an axonal chemoattractant protein, is involved in axonal growth during the embryonic development. This protein drives the corticospinal tract growth and its navigation across the pyramidal decussation to the lowest part of spinal cord by a haptotaxis phenomenon. As I described previously, Netrin-1 treatment promotes a significant recovery of locomotor activity in rats with a complete SCI at thoracic level 10, assessed by using BBB score. Moreover, this result correlated with an improvement in the control of voluntary locomotion assessed with the ladder rung test. Rats treated with Netrin-1 showed a decreased number of missed steps and slipped steps in the ladder compared to controls. In line with this, a stereotaxic surgery to

trace the corticospinal tract was carried out in the treated rats. Using "clearing technique" plus ultrathin sectioned; it was observed a significant regeneration of corticospinal tract at the injury site in Netrin-1 treated animals. Moreover, a significant maintaining in the number of synaptic contacts was observed downstream of lesion site only in rats that received Netrin-1 treatment. Besides, the sensitive tracts were preserved of dying back only in rats treated with Netrin-1. In conclusion, Netrin-1 administration after SCI promotes corticospinal tract regeneration avoids the dying back in the sensitive axonal shaft structure and stimulated the synaptic contact re-arrangement. All of these processes could partially explain the mechanism by which Netrin-1 treatment recover locomotor abilities after injury.

0212 - BRAIN MITOCHONDRIAL FUNCTION IN GLAUCOMA

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Glaucoma is the second leading cause for blindness worldwide and damages structures in the brain, including the primary visual cortex. The aim was to evaluate the alterations of mitochondrial function in an experimental glaucoma model. Three-month female Wistar rats were divided in two groups (n= 8): glaucoma (GG) in which rats were operated under a microscope by cauterized two of the episcleral veins and control (CG) which received a sham procedure. Seven days after surgery rats were euthanized, brain cortex was separated, and mitochondria were isolated (CICUAL FFyB n° 3314). The following markers were evaluated: oxygen consumption (OC), ATP production, hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) production, the activities of superoxide dismutase (SOD), complex I-III and complex II-III, cardiolipin content, mitochondrial membrane potential, and NADPH oxidase-4 (NOX4) expression. Comparing to CG, GG showed a decrease in ATP production (23 %, $p<0.05$) and an increase in H_2O_2 as well as O_2^- production (28and 30%, respectively, $p<0.05$). There were no significant differences in complex I-III activity, whereas complex II-III activity was 50 % lower in GG compared to CG ($p<0.05$). SOD activity was increased in GG compared to CG (33 %, $p<0.05$). NOX4 expression was increased in GG compared to CG (27 %, $p<0.05$). No significant differences were found in OC, cardiolipin content, and mitochondrial membrane potential. These results suggest that mitochondrial function is altered in glaucoma, resulting in a decreased capacity to produce ATP and an increased production of H_2O_2 and O_2^-. Increase in SOD activity contributes to the increase in H_2O_2 levels. As NOX4 expression was higher, it could be one of the sources of the increase of reactive oxygen species. The understanding of the role of mitochondria in this pathology is important since its function is essential for neurotransmission and impact on the neuronal survival pathways.

0232 - METHYLENE BLUE PREVENTS RETINAL DAMAGE CAUSED BY PERINATAL ASPHYXIA IN THE RAT

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IBCN, FACULTAD DE MEDICINA, UNIVERSIDAD DE BUENOS AIRES (1); CENTER FOR BIOMEDICAL RESEARCH OF LA RIOJA (CIBIR) (2); CEBBAD, UNIVERSIDAD MAIMÓNIDES (3)

Introduction. Perinatal asphyxia (PA) is responsible for a large proportion of neonatal deaths and numerous neurological sequelae, including visual dysfunction and blindness. In PA, the retina is exposed to ischemia/reoxygenation, which results in nitric oxide overproduction and neurotoxicity. We hypothesized that methylene blue (MB), a guanylylcyclase inhibitor and free-radical scavenger currently used in the clinic, may block this pathway and prevent PA-induced retinal degeneration. Materials and methods. Male rat pups were subjected to an experimental model of PA. Four groups were studied: normally delivered (CTL), normally delivered treated with 2 mg Kg-1 MB (MB), exposed to PA for 20 min at 37ºC (PA), and exposed to PA and, then, treated with MB (PA-MB). Forty five days after birth rats were subjected to electroretinography, sacrificed, and the eyes were studied by histology, TUNEL assay, and gene expression analysis. Results. Electroretinography showed that PA animals had significant defects in the a- and b-waves and oscillatory potentials. The same animals presented a significant increase in the thickness of the inner retina and a large number of TUNEL-positive cells. All these physiological and morphological parameters were significantly prevented by the treatment with MB. Gene expression analysis demonstrated significant increases in iNOS, MMP9, and VEGF in the eyes of PA animals, which were prevented by MB treatment. Conclusions. MB regulates key players of inflammation, matrix remodeling, gliosis, and angiogenesis in the eye and could be used as a treatment to prevent the deleterious visual consequences of PA. Given its safety profile and low cost, MB may be used clinically in places where alternative treatments may be unavailable.

0253 - REDOX IMBALANCE IN LATERAL GENICULATE NUCLEUS IN AN EXPERIMENTAL GLAUCOMA MODEL

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Antioxidant system is needed to respond to oxidative insult and to maintain redox homeostasis. Redox imbalance plays a significant role in development of glaucoma. The aim of this work was to evaluate the changes that occur in redox imbalance in lateral geniculate nucleus (LGN) of rats subjected to an experimental glaucoma model. Wistar rats (3 months) were divided in two groups (n= 12): glaucoma in which rats were operated by cauterized two of the episcleral veins (G) and control which received a sham procedure (C)(CICUAL FFYB 3314). Seven days after surgery rats were euthanized, brains were removed and LGN were separated. The markers evaluated were: thiobarbituric acid reactive substances (TBARS), protein carbonylation (PC), nitrites levels (NO), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), thioredoxin reductase (TrxR), total reactive antioxidant potential (TRAP) and glutathione (GSH). Comparing G to C: there were an increase in TBARS (34 %, $p<0.05$), PC (99 %, $p<0.001$), NO (32 %, $p<0.01$), SOD (20 %, $p<0.05$) and GPx (72 %, $p<0.01$); and a decrease in GR (49 %, $p<0.01$), TRxR (46 %, $p<0.001$), TRAP (34 %, $p<0.05$) and GSH (44%, $p<0.01$). No significant changes in CAT levels were observed. There were a positive correlation between a- TBARS and PC (R^2= 0.8093, $p<0.001$) and b-TrxR and GR (R^2=0.8973, $p<0.001$). The decrease in TRAP and GSH levels and a compensatory increase in SOD and GPX activities may have been consequences of an increase in oxidative process. The decay in GR activity as well as the increase in GPx activity would lead to a decrease in GSH levels. The decrease in TrxR activity could lead to a deficient recycling of thioredoxin. Results allow concluding that redox imbalance occurred in LGN in the glaucoma model that leads to an increase in lipid and protein oxidation.

0271 - AMPHETAMINE INDUCED DIFFERENTIAL EFFECTS IN VASCULAR AND GLIAL COMPONENTS AT SOMATOSENSORY CORTEX: WHY TO FOCUS ON AT1 RECEPTORS

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Amphetamine (Amph), is associated with inflammatory processes, involving glial and vascular alterations. Brain Angiotensin II, through AT1-receptors (AT1-R), modulates dopaminergic neurotransmission and plays a crucial role in inflammatory responses. Our aim was to evaluate the role of AT1-R in long-term alterations induced by repeated exposure to Amph. Astrocyte and microglia reactivity, and brain microvascular network were analysed at the somatosensory cortex (S1 Barrel and S1 Trunk area). Male Wistar rats (250–320 g) were administered with AT1-R antagonist Candesartan/vehicle (3 mg/kg p.o., days 1–10) and Amph/saline (2.5 mg/kg i.p., days 6–10). The four experimental groups at the two times evaluated (17 and 31 days) were: Veh-Sal, CV-Sal, Veh-Amph, CV-Amph. On days 17 and 31 the animals were sacrificed and their brains were processed for immunohistochemistry against GFAP (astroglial marker), CD11b (microglial marker) and von Willebrand factor (vascular marker). Data were analysed with factorial ANOVA followed by Bonferroni test. Our results indicate that Amph exposure induces an endurable increase in astrocyte and microglia reactivity at S1 Barrel and S1 Trunk area. Although, the microvascular rearrangement (evaluated as vascular area density, branching points and tortuosity) showed time dependant differential response to Amph, since at day 31 these parameters return to basal conditions at S1 Barrel. Meanwhile, at S1 Trunk the vascular changes were observed only at day 31. Pretreatment with the AT1-R blocker prevented the described alterations induced by Amph. We conclude that neuroplastic changes induced by Amph demand an AT1-R active role showing a regional susceptibility at vascular level.

0289 - ROLE OF PRO-INFLAMMATORY FACTORS ON THE SURVIVAL AND DIFFERENTIATION OF DOPAMINERGIC PRECURSORS

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LELOIR INSTITUTE FOUNDATION - IIBBA CONICET (1); INSTITUTE OF BASIC SCIENCE AND EXPERIMENTAL MEDICINE (ICBME), ITALIAN HOSPITAL (2); BUCK INSTITUTE FOR RESEARCH ON AGING (3)

Parkinson's disease (PD) is a neurodegenerative disease characterized by the progressive loss of dopaminergic neurons (DAn) of the nigrostriatal system. Studies in animal models of PD have provided proof of concept that transplantation of DA precursors can relieve parkinsonian symptoms. However, a major limiting factor of this strategy is the poor survival rate of grafted DAn. This could be due to host inflammatory response, among other factors. Our previous results demonstrated a host primary response related to the graft of human dopaminergic precursors (DA14), with an increase of host-MHCII positive cells. Expression of tumor necrosis factor-alpha (TNF-alpha) was also detected on host-ED1 positive cells. We aim to study the impact of the pro-inflammatory environment on DA14 cells and the effect of a TNF-alpha inhibitor on an in vitro approach. DA14 cells were exposed to conditioned media (CM) from basal or activated BV2 microglial cells during 4 days. A significant increase in cell death was observed by fluorescence microscopy after Hoechst staining in DA14 cultures exposed with CM from activated microglia ($p<0.05$). This result was in accordance to a decrease in the number of Tyrosine hydroxylase (TH) positive cells detected by immunofluorescence ($p<0.01$). Neurite length measurement was performed to evaluate the differentiation process. A decrease in neurite length of TH positive cells was detected in DA14 cultures incubated with CM from activated microglia ($p<0.05$). In order to study the relevance of TNF-alpha, DA14 cells were co-incubated with CM from basal or activated BV2 cells and the TNF-alpha inhibitor, Etanercept. Inhibition of TNF-alpha was able to avoid morphological alterations ($p<0.05$) and diminution of DA cells ($p<0.05$). Our results suggest that the pro-inflammatory microenvironment has a negative impact on survival and differentiation of DA14 cells. TNF-alpha inhibition could be an interesting strategy in order to improve survival of DA precursors.

0393 - NEW HYPERGLYCOSYLATED HUMAN ERYTHROPOIETIN-DERIVED MOLECULES AS THERAPEUTIC CANDIDATES FOR THE TREATMENT OF THE CENTRAL NERVOUS SYSTEM-AFFECTING DISEASES

María de Los Milagros BÜRGI (1) | Gabriela APARICIO(2) | Ricardo KRATJE(1) | Camila SCORTICATI(2) | Marcos OGGERO(1)

UNL, CONICET, FBCB, CENTRO BIOTECNOLÓGICO DEL LITORAL (1); UNSAM, CONICET, IIB-INTECH, LABORATORIO DE NEUROBIOLOGÍA (2)

Neurodegenerative diseases affect millions of people around the world causing cognitive and behaviour disorders. Nevertheless, does not exist any effective treatment for them nowadays. In this sense, human erythropoietin (EPO) has an important role considering its antiapoptotic, cytoprotective, angiogenic and antioxidant properties. However, its erythropoietic activity (EA) should be considered as a side effect. Thus, we proposed the development of new EPO analogues using an approach of N-glycoengineering by hyperglycosylation to preserve its neuroprotective and neuroplastic action but blocking the EA. New EPO muteins were obtained by adding one extra N-glycosylation site per molecule using site–directed mutagenesis. Then, they were produced in transduced CHO.K1 cells and purified by immunoaffinity chromatography. Primary cultures from hippocampal neurons were used to measure apoptosis inhibition, neuritogenesis, filopodia density and synapsis formation. In vitro and in vivo EA was also carried out. Three EPO variants were produced and one-step purified with a purity level higher than 89%. They presented an apparent molecular mass higher than EPO and a superior number of acidic isoforms as result of the increased glycosylation degree. The in vitro and the in vivo haematological activity of each EPO analogue was abolished ($p<0.001$). Nevertheless, all of them preserved the neuroprotective and neuroplastic activity as they prevented staurosporine-induced apoptosis ($p<0.001$) but promoted neuritogenesis ($p<0.05$ and $p<0.001$), filopodia density ($p<0.05$ and $p<0.001$) and synapsis formation ($p<0.01$ and $p<0.01$). Thus, blocking the erythropoiesis-stimulating activity of hEPO and retaining its neuroprotective and neuroplastic action, potentiate these new hyperglycosylated EPO entities as novel neurobiopharmaceutics useful for the treatment of those diseases affecting the central nervous system.

Infectología y Parasitología / Infectology and Parasitology II

Chairs: Guillermo Alonso | Vanina Álvarez | Javier de Gaudenzi | Natalia de Miguel | Alicia Graciela Fuchs

0043 - TRYPANOSOMA CRUZI PROLINE PERMEASE HAS A UNIQUE POLYAMINE CO-TRANSPORT ACTIVITY

Melisa MARTINEZ SAYE | Chantal REIGADA | Edward VALERA VERA | Mariana MIRANDA | Claudio PEREIRA

INSTITUTO DE INVESTIGACIONES MÉDICAS ALFREDO LANARI - UNIVERSIDAD DE BUENOS AIRES

Polyamines are essential compounds to all living cells and in T. cruzi, the causative agent of Chagas disease, besides their participation in cell growth and differentiation, its acquisition relies exclusively on transport processes since the parasite is unable to de novo synthesize them. We have previously characterized the proline permease TcAAAP069 and we have identified inhibitors of this transporter that possess trypanocidal activity. In T. brucei the TcAAAP069 orthologue (TbAAT6) mediates the transport of proline and neutral amino acids. In addition, it is responsible for the uptake of the drug eflornithine, a fluoroamino acid containing a putrescine structure, used to treat the human African trypanosomiasis. Also, mutant T. cruzi parasites lacking the TcPAT12, a T. cruzi polyamine permease, retain the capacity to transport diamines via a low-affinity mechanism suggesting that there exists another uptake system. Taking all this information into account, we postulated that the proline permease TcAAAP069 could also be responsible for polyamines uptake. In order to test this hypothesis, proline incorporation was competed with increasing concentrations of putrescine. Proline uptake was significantly reduced in the presence of putrescine and this inhibition was dose-dependent. Reciprocally, putrescine incorporation was evaluated in the presence or absence of proline. The uptake was significantly inhibited both in parasites overexpressing TcAAAP069 and wild-type parasites. In silico analysis showed that TbAAT6 and TcAAAP069 have a very similar topology including 11 transmembrane spans, but completely different to that predicted for TcPAT12. Altogether the results suggest that TcAAAP069 could transport not only proline but also the essential polyamine putrescine, increasing its potential as a target of trypanocidal drugs. However, we cannot rule out the possibility that instead of a co-transport there is a crosstalk that allows a co-regulation of both transporters.

0128 - NOVEL FUNCTIONS OF TRYPANOSOMA CRUZI NDPK ON PARASITE DNA INTEGRITY

Melisa SAYE | Fabio DIGIROLAMO | Chantal REIGADA | Valera-vera EDWARD | Claudio A. PEREIRA | **Mariana MIRANDA**

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Nucleoside diphosphate kinases (NDPK) are housekeeping enzymes that maintain the intracellular pools of di and tri phosphate nucleotides. These are multifunctional enzymes which are involved in diverse processes, such as signal transduction and DNA repair, among others. TcNDPK1 is a canonical NDPK of Trypanosoma cruzi, the parasite that causes Chagas disease. Several evidences associate TcNDPK1 with DNA-processing mechanisms, for example, the in vitro ability of binding nucleic acids and nuclease activity. In the present work we evaluate the role of TcNDPK1 in DNA-damage responses using heterologous gene expression systems and over-expression in epimastigote cells. We found that different strains of bacteria, WT and ndk- mutants, expressing the enzyme decreased about 5 folds and 18 folds the spontaneous mutation rate, respectively. In addition, yeasts lacking the endogenous gene YNK1 (YNK1-) and expressing TcNDPK1, were significantly more resistant to 10 and 25 mM hydrogen peroxide and were less sensible to UV irradiation than controls. Parasites over-expressing TcNDPK1 were able to withstand different genotoxic stresses caused by hydrogen peroxide, phleomycin and hidroxyurea, being statistically more resistant than control at different concentrations. In addition, under oxidative damage, over-expressing parasites presented lesser genomic damage assessed by agarose gel electrophoresis and augmented levels of PARP, an enzyme involved in the DNA repair machinery. Furthermore, TcNDPK1 was found to have nuclear, peri-nuclear and cytosolic localization. These results strongly suggest that TcNDPK1 is involved in the maintenance of parasite genomic-DNA integrity, thus, giving rise to a novel function.

0228 - IN SILICO ANALYSIS OF PHOSPHOLIPASE A1 GENE IN LEISHMANIA SPP.

Guadalupe GIMENEZ | Emanuel BOTT | Estela Maria LAMMEL | Paula* RUYBAL | **Maria Laura BELAUNZARAN**

IMPAM (UBA-CONICET)

Leishmaniasis, caused by several species of protozoan parasites of the genus Leishmania, is an important global health problem. L.braziliensis is the main species responsible of cutaneous and mucocutaneous clinical forms in Latin America. Phospholipase A1 (PLA1), lipolytic enzyme involved in phospholipid metabolism, can act as a virulence factor for many pathogens including protozoans. Putative PLA1 was initially explored by Trypanosoma cruzi PLA1 orthologs search in Leishmania spp. L. braziliensis PLA1 (LbPLA1) gene was identified, cloned and expressed from MHOM/BR/75/M2904 gDNA (GenBank ACCN KJ957826). Analysis of amino acid (aa) PLA1 sequences showed 98.1 % of identity and 98.7 % of consensus positions between cloned LbPLA1 and LbrM.31.2750 (reference strain). Detailed analyses evidenced substitution of 7 aa: 2 conservative, 2 neutral, 2 semi-conservative and 1 non-conservative. Comparison of the deduced aa sequences of LbPLA1 with T. cruzi PLA1 (GenBank ACCN AEX65839) and T.brucei PLA1 (GenBank ACCN CAG29794) indicated 30.9 % identity and 44.3 % consensus positions with T.cruzi PLA1 and 11.5 % identity and 22.0 % consensus positions with T. brucei PLA1; class 3 lipase domain (GXSXG motif) was highly conserved. PLA1 orthologs search in 12 species genomic-sequences showed that in Viannia subgenus the protein length was 373 aa while in 9 species of Leishmania subgenus it ranged from 363 to 367 aa with the GXSXG motif highly conserved throughout the genus. Phylogenetic analysis of these sequences resulted in a tree topology congruent with the genus taxonomy. Two main clusters were obtained according to subgenus classification while each species/species complex clustered together with high boostrap support values. As expected, nucleotide phylogenetic analysis revealed more accurate cluster organization according to the genus taxonomy. Our results contribute to increase the knowledge of trypanosomatids PLA1 as novel potential targets to fight neglected diseases.

Supported by UBA and CONICET grants. *RP and BML equally contributed to this work.

0334 - CHARACTERIZATION OF TRYPANOSOMA CRUZI APHA-TUBULIN ACETYLTRANSFERASE (TCATAT)

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Microtubules are essential cellular structures built from tubulin that show promise as antimicrobial drug targets. The arrangement of trypanosomatid cytoskeleton is simpler than those of other eukaryotic cells but it is precisely organized and constituted by stable microtubules. Such microtubules compose the mitotic spindle, the basal body, the flagellar and the subpellicular microtubules, which are connected to each other and to the plasma membrane forming a helical arrangement along the cell body. Acetylation on K40 of a-tubulin is conserved from lower eukaryotes to mammals and is associated with microtubule stability. It is also known that K40 acetylation occurs significantly on flagella, centrioles, cilia, basal body and the mitotic spindle. The primary acetyltransferase that delivers this modification was recently identified as Mec-17/ATAT, a Gcn5-related N-acetyltransferase. Despite evidence supporting a role for K40 acetylation in microtubule stability, its biological function in vivo is unclear. We

have expressed T. cruzi ATAT with an HA tag in epimastigotes using the inducible vector pTcINDEX-GW. Over-expressing parasites present a growth defect and also, we observed a diminished infectivity and an alteration in the differentiation from amastigotes to trypomastigotes. TcATAT is located in the cytoskeleton and flagella of T. cruzi as determined by Western blot and confocal microscopy. Moreover, TcATAT colocalizes with acetylated alpha-tubulin in these structures and over-expression causes increased levels of the acetylated isoform. Also, over-expression causes a halt in the cell cycle progression of epimastigotes determined by flow cytometry and SEM. Finally, when this ATAT is over-expressed we observed that parasites become more resistant to microtubule depolymerizing drugs. These evidence supports the idea that tubulin acetylation is crucial for T. cruzi replication and differentiation and that TcATAT is responsible for this postranslational modification.

0355 - EGPE CELLS, ANALYTICAL LABORATORY SOURCE FOR ECHINOCOCCUS GRANULOSUS ANTIGENS.

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Cystic echinococcosis (CE) is a zoonoses worldwide distributed produced by Echinococcus granulosus sensu lato. In Argentina, CE is an endemic disease with active dissemination reporting more than 450 human cases per year. Disease diagnosis is performed by ultrasound. Serology tests for diagnosis, population screening and patient follow-up have poor or variable sensitivity and specificity. A cell line from E. granulosus G1 (EGPE cells) obtained in our laboratory (Echeverría et al., 2010) expresses antigen B and protein extracts from EGPE in different culture stages were recognized by sera from CE patients with high sensitivity by Western blot. The aim of this study was to identify, by proteomics, the CE antigens present in EGPE from 20-day-old culture. We tested sera from 34 CE patients (Tandil, Chubut and Perú), 21 healthy donors (Tandil), 5 cysticercosis (Perú) and 3 fascioliasis (Perú). Protocols were approved by the UAI Ethical Committee. Protein extracts were obtained with ice-cold lysis buffer, after 20 days of cell culture. Sera reactivity was detected with AP- goat anti-human IgG and BCIP/NBT, bands were analyzed with GelAnalyzer software. Protein extracts were separated through Sephacryl S-200 and further by affinity column performed with a pool of antibodies from sera of CE patients (CE column) or patients with other parasitoses (OP column). Eluted proteins from affinity columns were identified by proteomics (CEQUIBIEM - FCEyN, UBA). CE patient sera recognized bands from 12 to 94 kDa, few bands were also recognized by sera from healthy donors or from patients with other parasitoses. Elution profile of the gel filtration column showed three peaks, all of them recognized by CE patients´ sera. Proteins obtained from CE column allowed the identification of 15 proteins from E. granulosus. No detectable proteins were identified from OP column. EGPE cells can be used as a laboratory tool for identification of epitopes involved in the immune response.

0412 - TCVPS34-VPS15 COMPLEX COORDINATES THE CROSSTALK BETWEEN AUTOPHAGY AND METACYCLOGENESIS IN TRYPANOSOMA CRUZI

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Autophagy is a conserved process along evolution and is essential for the maintenance of cellular homeostasis. However, trypanosomatids seem to possess a less complex route, or different proteins not yet identified. This process can be up-regulated during stress, starvation, or infection. In mammals, two kinases differentially regulate the process of autophagy: mTor and a phosphatidylinositol 3-kinase, Vps34, which interact with a regulatory subunit, Vps15. Metacyclogenesis is a fundamental process in the life cycle of the protozoan Trypanosoma cruzi, the etiological agent of Chagas disease. This process involves the transformation of non-infective epimastigotes into infective metacyclic trypomastigotes. Several changes occur during metacyclogenesis, and autophagy was found to be strongly upregulated during this process. In this work, we demonstrate that parasites overexpressing TcVps34 or TcVps15 proteins enhance both, autophagy and metacyclogenesis. TcVps34 or TcVps15 overexpressing epimastigotes were able to differentiate to metacyclic forms in a higher proportion than wild-type cells. Parasites overexpressing these proteins showed a more intense labeling with the autophagosome marker Atg8.1 and higher levels of monodansycadaverine (MDC) staining, a specific in vivo marker for autophagic vacuoles, in the intermediate forms of differentiated parasites, in comparison to control parasites. To extend this study we are also performing assays with DQ-BSA, to evaluate degradative compartments, since the induction of autophagy is characterized by an increase in the number of lysosomes/autolysosomes required for the lysis of trapped components. In addition, we will study the effect of autophagy regulatory drugs such as rapamycin and wortmanin in transgenic parasites during metacyclogenesis. Taken together, these data demonstrate the key role of phosphatidylinositol 3-phostate pathway in autophagy, T. cruzi differentiation and cell cycle progression.

0438 - BIOCHEMICAL CHARACTERIZATION OF DIHYDROXYACETONE KINASE FROM TRYPANOSOMA CRUZI (TCDAK)

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Dihydroxyacetone (DHA) is used as carbon source by many cell types; however, at high concentrations, it is toxic and some microorganisms are susceptible to this compound. DHA detoxification is dependent on a functional dihydroxyacetone kinase (DAK) which converts DHA to DHA-phosphate (DHAP). Trypanosoma cruzi, the etiological agent of Chagas disease, possess two genes encoding for putative ATP-dependent DAKs (TcDAKs). Previous studies have shown that T. cruzi epimastigotes are able to grow in the presence of DHA and DAK activity was found in their lysates. Nevertheless, TcDAK has not been characterized so far. With this aim, recombinant TcDAK, expressed in E. coli by pJexpress vector and purified by IMAC, was used to determine kinetic properties of this enzyme. DAK activity was measured using DHA and ATP as substrates in the presence of 2 mM $MgCl_2$ in a coupled assay by enzymatic reduction of DHAP with NADH in the presence of glycerol-3-P dehydrogenase. TcDAK exhibited Michaelis-Menten kinetics for DHA (Km= 6.3 µM and Vmax= 12.79 µmoles/min/mg of protein). On the other hand, it showed sigmoideal kinetics for Mg-ATP (S0.5 =125.03 µM, Vmax= 1.18 µmoles/min/mg of protein and Hill coefficient= 1.99). TcDAK activity in the presence of other metals different than Mg^{++} was also evaluated. All of them gave lower activities than that observed with Mg-ATP (Ca^{++}> Cd^{++}>

Mn^{++}> Co^{++}> Zn^{++}). Additionally, TcDAK expression was evaluated in epimastigote and trypomastigote lysates by western blot using an anti-recombinant TcDAK serum. Epimastigotes and trypomastigotes showed the presence of two proteins recognized by the specific antiserum of molecular masses of 60 and 49 kDa, respectively, suggesting that trypomastigotes might have a TcDAK cleaved isoform. These results endorse the hypothesis that T. cruzi possess an active TcDAK which enables the parasite to use DHA as carbon source.

0509 - A COLLABORATIVE TRANSLATIONAL RESEARCH FRAMEWORK TO STUDY THE ASSOCIATION BETWEEN THE ALTERATION OF THE VAGINAL MICROBIOTA AND PRETERM BIRTH: PRELIMINARY RESULTS.

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Preterm birth (PTB) constitutes a major health problem in our country. There is a variety of potential etiological mechanisms for spontaneous PTB, including infectious and inflammatory pathways, stress-related influences and genetic predispositions. Uncovering the multifactorial processes and the interplay of risk factors that lead to spontaneous birth is necessary to identify effective strategies for preventing PTB. Our study analyzes the possible association between the vaginal microbiota and preterm birth. We conducted a prospective cohort study on 163 pregnant women treated in Hospital Dr. Penna Bahia Blanca (October, 2017- August, 2019), to analyze the alteration of vaginal microbiota by Nugent score (dysbacteriosis/ vaginosis), the assessment of Candida spp. and other pathogenic microorganisms at weeks 24/28 of pregnancy. Follow-up until delivery, with identification and recording of PTB or premature membrane rupture (PMR) events. The average age was 26 ± 6.1. Sixty seven % of women had a vaginal microbiota alteration: 35 % dysbacteriosis-vaginosis, 27 % candidiasis, and 41 % a sexual transmitted pathogen like Chlamydia trachomatis, Mycoplasma genitalium or Trichomonas vaginalis. Mixed infections were detected in 33 % of women and 28 % were positive for HPV. Sixty nine % concluded the follow-up until delivery and 14 % presented at least one of the outcomes of interest: 7 % PTB; 6 % PMR and 1 % PTB and PMR. The frequency of PTB was higher in the presence of some types of infections, however, a higher number of cases is still required for a statistical significance analysis. These results are of clinical and public health relevance due to high rates of vaginal microbiota alteration found in the analyzed population of pregnant women. In addition, the implementation of a translational research represented a huge challenge that impacted positively on public health by improving the systematizing care processes in and between hospital units as well as on primary health care attention.

0534 - PROTEINS BEARING CYCLIC NUCLEOTIDE BINDING DOMAINS IN TRYPANOSOMA CRUZI.

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Cyclic AMP signaling have shown to be involved in Trypanosoma cruzi biology. However, little is now about the pathway in the parasite. Previous reports show that at the genomic level this parasite has several proteins that possess cyclic nucleotide binding domain (CNB), such as protein kinase A. It has also been published that, unlike its mammalian counterpart, the trypanosomatid PKA does not bind cAMP and seems not to have the regulatory function of kinase activity. In order to increase the knowledge about the cAMP mediated pathway in T. cruzi, we have cloned and biochemically characterized cAMP binding proteins in T. cruzi. At the bioinformatic level, several proteins were found in the parasite's genome. Six candidates, TcCLB.418221.20, TcCLB.504153.20, TcCLB.504449.30, TcCLB.508523.80, TcCLB.510691.30 and TcCLB.508273.30 were selected to cloned and fusion proteins to a his-tag and expressed in E. coli. Bacterially expressed proteins and a cAMP-agarose resin, were used in pull down and displacement experiments, in order to confirm cAMP binding. Interaction assays were then performed using trypomastigote lysates obtained from infected Vero cell cultures. The eluted proteins were analyzed by mass spectrometry obtaining potential partners candidates that will be futher studied.

0535 - STRUCTURAL AND FUNCTIONAL ANALYSIS OF TCCARP1 AND TCCARP3 IN TRYPANOSOMA CRUZI

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Chagas disease is a chronic life-threatening disease caused by the protozoan parasite Trypanosoma cruzi, which represents a significant health burden in Latin America. For a targeted therapy of this disease, new antiparasitic drugs should be specifically directed against essential pathways in the parasite life cycle. Among these potential targets are signal transduction pathways, which have remained largely unexplored in trypanosome species. Of special interest is cAMP-mediated signaling, since cAMP has been shown to play critical roles in the life cycle of T. cruzi. The present research focuses on the identification and characterization of TcCARP1 and TcCARP3, cAMP Related Proteins (CARPs) in T. cruzi recently described by Gould et al, while investigating the mechanism of resistance of the parasite to the CdpA, an inhibitor of phosphodiesterases. Within CARPs, it has been shown that TcCARP1 and TcCARP3 presented a significant increase in both gene expression and protein translation during host cell invasion, only in virulent strains parasite. The aim of this research was to elucidate CARP mediated signaling in T. cruzi, through protein-protein studies, subcellular localization and oxidative stress assays; providing structural and functional analysis of CARPs proteins, not only to increase our knowledge about T. cruzi biology but also to target CARPs for the design and development of novel therapeutic agents against Chagas disease.

0554 - TRYPANOSOMA CRUZI TCHTE PROTEIN EXPRESSION IS REGULATED BY INTRACELLULAR HEME LEVELS

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Trypanosoma cruzi is a heme auxotroph, therefore it must scavenge heme (as free heme or as hemoglobin) from their hosts.

The protein TcHTE (T. cruzi heme transport enhancer) is involved in the uptake of this cofactor. It is localized to the flagellar pocket of the parasite and there is evidence that it forms a homotrimer. Conversely to other trypanosomatids, no hemoglobin (Hb) receptor has been found in T. cruzi yet. Given these precedents, we investigated if TcHTE has a role in Hb uptake from the extracellular medium or in Hb-derived heme transport. At mRNA and protein level, TcHTE is higher in heme starved Wild Type epimastigotes, and it gradually decreases when increasing amounts of a heme source (hemin or Hb) are added to the media. However, this response is faster when hemin is used as heme source, which may be related to the different biodisponibilities and/or uptake mechanisms of both heme sources. Surprisingly, epimastigotes that overexpress rTcHTE.His-GFP incubated in media supplemented with Hb have a significantly higher intracellular heme content compared to control epimastigotes; as previously reported using hemin as heme source. Altogether, these results mean that TcHTE is also involved in Hb uptake. Conversely to Trypanosoma brucei ortholog rTbHRG, rTcHTE.His-GFP does not change its localization when Hb is used as heme source, which discards that TcHTE has a role in the salvage of Hb-derived heme in internal compartments. We concluded that T. cruzi is able to sense intracellular heme level and regulates TcHTE expression according to it. Based on these and our previous results we propose two models of heme uptake in T. cruzi. In the first one, Hb is endocyted via a non-canonical Hb receptor and Hb-derived heme is transported through an unknown protein, meanwhile heme enters the cell via TcHTE. In the other model, Hb is degraded by external proteases in the parasite's surface, heme is released and enters the cell via TcHTE.

0563 - MOLECULAR CHARACTERIZATION OF TRITRICHOMONAS FOETUS SURFACE PROTEINS

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Bovine Trichomonosis, whose causative agent is the protozoan Tritrichomonas foetus (T. foetus), is one of the most common endemic infections of the reproductive tract of livestock. Its diagnosis is difficult due to lack of trained people and effective tools that allow early detection to prevent its spread. Little is known about the molecular biology of this parasite and its potential antigen targets. In this sense, the study of its surface proteins is of great interest to identify new diagnostic targets as well as to elucidate possible mechanisms of evasion of the immune response. The aim of this work was to characterize different surface antigens of T. foetus. Six candidate proteins for surface proteins were selected and the search for these genes in the genome of T. foetus was performed. Their presence in the genome of the isolates and that of their transcripts was confirmed by the use of specific primers by PCR and RT-PCR respectively. To study the presence of the translation products, plasma membrane proteins were purified by subcellular fractionation and analysed by mass spectrometry (MALDI-TOF). The 6 selected genes were found in the genome of the isolates as well as their transcripts. Membrane protein analysis showed the presence of only 3 of them. They represent useful tools for the design of new diagnostic methods through the evaluation of their antigenic characteristics and the development of antibodies that allow them to be detected quickly and specifically.

0601 - CONTRACTILE VACUOLE COMPLEX AS PART OF THE CLASSIC SECRETORY PATHWAY IN TRYPANOSOMA CRUZI

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T. cruzi has glycosylphosphatidylinositol (GPI) anchored virulence factors, such as trans-sialidase (TS) and mucins which are transported to the membrane through a non-conventional pathway involving the contractile vacuole complex (CVC), an organelle absent in mammals. There is still scarce information concerning the role of CVC in the general transport of proteins to the surface. To analyze whether CVC is part of the global membrane protein traffic or is only involved in the transport of proteins anchored by GPI, we studied the intracellular traffic of proteins with different membrane anchorage according to bioinformatics predictions (myristoyl and palmitoyl, direct interaction with lipids, and GPI). Calpain, KMP and TolT3 were selected as model proteins, respectively, three flagellar proteins present in the trypomastigote stage of T. cruzi. Traffic signals predicted in silico were confirmed by treatment with PI-PLC or protein overexpression with their anchor sites mutated. The analysis of intracellular traffic was performed by confocal fluorescence microscopy in the intermediate stage during the differentiation of amastigotes to trypomastigotes when flagellar protein transport is increased. For this we obtained a population of transfected parasites that express a CVC protein fused to GFP (the GFP-GTPase Rab11) as a marker of this organelle. Finally, to become independent of the protein cores, the traffic of GFP fused to different traffic signals was analyzed. For this we used a tet-on inducible expression system and we developed anti-GST-Rab11 antibodies to label the CVC. We observed that both TolT3 and KMP colocalized with the CVC marker while Calpain did not. Same results were obtained with the GFP fused to the different traffic signals. Thus, our results indicate that CVC does not participate in a general route of protein traffic to the cell surface, but rather is part of the classic secretory pathway in T. cruzi.

0613 - THE EPIGENETIC MODULATOR BDF2 IS INVOLVED IN THE NORMAL PROGRESSION OF TRYPANOSOMA CRUZI LIFE CYCLE.

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Trypansoma cruzi is the causative agent of Chagas' disease. In this parasite, protein-coding genes are transcribed polycistronically without canonical promoters or typical gene transcription initiation control. In this context, epigenetic regulation and post-trancriptional regulating mechanisms become crucial for the normal physiology of the parasite. Bromodomains are protein interaction modules that selectively recognize and bind acetyl-lysine residues present in histone and non-histone proteins. The bromodomain factor 2 of T. cruzi (TcBDF2) is a nuclear protein containing a bromodomain on its N-terminal portion. This domain enables the protein to interact whit acetylated lysine on chromatin proteins, like H4 histones, becoming an important factor on epigenetic regulation. Working with truncated mutant version of BDF2, we determine the nuclear localization signal of the protein by inmunofluorescent microscopy. In addition, we constructed mutant versions of the protein and corroborates by in vitro assays that these punctual modifications impaired the ligand binding. We analyzed the inducible overexpression of the wild type and mutant proteins over the life cycle of the parasite determining that BDF2 is an important factor for the normal growth of the epimastigotes. Moreover, this protein is involved on the metacyclic trypomastigote differentiation and is crucial for the tripomastigotes release from VERO cells once the infection is established. Finally, using a fluorescence quenching assay we determined the ability of BDF2 to bind to commercial

bromodomain inhibitors. We determined the dissociation constant for bromosporine and I-BET151.

0653 - EFFECT OF THE CALCIUM BINDING PROTEIN TCCAL1 ON THE PROLIFERATION OF TRYPANOSOMA CRUZI

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Calcium homeostasis is implicated in essential processes of T. cruzi life cycle. Although several calcium binding proteins have been identified in T. cruzi genome, their role in Ca^{2+} signaling remain still unknown. TcCAL1 is a hypothetical protein identified through proteome analysis of T. cruzi, which has two EF-Hand domains involved in Ca^{2+} binding. The hypothesis to be tested in this study was that TcCAL1 overexpression could trigger alterations in the intracellular calcium homeostasis leading an effect on epimastigote proliferation. For this purpose, the gene encoding tccal1 was amplified by PCR and cloned fussed to a 6x histidine tag into the pTREX expression vector. The recombinant plasmid pTREX/tccal1x6His was used to transfect epimastigotes from T. cruzi Y or CL Brener strains. Selection of transgenic cultures was carried out growing parasites in presence of increasing concentrations of Geneticin. Overexpression of TcCAL1x6His fusion protein was confirmed through western blot and affinity chromatography. Growth curves of recombinant cultures from Y or CL Brener strains were performed in triplicate, counting the parasites in a Neubauer chamber. Parasite cultures carrying the empty vector pTREX were used as controls. Data obtained were processed using GraphPad Prism 6.0 Software and subjected to statistical analysis by two-way ANOVA test. The preliminary results showed that the overexpression of TcCAL1x6His affects the growth curve of CL Brenner strain at the stationary phase but the proliferation Y strain is not affected. Additional experiments are being carrying out to validate these results and to study the effect of TcCAL1 overexpression on T. cruzi differentiation. Future assays including parasites carrying the tccal1 gene silenced will contribute to validate our hypothesis and to reveal the function of TcCAL1 in calcium homeostasis of T. cruzi.

0673 - IDENTIFICATION AND QUANTIFICATION OF PROTEASES IN GERMINAL CELLS OF ECHINOCOCCUS GRANULOSUS

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The cestode Echinococcus granulosus (Eg) is the causative agent of cystic echinococcosis, a severe neglected zoonosis which has important medical and economic impact. The inner layer of the hydatid cyst or "germinative layer" contains a group of cells called germinative cells (GC). These cells are responsible for cell proliferation and differentiation. Eg has the capability to survive for long periods of time in many mammalian host species. The prolonged survival indicates that the parasite is able to evade host attack for example through the production and release of proteases to digest host proteins. The aim of the present work was to identify and to quantify proteases in Eg GC grown in different conditions using a proteomic strategy. Eg primary cell culture was obtained from hydatid cysts and maintained with weekly splitting during 1-4 month. Cells were culture in normal media and conditioned media (resembling host environment) for different time periods. Then, 50 µl of cells were homogenized in lysis buffer and the peptide content was estimated. Aliquots of 100 µg protein per sample were used for filter-aided sample preparation and the resulting peptides were subjected to nano-LC-MS/MS-analysis. A combined library was set-up by combining the different runs using the Protein Pilot-software and Uniprot database. We identified a total of 455 proteins in all the studied conditions. We identified and quantified 7 different proteases which were differentially represented in the studied conditions. For example, Calpain A (Accession U6J063) and Mitochondrial processing peptidase beta subunit (Accession U6JE67) were found overrepresented in cells cultured in normal media with a fold change 12 and 2.8 respectively ($p<0.05$). Through the methodology used, it was possible to describe the presence of several proteases in the GC of Eg, suggesting that some evasion mechanisms are present.

0684 - EXPRESSION PROFILING OF MIRNAS THROUGH METACESTODE DEVELOPMENT IN ECHINOCOCCUS MULTILOCULARIS

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The tapeworm (cestode) Echinococcus multilocularis is the causative agent of alveolar echinococcosis, a neglected zoonotic disease. MicroRNAs (miRNAs), a class of small non-coding RNAs, are principle regulators of gene expression at the post-transcriptional level and are involved in many different biological processes. In previous work, we described the miRNA repertoire of E. multilocularis in vivo metacestode, the stage of sanitary relevance. In this work we described for the first known time the expression profile of the miRNA repertoire through metacestode development in E. multilocularis. Small RNA libraries from E. multilocularis in vivo metacestodes, in vitro metacestode vesicles and different stages of primary cells were sequenced. Then, miRNA prediction and differential expression analysis were performed. We found a high expression of a few miRNAs, such as miR-71 and miR-9, in all sequenced samples of E. multilocularis. The high expression of these miRNAs was conserved in other cestodes, suggesting that these miRNAs may play essential roles in development and survival. Differential expression analysis showed highly regulated miRNAs through metacestode development, suggesting a role in the regulation of developmental timing and/or host-parasite interaction. Some E. multilocularis miRNAs are protostome-specific or bilaterian-specific but divergent from host orthologs, and therefore could represent novel biomarkers and/or selective drug targets for echinococcosis infection. The comprehensive identification and expression analysis of E. multilocularis miRNAs can help to analyze miRNA function and identify miRNAs potentially useful for the control of alveolar echinococcosis.

0687 - DECIPHERING THE ROLE OF MICRORNAS IN TAPEWORM BIOLOGY: MIR-71 KNOCKDOWN INHIBITS ECHINOCOCCUS MULTILOCULARIS EARLY DEVELOPMENT IN VITRO.

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Echinococcosis represents a major public health problem worldwide and is considered a neglected disease by the World Health Organization. The etiological agents are Echinococcus tapeworms, which display particular developmental traits that imply a complex control of gene expression. MicroRNAs (miRNAs), a class of small regulatory RNAs, are involved in the regulation of many biological processes such as development and metabolism. They act through the repression of messenger RNAs (mRNAs) by

binding to the 3' untranslated region (3'UTR). Previously, we described the miRNome of several Echinococcus species and found that miRNAs are highly expressed in all life cycle stages, suggesting an important role in gene expression regulation. However, studying the role of miRNAs in helminth biology remains a challenge. To provide a means of functional analysis of miRNAs in tapeworms, we performed miRNA knockdown experiments in primary cell cultures of Echinococcus multilocularis, which mimic the development of metacestode vesicles from parasite stem cells. We first analysed the miRNA repertoire of E. multilocularis primary cells by small RNA-seq and found that miR-71, a bilaterian miRNA absent in vertebrate hosts, is one of the top five most expressed miRNAs. Then we predicted miR-71 mRNA targets using genomic information and observed a high number of predicted targets. The inhibition of miRNAs can be achieved by transfection of antisense oligonucleotides (anti-miRs) that block miRNA function. Here we evaluated a variety of chemically modified anti-miRs for miR-71 knockdown. Electroporation of primary cells with 2'-O-methyl modified anti-miR-71 led to a significant reduction of miR-71 levels. Transcriptomic analyses showed that several predicted miR-71 targets were up-regulated in anti-miR-treated primary cells, including genes involved in parasite development such as a frizzled ortholog, a GPCR receptor presumably acting in the Wnt signaling pathway, and genes involved in parasite-host interplay such as EmTIP, as well as genes coding for tapeworm-specific proteins of unknown function. Notably, miR-71-silenced primary cells showed a different phenotype from control cells and were not able to develop metacestodes. These findings indicate an important function of miR-71 in Echinococcus development and provide methodology for miRNA functional analysis in this parasite that could be applied to related tapeworms.

0688 - THE GENOME OF THE SYLVATIC SPECIES ECHINOCOCCUS OLIGARTHRUS: PHYLOGENETIC HISTORY OF ECHINOCCOCUS THROUGH WHOLE GENOME VARIANTS ANALYSIS

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The first parasitic helminth genome sequence was published in 2007, since then only ~200 genomes have become available, most of them being draft assemblies. Nevertheless, despite the medical and economical global impact of helminthic infections, parasites genomes in public databases are under-represented. Recently, through an integrative approach involving morphological, genetic and ecological aspects, we have demonstrated that the complete life cycle of Echinococcus oligarthrus (Cestoda: Taeniidae) is present in South America. The neotropical E. oligarthrus parasite is capable of developing in any felid species and producing human infections. Neotropical echinococcosis is poorly understood yet and only a few cases of echinococcosis have been unequivocally identified as consequence of E. oligarthrus infections. Regarding phylogenetics, the analyses of mitogenomes and nuclear data sets have resulted in discordant topologies and there is no unequivocal taxonomic classification so far. In this work, we sequenced and assembled the genome of E. oligarthrus that was isolated from agoutis (Dasyprocta azarae) naturally infected and performed the first comparative genomic study of a neotropical Echinococcus species. The E. oligarthrus genome assembly consisted of 86.22 Mb which showed ~90 % of identity and 76.3 % of coverage with Echinococcus multilocularis and contained the 85.0 % of the total expected genes. Genetic variants analysis of whole genome revealed a higher rate of intraspecific genetic variability (23,301 SNPs; 0.22 SNPs/Kb) rather than for the genomes of E. multilocularis and Echinococcus canadensis G7 but lower with respect to Echinococcus granulosus G1. Comparative genomics against E. multilocularis, E. granulosus G1 and E. canadensis G7 revealed 38,762; 125,147 and 170,049 homozygous polymorphic sites respectively, indicating a higher genetic distance between E. oligarthrus and Echinococcus granulosus sensu lato species. Phylogenetic analysis using whole genome SNPs demonstrated that E. oligarthrus is one of the basal species of the genus Echinococcus and is phylogenetically closer to E. multilocularis. This work sheds light on the Echinococcus phylogeny and settles the basis to study sylvatic Echinococcus species and their developmental evolutionary features.

0698 - CHARACTERIZATION OF NUCLEASE ACTIVITY PRODUCED AND SHEDDED BY TRYPANOSOMA CRUZI TRYPOMASTIGOTES

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IIBIO-UNSAM

In several pathogenic microorganisms, since bacteria to protozoos, production of nucleases was described as a virulence factor that, among other functions, would allow them to escape from Neutrophil-produced NETs (neutrophil extracellular traps), DNA tramps produced as part of the natural immune response. Recently, Trypanosoma cruzi, the etiological agent of Chagas disease, was observed to induce NETs where, interestingly, parasites persist alive. Herein we describe the production, shedding, and the first characterization of a nuclease activity by means of T. cruzi trypomastigotes (CL Brener strain). For these studies, cells-free parasites were incubated in CO_2 atmosphere from hours to several days and their supernatant was tested for nuclease activity. Through DNA degradation studies we observed that nuclease shedding, and its activity, depends on incubation temperature. Moreover, nuclease activity was observed both on circular and lineal plasmid, as well as on human genomic DNA. By means of zymography approaches (SDS-PAGE with DNA from salmon testes), at least two proteins, of 40 and 55 kDa approximately, with nuclease activity were observed. Among cofactors, activity depends on Mg^{++} presence but not Ca^{++} cations, and is inhibited by Zn^{++} and EDTA. Moreover, it is no longer observed in samples heated above 70 ºC. Our observations describe, by the first time, the production and shedding of nuclease activity by T. cruzi trypomastigotes, which could constitute a new parasitic virulence factor. Protein purification and identification is under process.

0733 - DEVELOPMENT OF A COLORIMETRIC RT-LAMP AMPLICATION ASSAY ADAPTED TO AN EARLY AND EASY DETECTION OF ZIKA, CHIKUNGUNYA AND THE FOUR SEROTYPES OF DENGUE VIRUS

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The global expansion of dengue (DENV), chikungunya (CHIKV), and Zika viruses (ZIKV) is having a serious impact on public health. These arboviruses are transmitted by mosquitoes, and cause diseases having similar symptoms in human patients, but requiring different immediate management steps. Therefore, rapid and specific discrimination of these three viruses in patient samples is needed. The aim of this work was to develop a simplified test for DENV serotypes, CHIKV and ZIKAV detection in early stages of infection. We developed a colorimetric reverse transcriptase loop-mediated isothermal amplification assay (COLOR-RT-LAMP), an alternative method of polymerase chain reaction (PCR) that, as it works at a fixed temperature, not require thermocyclers and, also, can be performed in short times, showing accurate and reliable results. A single-tube was developed with specific primers, RT and DNA

polymerase together with the tested samples (RNA genomes references). Ranges of temperature, pH, Mg and time were evaluated. The readout of the test was defined as a color change - visible to a naked eye-, by the addition of neutral red dye, or HNB dye (Hydroxy naphthol blue) prior to the amplification; these dyes changes its color as a result of the amplification. The sensitivity and specificity of the RT-LAMP were evaluated. Here we described the achievement of an early and easy method to detect and diferentiate DENV serotypes, ZIKAV and CHIKV. The results showed that our ColorRT-LAMP test is highly sensitive and specific. No cross-reactivity was observed with all other three closely related arboviruses. The COLOR-RT-LAMP method used in our study is specific, sensitive, and suitable for further investigation as a useful alternative to the current methods used for clinical diagnosis and diferentiated of DENV1-4, ZIKAV and CHIKV, especially in hospitals and laboratories that lack sophisticated diagnostic systems.

0745 - BIOGENESIS OF EXTRACELLULAR VESICLES RELEASED BY THE PARASITE TRICHOMONAS VAGINALIS

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T. vaginalis is a parasite that causes trichomoniasis, the most common non-viral sexually disease worldwide. Given it is an extracellular parasite, adhesion to host cells is one of the key processes for the development of infection. In recent years, various factors that influence this process have been identified, including extracellular vesicles (EVs). Previously in our laboratory we demonstrated that both exosomes (vesicles with a size range of 40 - 100 nm) and ectosomes (vesicles with a size of 100 - 1000 nm) are involved in the process of interaction of T. vaginalis with host cells. It is currently known that ESCRT-III subunit of to the ESCRT complex is involved in membrane cleavage being the VPS32 protein the key effector during membrane scission. These antecedents lead to our hypothesis that VPS32 might be regulating the process of extracellular vesicles formation in T. vaginalis. Based on this, we transfected the protein VPS32 and an empty vector as a control (EpNEO) in a poorly-adherent parasite strain and evaluated the amount of EVs released by these parasites by TEM, NTA and Western blot using Evs markers. Our results demonstrated that VPS32 transfected parasites released more EVs than EpNEO parasites. The amount of both EVs populations is affected; suggesting that VPS32 is involved in the biogenesis of exosomes as well as ectosomes. As our previous results demonstrated that EVs are regulating parasites adhesion and now, we observe that VPS32 parasites produces more EVs, we performed an adhesion assay to host cells to evaluate the adherence capacity of VPS32 and EpNEO parasites. Interestingly, our results indicate that parasites transfected with VPS32 are ~40 times more adherents than EpNEO parasites. In summary, our results demonstrated that VPS32 is a key player in the regulation of the adherence process; provably due to its role in the increased biogenesis of extracellular vesicles.

0751 - ESCRTIII COMPLEX IN TRYPANOSOMA BRUCEI: FUNCTIONAL CHARACTERIZATION OF TBVPS32

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The ESCRT (endosomal sorting complex required for transport) drives a diverse collection of membrane remodeling events such as endocytosis,autophagy, release of enveloped viruses, reorganization of the nuclear envelope and cytokinetic abscission.ESCRTIII isthe effector sub-complex for the reason that is capable to form filaments and spirals, which produces membrane constrictions. Vps32 is the most abundant protein in ESCRTIII and its dynamic over membranes is given for its molecular structure that alternates between a monomeric-closed state to polymeric-open state.Here we investigate the conservation of Vps32 in Trypanosoma brucei and we found an orthologue sequence (TbVps32) that shows Snf7 domain and the characteristic secondary structure which gives evidence of a high conservation among eukaryotic cells. For T. brucei procyclic form (PCF) we designed an RNAi strategy where a 405 bp of TbVps32 was cloned into the p2T7 vector (TbVps32-RNAi) allowing a tetracycline inducible downregulation. We confirmed the silencing of TbVps32 by RT-PCR. Moreover, after 72h the viability of the parasites was severely affected with a decrease cell growth and abnormal nucleus-kinetoplast configurations observed by fluorescence microscopy. To further understand the defects in cell cycle progression, knockdown cultures of TbVps32-RNAi were synchronized with HU (hydroxyurea) and then evaluated by propidium iodide (PI) staining showing an increase of cells in G1 phase. On the other hand, we evaluated Vps32 role in vesicular trafficking by receptor mediated endocytosis of transferrin and fluid phase uptake of dextran and the results are under analysis.To perform a functional characterization, TbVps32coding sequence was amplified fused to a hemagglutinin tag at N-terminal (HA-TbVps32) and subcloned into pLew100v5, an inducible overexpression vector. Until now, PCF 29-13 cell line and bloodstream form (BSF) single marker cell line were transfected and are under clonal selection.

0758 - GENETIC DIVERSITY OF NATURAL POPULATIONS OF TRYPANOSOMA CRUZI IN CLINICAL SAMPLES FROM PATIENTS WITH ORAL CHAGAS DISEASE IN VENEZUELA: FOLLOW-UP AFTER TREATMENT WITH TRYPANOCIDAL DRUGS

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Oral transmission of Chagas disease (OChD) is an increasingly important aspect in the epidemiology. Venezuela has reported the two largest outbreaks described so far, affecting a total of 192 people mostly children. The long-term impact after treatment on the dynamics of infection in natural populations of T. cruzi is still unclear. In this sense, we proposed a genetic characterization of T. cruzi populations present in peripheral blood in order to differentiate responder or non-responder patients and predict the response to treatment (Tmt). We performed quantification of the parasitic load by qPCR, genetic typing of T. cruzi populations by multiplex qPCR of nuclear genome markers and RFLP-PCR for the hypervariable region of T. cruzi kDNA to demonstrate changes in Minicircle signatures (Ms) of the parasite populations present in 41 clinical samples from 15 patients. To reflect the genetic diversity found, Jaccard distances (Jd) values were compared. This clinical monitoring confirmed the presence of T. cruzi DNA in 26 post-treatment samples up to 9 years after Tmt. These results reveal 100 % therapeutic failure for both outbreaks of OChD, classifying these patients as non-responders to Tmt. All samples showed homogeneity at the DTU level, being typified as TcI. The Ms showed a high degree of polymorphism, with 73 % of total post-Tmt samples with Jd values close to 1. Analyzing the dynamics of each patient`s population separately, in all post-Tmt samples the change in Ms variability respect to pre-Tmt sample was not statistically significant. This variability does not reflect a natural or induced clonal selection process driven by the etiological Tmt; on the contrary, it could be associated with the clonal histotropism process evidenced in natural T. cruzi infections. In conclusion, these strategies of molecular characterization of parasite DNA were useful to detect Tmt failure and find out the lack of parasite population selection with Tmt in these OChD settings.

0778 - FUNCTIONAL ROLES OF AMP-ACTIVATED PROTEIN KINASE (AMPK) COMPLEXES

CONTAINING TCAMPKA1 OR TCAMPKA2 IN ENERGY HOMEOSTASIS REGULATION AND CELL CULTURE PROGRESSION IN TRYPANOSOMA CRUZI

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The AMP-activated protein kinase (AMPK) is a heterotrimeric enzyme involved in maintaining energy homeostasis in response to different stresses in many organisms. During the transition between the mammalian host and the insect vector, Trypanosoma cruzi, the causative agent of Chagas disease, faces different types of environmental fluctuations, all of which prompt the parasite to remodel its metabolism. Recently, it was shown that Trypanosoma brucei AMPK is involved in the differentiation from the bloodstream slender to stumpy stage and in surface protein expression changes in response to nutritional stress. This underscores the relevance of AMPK for parasite life cycle progression. We identified four candidate genes for the AMPK subunits of T. cruzi (alpha1 and alpha2 catalytic subunits, beta and gamma regulatory subunits). The beta and gamma subunits are largely conserved in their domain structure relative to the mammalian orthologs. However, the alpha subunits show significant sequence and structure differences from the human counterparts. The presence of these subunits in T. cruzi epimastigotes was confirmed by RT-PCR, Western blot using a phospho-AMPKa specific antibody, mass spectrometry and by kinase activity assays using the specific AMPK substrate SAMS. TcAMPKa1 over-expressing epimastigotes showed a lower growth rate in basal culture conditions compared to the control. On the other hand, alpha2 over-expression had the opposite effect. Additionally, we observed upregulation of AMPK activity under epimastigote starvation, and that dorsomorphin, a specific AMPK inhibitor, also inhibits T. cruzi AMPK. Moreover, each of these subunits could complement S. cerevisiae conditional mutants lacking the respective subunit of the AMPK ortholog SNF1. Finally, starving assays with AMPKa over-expressing parasites also showed a possible role of AMPK in autophagy. Overall, our results show for the first time, the presence of a functional AMPK orthologue in Trypanosoma cruzi.

0784 - MOLECULAR CHARACTERIZATION OF MRE11-RAD50 PROTEINS DURING HOMOLOGOUS RECOMBINATION REPAIR (HRR) IN TOXOPLASMA GONDII

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The homologous recombination repair (HRR) is critical to genome integrity maintenance during cell replication (late S/G2) but it has not been elucidated in T. gondii. HRR starts with recognition of DNA damage follow by generation of resection ends for which Mre11-RAD50-Nbs1 (MRN) complex is required. In our research group was observed that this parasite´s genome only encodes 50% of HRR proteins described in yeast and mammals suggesting either divergence between these homologues or the presence of parasite-specific components functional in this essential pathway. In fact, T. gondii harbours homologues of Mre11 and RAD50 proteins although no coding sequence to Nbs1 was found. In humans, the complex is composed by a homodimer of Mre11 with endo/exonuclease activity, two RAD50 ATPase and Nbs1. The gene of T. gondii Mre11 (TgMre11) contains an open reading frame encoding a polypeptide with 38.41 % of identity to its human homologue and 535 residues longer. Superimposition between TgMre11 model and HuMre11 crystal structure (RMS 0.84) showed at least three insertions with more than 20 amino acids each on relevant regions for both dimerization and interaction with other proteins or DNA. Then, taking into account that Mre11 and RAD50 are predicted as essential genes in T. gondii, disclosing structural-functional differences between these proteins and their human counterpart as well as characterizing novel T. gondii HRR components might give insights into evolution of this pathway, identify novel drug targets and help to elucidate orthologues in other Apicomplexa.

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0796 - DEPOLYMERIZATION OF SUMO CHAINS IN T. BRUCEI BLOODSTREAM PARASITES AS A SIGNAL TO CONTROL GROWTH DURING INFECTIONS.

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IIBIO-UNSAM

SUMOylation is a reversible post-translational modification (PTM) that involves the attachment of one SUMO protein or SUMO chains to internal lysines in target proteins. This PTM enables rapid cellular responses, which are essential to pathogenic microorganisms that undergo complex life cycles involving several hosts, as is the case with trypanosomatids. Since we have previously shown that T. brucei is capable of forming SUMO polymeric chains in vitro, our next goals were to determine if this also occurs in vivo and to study their physiological relevance. To achieve this, we generated SUMO chain mutant parasites by replacing the endogenous SUMO alleles with a lysine deficient variant unable to polymerize. This transgenic cell line did not exhibit any evident phenotype and grew equivalent to WT parasites when culture in vitro. However, there were striking differences when using a mouse model of infection. While WT parasite grew uncontrollably killing the host 5-6 days after infection, SUMO mutant parasites limited their growth, generating oscillating parasitemia with prolonged host survival. Knowing that naturally occurring parasites achieve long-term infections inducing differentiation to a quiescent stage, we next examined differentiation kinetics of these parasites by an in vitro approach exposing them to cis-aconitate (CA) at low temperatures. Differentiation from BF to procyclic form (PF) was evaluated by immunofluorescence visualizing the switching from VSG to procyclin. SUMO mutant parasite showed accelerated differentiation kinetics, suggesting that the absence of SUMO chains favors differentiation of the parasite and allows it to successfully establish and maintain an infection.

0805 - CHARACTERIZATION OF THE TCDOT1A AND TCDOT1B ISOFORMS: IMPLICATION OF H3K76 DIFFERENTIAL METHYLATION DURING TRYPANOSOMA CRUZI LIFE CYCLE.

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Trypanosoma cruzi, the etiologic agent of Chagas Disease, affects a large number of the population in Latin America. It has a complex life cycle alternating between a mammalian host and the vector insect, Triatoma infestans. This cycle consists of three well-defined stages: amastigotes, epimastigotes and trypomastigotes. As the parasite faces different environments, it requires changes in gene expression in order to survive. Hence, gene expression regulation might be a key aspect to understand adaptation. Despite Trypanosomes gene expression is mainly regulated post transcriptionally, there are evidences that chromatin state influence it. Recent studies have shown that DOT1 methyltransferases homologues, called DOT1a and DOT1b, are involved in the methylation of lysine 76 of histone H3 in T. cruzi. In T. brucei, DOT1a mediates H3K76 mono and di-methylation, whereas DOT1b catalyzes H3K76 tri-methylation. However, these two enzymes remain poorly characterized. In this project, we investigated the relevance of TcDOT1a and TcDOT1b during cell

cycle and the metacyclogenesis process. Therefore, to evaluate the catalytic activity using heterologous complementation, we have successfully cloned and transformed a null DOT1 yeast strain with TcDOT1a. Additionally, to analyze the isoforms subcelular location and their effects on cell cycle progression and differentiation, we have cloned the TcDOT1 isoforms in a pRibotex vector with an N-terminal HA-tag and transfected epimastigotes of CL Brener strain. Nevertheless, the overexpression was toxic for the cell. Therefore, we decided to switch to an inducible vector. Overall, our data will be useful to further understand the role of the DOT1 isoforms and the differential methylation of H3K76 in T. cruzi. In the long term, our findings might unravel new targets for antiparasitic drugs.

0808 - CHARACTERIZATION OF HEPATITIS C VIRUS INFECTIONS IN MAR DEL PLATA AND ANALYSIS OF POTENTIAL DIRECT ANTIVIRAL RESISTANCE

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About 71 million people are chronically infected with hepatitis C virus (HCV), at risk to evolve to cirrhosis and hepatocellular carcinoma. HCV is distributed in genotypes (gts), associated with the natural history of infection and antiviral response. Direct-acting antivirals (DAAs) are the current drugs used for the treatment of chronic infections, curing 95 % of them. Daclatasvir inhibits the multifunctional NS5A protein, essential component of the replication complex. The aim of this study was to extend the characterization of HCV infections in Mar del Plata and determine the presence of mutations associated to daclatasvir resistance. Treatment-naïve chronically HCV infected patients, were included, and their clinical information was analyzed. The HCV NS5A gene was amplified by RT-nested PCR from serum samples and sequenced. Genotypes were determined by phylogenetic analysis and the presence of mutations was determined. The characteristics of the patients in this cohort were: mean age 55.25 years; 71.43% males; 42.86 % coinfected with HIV and HCV viral load mean 6.61 log. The phylogenetic analysis showed that most samples (85.71 %) were subgt 1b and 14.29 % was 1a, in agreement with the reported most prevalent subgts in the region. All the samples showed mutations in the NS5A gene nucleotide sequences. The analysis of NS5A proteins showed that all samples presented aminoacidic substitutions, but most of them were not associated to resistance. Only a few samples presented mutations that can be associated to antiviral resistance (Y93D and P58R), because they are localized at the binding site of the antiviral. The presence of other aminoacids at this site was reported to reduce daclatasvir activity. In conclusion, new HCV infections in Mar del Plata are produced by the already circulating most prevalent subgts in the region. Actually, local HCV does not exhibit significant mutations related to resistance to one of the most used DAAs.

0827 - FUNCTIONAL CHARACTERIZATION OF BABESIA BOVIS PLP1 THROUGH HEMOLYSIS ASSAYS AND GENERATION OF A KNOCK OUT STRAIN

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Bovine babesiosis is a tick-borne disease caused by parasites of the Babesia genus affecting livestock production worldwide. Proteins implicated in life cycle progression of parasites are key factors in host-pathogen interaction. We have previously identified in B. bovis a family of Perforin-Like Proteins (PLP) involved in pore formation and erythrocyte damage. One member, PLP1, is expressed and exposed to the host immune system during infection. The aim of this study was to determine the function of PLP1 and its contribution to parasite's pathogenesis. The recombinant MACPF domain (responsible for pore formation in PLPs) of PLP1 was expressed and hemolysis assays were done incubating this protein with bovine RBCs. Cell lysis was expressed as a percentage of maximum hemoglobin release with Triton X-100 treatment. High hemolysis levels (> 80 %) were obtained at [rMACPF] >80 nM, and pH >5. The hemolysis activity was not affected by changes in [Ca^{2+}]. A B. bovis knock out (KO) strain was generated by disruption of plp1. Parasites were transfected with a plasmid to guide replacement of plp1 with an egfp-bsd fusion gene. Integration and disruption of the plp1 gene was confirmed by PCR and Southern blot analysis. The KO phenotype was evaluated by observation of in vitro replication in bovine RBC cultures. KO parasites showed normal rates of replication and development. Yet, an unusual phenotype of multiple parasites accumulated within a single RBC was observed, suggesting a possible defect in egress. This phenotype was already reported for T. gondii plp1 KO. Current studies are aimed to compare transcription levels of other members of the plp family between KO and WT strains. Future studies of in vivo replication of the KO strain in experimental bovine and tick infections will help to determine if plp1 plays a role on another stage of parasite's life cycle.

0832 - OVER-EXPRESSION OF TBRRM1 LEADS TO ABERRANT PHENOTYPES AND CELL DEATH IN TRYPANOSOMA BRUCEI PROCYCLIC CELLS

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Since transcription in trypanosomatids is polycistronic, regulation of gene expression occurs mainly at the post-transcriptional level by RNA binding proteins. In our lab we study the gene expression regulation in T. brucei, particularly we focus on elucidating the function of the RNA binding protein, TbRRM1. TbRRM1 has three RRM domains in the amino-terminal region followed by two zinc finger domains and a region rich in dipeptides (Asp/Glu)-Arg which precedes the RS domain characteristic of the SR protein family. Previously, we have demonstrated that TbRRM1 is essential for survival in procyclic and bloodstream form stages of T. brucei since its silencing by RNAi affects the growth curve, produces aberrant phenotypes and promotes cell death by a mechanism compatible with apoptosis. The aim of the present work was to contribute to the elucidation of TbRRM1 function through the study of the effects of its over-expression. For that purpose, we have established a system for the inducible over-expression of a FLAG-tagged TbRRM1 and different mutants. Results showed that over-expression of 3xFLAG-TbRRM1 was deleterious for parasite survival. Surprisingly, the parasites displayed an aberrant morphology that was previously observed when TbRRM1 was depleted, suggesting that both silencing and over-expression of TbRRM1 produce a similar phenotype. In addition, since both silencing and over-expression of TbRRM1 are lethal for parasites, it could be concluded that TbRRM1 is a relevant protein whose levels must be finely regulated.

0842 - MONOSISTRONIC EXPRESSION SYSTEM AND ENDOGENOUS GENE LABELING THROUGH THE T2A STRATEGY IN TRYPANOSOMA CRUZI

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To date, most gene expression systems available for T. cruzi are based on multicistronic vectors. In these, messenger RNAs for both, the gene of interest and the selectable marker, mature independently from the same primary transcript. Due to this independence, a common disadvantage of this configuration is the selection of resistant cells lacking expression of the gene of interest. In this sense, the selective agent only assures the expression of the resistance gene. To overcome this limitation, we devised a monocistronic vector in which the gene of interest and the selectable marker are coded on the same mRNA linked in frame by the sequence for a 2A peptide. To test this approach, we introduced the Thosea asigna virus self-cleaving 2A peptide between the eGFP reporter and the neomycin resistance gene. For comparative purposes we derived an additional plasmid with a bicistronic configuration by insertion of a GAPDH intergenic sequence in place of that for the 2A and transfected three different strains of T. cruzi. Flow cytometry analysis showed higher eGFP expression levels and more homogeneous populations when comparing cells obtained with the monocistronic vector than those transfected with the bicistronic one. Moreover, fusion protein dissociation occurred with high efficiency (>94 %) as determined by Western blot. In addition to that, this system is highly stable and is active in all stages of the parasite. Given the resulting efficiency, we successfully adapted the monocistronic vector to easily introduce a hemagglutinin tag to the actin gene by ends-in homologous recombination. In conclusion, the use of ribosome skipping peptides in the context of a monocistronic expression vector, can be used as a cost-efficient alternative not only to improve protein expression in T. cruzi, but also to label endogenous genes without needing complex systems, such as the CRISPR/Cas9.

0859 - ROLE OF THE MOB PROTEIN IN THE CELL DIVISION OF TRITRICHOMONAS FOETUS

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The flagellated protist Tritrichomonas foetus is a parasite that causes bovine trichomonosis, a major sexually transmitted disease in cattle. T. foetus has a worldwide distribution and causes significant economic losses. Cell division has been described as a key player in controlling cell survival in other cells, including parasites but there is no information about the regulation of this process in trichomonads. MOB proteins have also been shown to regulate cell division in plants and trypanosomes. Two MOB1 genes, MOB1A and MOB1B, were identified in T. foetus genome. We report that MOB1A is localized on cell cytoplasm and nucleus of T. foetus and deletion of TfMOB1A-N terminal (with sites of phosphorylation necessary for activation) leads to abnormal T. foetus growth and an increase in the percentage of multinucleated parasites. These results suggest that MOB1 protein would be important for a correct parasite division.

0866 - REMODELING OF AN RNA REGULON FOR A CELL-SURFACE ASSOCIATED TRYPANOSOMA CRUZI GLYCOPROTEIN DURING PARASITE DIFFERENTIATION

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RNA regulons, ribonucleoprotein complexes composed by functionally related mRNAs together with RNA-binding proteins (RBPs), play an important role in the post-transcriptional regulation of gene expression in trypanosomes. The RNA-binding protein U-rich RBP 1 (TcUBP1) targets numerous mRNAs encoding cell-surface glycoproteins preferentially expressed in infective trypomastigotes of Trypanosoma cruzi, the agent of Chagas disease. All these mRNA targets have common 3'-UTRs with a 50-nt linear sequence motif proximately upstream of the signature RNA element for TcUBP1. Overexpression of TcUBP1-GFP in replicative epimastigotes resulted in changes in the subcellular localization of these transcripts from the posterior region to the perinuclear region of the cell, as is typically observed in infective trypomastigotes. We hypothesize that mRNA localization is a mechanism for stage-specific gene regulation in trypanosomes. To test this possibility, we used the wild-type T. cruzi CL-Brener strain and performed a trypomastigote-to-epimastigote differentiation in vitro, incubating the parasites in BHT media supplemented with BFS 10 %. During this differentiation process, the expression and localization of cell-surface associated glycoprotein transcripts were followed by RNA FISH, with a specific Cy3-oligo probe, at different time-points ranging from 1 to 42 days. After incubation and washing, the RNA signal changed from being uniformly distributed in the cytosol (in the trypomastigote form, day 1) to be preferentially restricted to the posterior region of the cell (in the epimastigote form, day 42). Indirect immunofluorescence labeling of cells with an anti-TcCruzipain polyclonal serum detected these mRNAs in a subcellular region that matches to reservosomes, suggesting that RNA localization mechanisms triggered by TcUBP1 might be involved in the regulation of stage-specific protein expression.

0867 - GENOTYPING FASCIOLA HEPATICA BY ITS1 AND RAPDS SUGGESTS DISTINCTIVE SOUTH AMERICAN GENETIC DIVERSITY AND HOST AFFINITY

Silvana SCARCELLA (1) | Florencia BIANCO(1) | Roberto MERA Y SIERRA(2) | Gisela NEIRA(2) | Maria Victoria SOLANA(1) | Hugo SOLANA(1) | Estefan MIRANDA MIRANDA(3)

CIVETAN CONICET (1); CIPAR (2); INIFAP- INSTITUTO NACIONAL DE INVESTIGACIONES FORESTALES AGRICOLAS Y PECUARIAS (3)

The common liver fluke (Fasciola hepatica) is a major cause of economic losses to agriculture all over the world, with cost estimated at US$ 2,000 million per annum, Given that the existence of genetically different populations of F. hepatica could allow, against any selection pressure, natural or artificial (for use fasciolicides products and/or control measures), one or more populations of F. hepatica to be able to survive and create resistance or adaptability to such selective pressure. It's important to characterize the different isolation of the liver fluke. The aim of the present work was to characterize genetically adult F. hepatica isolates from cattle, pigs, buffaloes and donkeys from different regions of South American, using sequence analysis of ribosomal ITS1 and RAPD-PCR. Genotyping of Fasciola hepatica DNA samples derived from, cattle, pig, buffalo, and donkey collected from different regions of South America, were performed using the F. hepatica Internal Transcribed Spacer (ITS) sequencing, as well as RAPDs-PCR. Phylogeny assessment derived from multiple sequence alignment (MSA) of ITS sequences, exhibit a distinctive South American geographical pattern compared against F. hepatica reported ITS sequences from around the world, MSA analysis of ITS sequences also showed the F. hepatica ITS haplotypes found in south America are consistent with other reported ITS haplotypes. Further phylogenetic assessment of the electrophoresis band pattern of RAPDs-PCR amplicons, suggest the parasite's genome contains markers that may reveal a host preference. Further assessment revealed two major F. hepatica groups within the South American isolates that clearly diverged from each other, one containing parasites obtained from swine and donkeys and other found in bovid with two additional branch subdivisions of the latter group one containing water-buffalos and a second containing only cattle.

0869 - MOLECULAR CHARACTERIZATION OF CIRCULATING TREPONEMA PALLIDUM CLUSTERS IN PEDIATRIC PATIENT

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Syphilis is a public health problem with a sustained increase being the incidence of congenital syphilis (Sc) of 1.7 ‰ live births. Currently there are no techniques with sufficient sensitivity and specificity for the diagnosis of Sc Our group recently started with maternities a multicenter study to evaluate the use of molecular biology techniques (MBT) such as PCR, for the diagnosis of Sc. In Argentina there aren't data about MBT in the diagnosis of syphilis or about the strains of Treponema pallidum pallidum (Tpp) in the pediatric population. Our objective was to evaluate the use of PCR in the diagnostic of syphilis in children and examine the clusters of Tpp by DNA sequencing. Pilot study in 5 pediatric cases of syphilis. Different lesion swabs (total= 8 samples) were processed for DNA extraction (QIAamp DNA Blood Mini Kit) followed by PCR for the genes of Tpp: Tp47kDA (conventional PCR) and dnaA using Taqman® probes (real time PCR - qPCR). Additionally, nested PCR for the TP0136 and TP0548 genes were performed in the samples and the sequences were subsequently purified and sequenced by commercial kit (BigDye™) in a genetic analyzer (3500 analyzer).Then, edition and alignment analysis were performed compared to the reference sequences of cluster SS14 (GenBank CP004011.1) and Nichols (GenBank CP004010.2). The different MBT (PCR, nested PCR and qPCR) concordance in an 88 % while 5 samples (at least one swab per patient) from different regions (soft palate, perianal, palms) were positive, 2 samples (perianal, tongue) were negative. Only 1 sample (perianal) was only positive by qPCR. In the sequencing analysis the predominant Tpp clade was Nichols (n= 4) over SS14 (n= 1). The results of PCR determination in blood of these patients is still in process. This study is the first one conducted in the pediatric population in our country for the detection of syphilis by BMT. As recently studies in Argentina reports, the Nichols clade in our study was greater as 10%, although in our pediatric population the prevalentce was higher (80 %) that in adults (26,8 %). The application of BMT in the detection of Sc is a promising alternative; the development of a large-scale and multicentric study will asses the effectiveness of the BMT and provide valuable information on local transmission networks of Tpp.

0870 - TRYPANOSOMA CRUZI EXPERIMENTAL INFECTION IN MICE DEFICIENT FOR MITOCHONDRIAL CYCLOPHILIN D

Natalia MILDUBERGER (1) | Carolina GONZALEZ(2) | Patricia BUSTOS(1) | Alina Elizabet PERRONE(1) | Miriam POSTAN(3) | Jacqueline BUA(4)

INP "DR M FATALA-CHABEN" ANLIS MALBRAN (1); INP "DR M FATALA-CHABEN" ANLIS MALBRAN; UNIVERSIDAD ABIERTA INTERAMERICANA (2); INP "DR M FATALA-CHABEN" ANLIS MALBRAN; CONICET (3); INP "DR M FATALA-CHABEN" ANLIS MALBRAN; UNIVERSIDAD ABIERTA INTERAMERICANA; CONICET (4)

Cyclophilins are chaperone enzymes involved in peptides and proteins folding. Cyclophilin D (CyPD), is localized to mitochondria and is a crucial component of the mitochondrial permeability transition pore, involved in cell death process. With the aim to study the role of CyPD in the experimental T. cruzi infection in CyPD deficient mice were inoculated with 100 trypomastigotes (Tulahuen strain). A lower initial parasitemia in mice deficient of CyPD was observed, but a very high parasite load was observed at 21 days post-infection, with a consequent higher mortality that wild type (WT) mice. Histopathological analysis in the acute phase of the infection did not show any significant differences in heart, liver and skeletal muscle damages between transgenic mice and their controls. Nevetheless, spleens from CyPD knocked out (KO) mice showed lost of the typical architecture that follicle present under T. cruzi infection. Ex vivo studies on mice macrophages and cardiomyocytes infected with T. cruzi, a decrease of around 50 % of infected cells were observed in transgenic mice compared to WT mice, which expressed CyPD. The levels of cytokines IFNγ, TNFα, IL-6, IL-10, IL-17, measured from mRNA of heart tissues of all groups of mice by qPCR were significantly different in CyPD KO mice and WT infected mice compared to their control groups. No differences were observed, however, between mice which expressed CyPD and the ones who did not. The levels of IL-4 y TGFβ cytokines did not show differences among the four group of animals, infected or not. Our results show that mice which do not express CyPD in their mitochondria, showed a differential course of the T. cruzi infection compared to WT mice, regarding parasitemia, survival and parasite load in organs and ex vivo cell cultures.

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0872 - BIOSYNTHESIS OF 5-AMINOLEVULIC ACID IN TRYPANOSOMA CRUZI

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Trypanosoma cruzi requires heme-compounds for growing, due to its partially or totally deficient biosynthetic pathway of heme. There are reports that support the functionality of mitochondrial enzymes involved in this pathway, such as 5-aminolevúlico synthetase (ALA-S) and Heme synthetase (Heme-S). T. cruzi genome is known and two homologous genes, Tc00.1047053511899.40 y Tc00.1047053511071.140, were identified by bioinformatic studies. Both of them are candidates to code with high score (50 %) for the ALA-S enzyme, responsible for synthesizing ALA from succinyl CoA and glycine. Our hypothesis is that the parasite is able to synthesize ALA (although it cannot be metabolized to heme) and the Tc00.1047053511899.40 y Tc00.1047053511071.140 sequences encodes for a protein with ALA-S activity. Using epimastigotes, we were able to detect and quantify, by spectrophotometric studies and HPLC chromatography, the presence of ALA in the parasite both intra and extracellularly. The mesasurements were made in 30ml of parasite culture which yielded about 608.31 ± 45.20 nmol of ALA. The extracellular content represents 96 % of the total synthesized. Such excretion would be avoiding the citotoxicity of ALA since it cannot be metabolized to heme. From bioinformatic studies using the Blast, ORF Finder, Mitoprop, Prosite and ClustalW platforms, it was determined that the above genes would code for a mitochondrial protein (98 %) which is dependent on pyridoxal phosphate and shown a KBL domain, which is characteristic of enzymes as ALA-S. Both, ALA detection and the computer analysis would support our hypothesis and encourages us to continue trying to confirm it.

0873 - COMPARISON OF THE DIAGNOSTIC ACCURACY OF A STANDARD RAPID TEST AND AN ALTERNATIVELY MANUFACTURED TEST FOR HUMAN LEPTOSPIROSIS SCREENING IN ARGENTINA.

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FACULTAD DE BIOQUÍMICA Y CIENCIAS BIOLÓGICAS- UNIVERSIDAD NACIONAL DEL LITORAL (UNL) (1); INER "DR. E CONI"-ANLIS (2); FBCB (UNL)- INER "DR. E. CONI" (ANLIS) (3)

Technical difficulties in methodologies used for human leptospirosis detection, limit diagnosis to high/moderate complexity laboratories, hindering accessibility, causing delays in results and putting patient's health at risk. In consequence, development of new rapid tests, technically simple and easily interpretable, is mandatory. Lateral flow immunoassays (LFIs) are suitable diagnostic tools considering the epidemiological features of Leptospirosis. In Argentina, the manufacture of these tests is difficult because of the imported supplies, not always available. In consequences, we developed an alternative device for LFI employing materials easily acquired in our country (LeptoLFI-1). The test yielded the best performance comparing with the current screening test for leptospirosis. Recently, we have developed a standard LFI device (LeptoLFI-0) then the aim of the present work is to evaluate its diagnostic accuracy and compare with LeptoLFI-1. A double-blind assay was perform using a randomly selected panel of 59 serum samples, with different days post infection (d.p.i), classified according to the Leptospirosis standards of Ministerio de Salud. Sensitivity (Se), specificity (Sp), positive and negative likelihood ratio (LR+, LR-) and Youden index (J) were estimated for both LFIs and for the screening tests. As expected, both tests resulted in high performances surpassing the current screening test, not only when data was analyzed globally but also grouping samples by d.p.i. But more importantly, LeptoLFI-1 yielded very similar diagnostic accuracy compared with the standard one. Results suggests that, beyond LeptoLFI-1 implies some extra steps during assay (respect to one-step standard test), the diagnostic performance is not affected. In conclusion, we achieved two accurate LFIs for leptospirosis screening in Argentina. Application of one or another could be subject to economic and import feasibilities according to health or general national state policies.

0915 - "SYNTHESIS AND EVALUATION OF NEO-GLYCOCONJUGATES AS TOOLS FOR THE SEROLOGICAL DIAGNOSIS OF CHAGAS DISEASE."

Ivana DUCREY (1) | María de Los Milagros CÁMARA(1) | Virginia BALOUZ(1) | Rosana LOPEZ(2) | María Eugenia GIORGI(2) | Linda TORO MELGAREJO(2) | Fernán AGÜERO(1) | Rosa M. DE LEDERKREMER(2) | Carla MARINO(2) | Carlos A. BUSCAGLIA(1)

INSTITUTO DE INVESTIGACIONES BIOTECNOLÓGICAS 'DR RODOLFO UGALDE' (IIBIO, UNSAM-CONICET) (1); UBA-CONICET (CIHIDECAR). FACULTAD DE CIENCIAS EXACTAS Y NATURALES. DEPARTAMENTO DE QUÍMICA ORGÁNICA (2)

The immunodominant glycotope alfa-Galp-(1-->3)-ß-Galp-(1-->4)-GlcNAc (also known as alfa-Gal), expressed in the mucins of the infective trypomastigote stage of Trypanosoma cruzi has been proposed for multiple clinical applications, from xenotransplantation or cancer vaccinology to serodiagnosis of protozoan caused diseases, including Chagas disease. However, methodological limitations have precluded its consistent clinical. It was previously shown that the trisaccharide analogue to alfa-Gal, with Glc in the reducing end, was as efficient as the natural trisaccharide for recognition of antibodies to alfa-Gal elicited during T. cruzi infections. We describe here the synthesis of α-Galp-(1-->3)-ß-Galp-(1-->4)-Glcp and α-Galp-(1-->3)-ß-Galp both functionalized as the 6-aminohexyl glycosides, and their conjugation to BSA. For the synthesis of the trisaccharide a lactose derivative, which already has the ß-Galp-(1-->4)-ß-Glcp motif, was used as starting material. For conjugation, the squarate method was chosen. The synthesized neoglycoconjugates were structurally characterized by biochemical and mass spectrometry studies and antigenically validated by conventional ELISA immunoassays. Both compounds were specifically recognized by serum samples of T. cruzi-infected patients. Moreover, competition assays allowed us to map the disaccharide α-Galp-(1-->3)-ß-Gal as the glycotope recognized by anti-α-Gal antibodies, thereby supporting the 'antigenic mimicry' between α-Galp-(1-->3)-ß-Galp-(1-->4)-Glcp and the natural α-Gal structure. The disaccharide was next conjugated to immunodominant peptides present in selected T. cruzi antigens. Further immunoassays using unconjugated peptides and 6-aminohexyl α-Galp-(1-->3)-ß-Galp as controls indicated that it is possible to develop bivalent serological reagents, able to display peptidic- and carbohydrate-based epitopes. Overall, these results indicate that our neo-glycoconjugates provide suitable, cost-effective and much needed tools for the improvement of currently used Chagas disease diagnostic applications.

0948 - PHOTODYNAMIC INACTIVATION OF GRAM POSITIVE AND GRAM NEGATIVE BACTERIAS BY THE AMINOPORPHYRIN A4 AND A4+

Lara Carolina GHIO(1) | Jimena MORA(2) | María Gabriela ALVAREZ(2) | Edgardo DURANTINI(2) | María Elisa LOMBARDO(3) | **Maria Del Carmen MARTINEZ** (3)

DEPARTAMENTO DE QUÍMICA BIOLÓGICA, FACULTAD DE CIENCIAS EXACTAS Y NATURALES, UBA. (1); DEPARTAMENTO DE QUÍMICA- FCEFQYN. UNIVERSIDAD DE RÍO CUARTO (2); CIPYP - UBA-CONICET Y FCEN, UBA (3)

Photodynamic inactivation (PDI) is an alternative therapy to antibiotics treatment against for localized infections, particularly those caused by conventional antimicrobial agents resistant organisms. PDI is based on a preferential accumulation of a photosensitizer (PS) by microbial cells; illumination induces photodynamic activity producing lethal damage in the cells. Porphyrins, due to their molecular structure, are used as PS in PDI. In this study the effect of the synthetic porphyrin 5, 10, 15, 20-tetrakis [4- (3-N, Dimethyl amino propoxy) phenyl] porphyrin (A4) and its protonated form (A4+) as PS to inactivate S. aureus and E. coli were studied. Microbial viability was determined by CFU/ml Petri dish counting. As light source, fluorescent tubes placed 20 cm from the sample (0.5 mW/cm^2) were use. Microorganisms were incubated with PSs and exposed 15 min in darkness and 15 min in light or 30 min in darkness. Inhibitory concentration 50 (IC50) values PS uptake by bacterias and oxidative damage were evaluated. To calculate selectivity index, cytotoxicity in Vero cells was studied. Porphyrins showed low cytotoxicity in mammal cells. IC50 values for to S. aureus in light condition were 2.4 ± 0.6 µM for A4 and 6.0 ± 0.7 µM for A4+. No inhibition was shown by E. coli with the concentrations studied. High uptakes levels of A4 and A4+ were observed independently of the illumination, around 2.0 µmol/ml of inoculum for S. aureus; while in E. coli the A4+ uptake was 3 times greater than for A4. PS uptake correlated with an increase in the cell internal oxidative status. Porphyrins plus light treatment increased 500 % the lipid peroxidation levels in S. aureus compared to controls, while in E coli TBARS levels were 300 % for A4 and 500 % for A4+. Both porphyrins were more effective against S. aureus than for E. coli, but A4+ showed a remarkable toxic effect in E. coli without reaching high mortality. In conclusion A4 and A4+ could be used for PDI against Gram positive bacteria.

0974 - PROLIFERATION OF TROPHOBLAST IN HUMAN TERM PLACENTAE CULTURED UNDER HIGH CONCENTRATIONS OF GLUCOSE (DIABETES IN VITRO MODEL) AND IN PLACENTAE FROM DIABETIC WOMEN, INFECTED WITH TRYPANOSOMA CRUZI (T. CRUZI)

Pablo B SALERA | María José MOREIRA ESPINOZA | Cintia M DÍAZ LUJÁN | Mariana PIEGARI | Daniela A EDELSTEIN |

Evangelina BENIZIO | Maria Fernanda TRIQUELL | **Ricardo Emilio FRETES** | Luciana MEZZANO

INSTITUTO DE BIOLOGÍA CELULAR (IBC), CÁTEDRA DE BIOLOGÍA CELULAR, HISTOLOGÍA Y EMBRIOLOGÍA, FCM UNC

Placental trophoblast maintenance involves a highly regulated cellular turnover dependent on a delicate balance between cells proliferation, fusion, differentiation and death. Structural alterations of the chorionic villi caused by T cruzi have been described in vitro, but little is known about trophoblast turnover in diabetic placentae infected. The aim of this study was to analyze if T. cruzi infection induces trophoblast proliferation changes in human placentae cultured under normal and high concentrations of glucose and in diabetic placentae.Paraffin-embedded blocks of diabetic and normal placentae (from archive) were cultured under normal and hyperglycemic conditions with 5.5 or 25 mmol/l D-Glucose, respectively (diabetes in vitro model) (COEIS 32/2016). Term human placentae were infected in vitro with $1x10^6$ trypomastigotes (Tulahuen strain) for 24 h. Immunohistochemistry to ki67 and Cytokeratin 7 (CTK7) were performed to evaluate trophoblast proliferation and integrity, and syncytiotrophoblast (STB) detachment, perivillous fibrin deposits and syncytial knots quantity were analyzed in Hematoxiline/Eosin stained sections. Fiji ImageJ was used for image quantification and ANOVA, Tukey's multiple comparison, and Student's t-test ($p<0.05$) were tested for statistical significance. Significant increase of syncytial knots quantity, intervillous fibrin deposits and STB detachment (%) in infected placentae under diabetic conditions (in vitro and in vivo), versus uninfected controls. CTK7 expression significantly increased in infected diabetic placentae compared to uninfected diabetic controls (no significant differences seen in vitro). Proliferation index (Ki67) was significantly higher in infected placentae, especially under diabetic conditions. Results suggest that trophoblast proliferation and integrity could be modified in diabetic and T. cruzi infected placentae, thus altering the effectiveness of the human placental barrier to protect against infections.

Oncología / Oncology III

Chairs: María Inés Díaz Bessone | Roxana Gorojod

0196 - PI3K MOLECULAR PATHWAY MODULATES P63 TRANSCRIPTION FACTOR PROTEIN LEVELS IN BLADDER CANCER (BC)

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INSTITUTO DE ONCOLOGÍA ANGEL H. ROFFO

Based in the need to develop more precise therapies, bladder cancer has been classified related to its gene expression. The biological and clinical significance of these signatures remains unclear. p63 characterize two important groups: Luminal Papillary and Basal/Squamous bladder tumors (Kamoun, 2018). Our hypothesis is that p63 acts as a protumoral factor, by activation of invasive signaling programs. Our previous results showed that p63 depleted bladder cancer cells have lower extracellular matrix degradation, migratory capacity, 3D and in-vivo growth rate than controls. In order to identify possible molecular pathways that modulate p63 and that could eventually be drug molecular targets, UMUC14 BC cells were treated with inhibitors of different pathways SB20358 (p38 pathway), PD98059 (MAPK pathway), LY29002 (PI3K pathway). p63 expression was tested by Western blot. 2D growth was measured by MTS. UMUC14 cells were grown in 3D conditions during 30 days treated with or without inhibitors and spheroids diameter was measured. All of the inhibitors showed a decrease in p63 expression, being LY29002 the one that showed the best and most sustained effect over time. This decrease in p63 expression (52 % less expression, $p<0.05$ unpaired t test) was accompanied by a decrease in the growth capacity in 2D (33 % less viability, $p<0.0001$ two way ANOVA) as in 3D (60 % less spheroid surface at day 27, $p<0.001$ two way ANOVA/Tukey) of UMUC14 cells. These results demonstrate that inhibition of the PI3K pathway could be an interesting therapeutic target for p63 positive bladder tumors.

0211 - NOVEL INTERACTION OF 14-3-3 ZETA/DELTA AND HO-1 IN PROSTATE CANCER

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Proteomic signatures of primary prostate cancer (PCa) and associated metastases may aid in the identification of key player proteins involved in progression. We have previously showed that heme-oxygenase 1 (HO-1), encoded by the gene HMOX-1, the rate-limiting enzyme in heme degradation, has a strong anti-tumoral effect in PCa. In an effort to identify HO-1 molecular partners which might collaborate with its biological function, we undertook an in-depth mass spectrometry-based proteomics study to find HO-1 interactors. We constructed a recombinant GSTHO-1 protein. PC3 cells were transiently transfected with GSTHO-1 or the respective control and treated with the stressor agent H_2O_2. Immunoprecipitated protein complexes were subjected to LC-ESI MS/MS. The proteomics analysis of HO-1 interacting factors revealed a list of 44 proteins including 14-3-3 zeta/delta, a protein encoded by YWHAZ, an androgen-responsive gene that activates proliferation, cell survival, and androgen receptor transcriptional activity. These results were validated by co-immunoprecipitation analysis. Immunofluorescence assays provided evidence that HO-1 and 14-3-3 zeta/delta co-localize in the cell nuclei under H_2O_2 treatment. Bioinformatics analysis were performed to investigate the clinical relevance of these two proteins using public database repositories. The Ross-Adams (GSE70769) dataset showed a negative correlation between HMOX-1 and YWHAZ expression ($r= -0.3286$; $p<0.0001$). When analyzing the ratio YWHAZ/HMOX-1 in patients with PCa, the relapse free survival increased almost 4 times when the ratio was ‹1.466 (HR= 3.76; p= 0.00023), i.e. when HMOX-1 expression increased. Moreover, high expression of HMOX-1 increased relapse free survival in patients with high YWHAZ expression (HR= 0.4; p= 0.025). In summary, our results, may cast a new light on PCa treatment highlighting a multifaceted role for HO-1 in inhibiting 14-3-3 zeta/delta function.

0213 - CROSSTALK BETWEEN ADIPOCYTES AND BREAST CANCER CELLS: MODULATION OF NUCLEAR RECEPTOR COACTIVATOR 3 (RAC3) IN FAT CELLS

María Cecilia LIRA | Francisco Damián ROSA | Mileni SOARES MACHADO | Alejandra PALMA | Gabriela MARINO | Mónica Alejandra COSTAS | Maria Fernanda RUBIO

INSTITUTO DE INVESTIGACIONES MEDICAS A LANARI (UBA - CONICET)

Adipocytes account for the largest proportion among the cells that comprise breast tissue. Although they are considered to be a critical cell type in the tumor microenvironment of breast cancer, there is still unclear the molecular mechanisms that control their behavior in this context. We have demonstrated that the transcriptional coactivator RAC3 decreases during adipogenesis and its high expression in adipose tissue adjacent to breast tumors correlate with different markers of tumoral progression in patient samples. Therefore, the aim of this work was to further study the role of this molecule in an in vitro model. Conditioned media (CM) were collected from non-tumoral or tumoral breast human cell lines with the following RAC3 mRNA levels: MCF10A: 1.55 ± 0.73, MDA-MB-231: 167.20 ± 6.91, T47D: 571.00 ± 12.59. 3T3-L1 derived-murine adipocytes were stimulated with CM or serum-free medium. RAC3

expression levels measured by qPCR resulted significantly higher in adipocytes stimulated with CM from high RAC3 levels-expressing T47D cells (9.25 ± 1.02) compared to the other conditions: basal (1.00 ± 0.00), MCF10A (2.33 ± 0.96), MDA-MB-231 (2.80 ± 1.19) ($p<0.05$). As RAC3 is a NF-kB coactivator we studied by immunofluorescence the presence of its phosphorylated subunit p65. We observed a greater fluorescence intensity in nucleus when adipocytes were stimulated with CM from tumoral cell lines (MDA-MB-231 or T47D) compared to the other conditions. Even more, since TNF is a NF-kB target gene, we evaluated by dot plot its levels in CM from adipocytes post-stimulation. Adipocytes secreted more TNF after being stimulated with CM from T47D (1.41 ± 0.03) compared to all other conditions: basal (1.00 ± 0.00), MCF10A (1.08 ± 0.11), MDA-MB-231 (1.02 ± 0.04) ($p<0.05$). The values were relativized to basal condition and results are shown as the mean ± SEM. These results validate our previous findings and suggest RAC3 as a molecular key to understand changes in the adipokines pattern in the tumor context.

0219 - GENE EXPRESSION AND CHIP-SEQ DATA ANALYSES CONFIRM FOXM1 AS A PLAYER IN PROSTATE CANCER BIOLOGY

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Dysregulation of the androgen receptor (AR) and glucocorticoid receptor (GR) expression is responsible, at least in part, for the development and progression of prostate cancer (PCa). GR might also have oncogenic or tumor suppressor activities depending on the presence/absence of AR and other regulatory cofactors such as the Forkhead Box (FOX). We previously showed the involvement of FOXM1 in cell migration, proliferation, viability, morphology, apoptosis, and the modulation of the transcriptional activity of GR and AR in PCa cells. The aim of this study was to validate the potential use of FOXM1 as a biomarker for PCa and understand the molecular mechanisms involved. We took advantage of public repositories to study the association between FOXM1 expression and the clinico-pathological characteristics of patients with PCa. We downloaded raw transcriptome data from 3 datasets GSE54460, GSE94767 and PRAD-TCGA. The expression of FOXM1 and AR were positively correlated with Gleason score ($p<0.05$). We also observed significant higher expression of FOXM1 and AR in patients who relapsed ($p<0.02$ and $p<0.05$, respectively). Since FOXM1 modulates the transcriptional activity of AR and GR, we analyze publicly available data from ChIP-seq studies: 1) LNCaP cells treated with Dexamethasone + IP-GR, 2) LNCaP cells treated with Testosterone + IP-AR, and 3) MDA-MB-231 and MCF7 cells + IP-FOXM1. We identified 439 gene promoters having binding sites for all three abovementioned factors. Thirty-eight of these genes have been previously related to PCa; and 5/38 have strong associations with FOXM1, AR and GR: BMP7, NCOA3, SMAD2, FAM120B and AHR. Altogether, the in-vitro and bioinformatic analyses demonstrate the involvement of FOXM1 in PCa. In addition, the identification of genes regulated by AR, GR and FOXM1 give new insights in the biology of PCa. Because there are approved drugs for all three factors, these results might lead to new potential therapeutic strategies to treat PCa.

0227 - EVALUATION OF LOCAL AND IMMUNOLOGIC EFFECTS OF BORON NEUTRON CAPTURE THERAPY (BNCT) COMBINED WITH BACILLUS CALMETTE-GUERIN (BCG) IN AN ECTOPIC COLON CANCER MODEL

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COMISIÓN NACIONAL DE ENERGÍA ATÓMICA (CNEA) (1); INSTITUTO DE ONCOLOGÍA ANGEL H. ROFFO (2); FACULTAD DE ODONTOLOGÍA-UBA (3)

BNCT combines selective tumor uptake of 10B compounds and neutron irradiation. The aim of the present study was to evaluate the local therapeutic efficacy, abscopal effect (out-of-field effect) and cytotoxicity of BNCT combined with Bacillus Calmette-Guerin (BCG) as immunotherapy in the BDIX rat ectopic colon cancer model. BDIX rats were inoculated subcutaneously with syngeneic colon cancer cells in the right hind flank. Four weeks post-inoculation the tumor bearing rats were treated, i.e. BNCT-group: BNCT mediated by borono-phenyl-alanine (BPA) at RA-3 Nuclear Reactor; BNCT+BCG-group: BNCT+three intratumoral applications of BCG; BCG-group: BCG only; Beam only-group (BO-group): irradiated without BPA; BO+BCG-group; Sham-group: same manipulation, no treatment. Two weeks post-BNCT, colon cancer cells were inoculated in the contralateral left hind flank to assess abscopal effect. Tumor volume was measured in both legs weekly. Seven weeks post-BNCT the animals were euthanized, samples were taken for histology and proximal lymph nodes were evaluated. A cytotoxicity test was carried out with splenocytes and colon tumor cells. BNCT, BCG and BNCT+BCG groups exhibited significantly greater local tumor response and abscopal effect vs Sham-group ($p<0.05$). The percentage of animals with metastatic spread in proximal lymph nodes was significantly lower in the BNCT-group (11 %) and the BNCT+BCG-group (10 %) vs. Sham-group (48 %, $p<0.05$). The BCG and BNCT+BCG groups exhibited the highest percentage (43 %) of animals that recover normal toxicity levels vs. Sham-group (28 %). The present study demonstrated that BNCT and BNCT combined with immunotherapy induce robust therapeutic and abscopal effects. A trend towards potential synergy between BNCT and immunotherapy was observed and warrants further study.

0233 - ANALYSIS OF CIRCULATING TUMOR DNA BY DIGITAL PCR IN LIQUID BIOPSIES FROM CUTANEOUS MELANOMA PATIENTS

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CENTRO DE INVESTIGACIONES ONCOLÓGICAS-FUCA (1); INSTITUTO ALEXANDER FLEMING (2); INSERM (3)

Cutaneous melanoma (CM) is the most dangerous skin cancer with increasing prevalence. A current challenge is to establish parameters that allow rapid monitoring of the clinical response in patients to different therapies. Analysis of liquid biopsies can allow the detection of biomarkers in body fluids from patients, related to their systemic tumor-load in real-time. The goal of this work was to analyze the correlation of circulating tumor DNA in liquid biopsies from CM patients with their clinical response. A prospective study was designed for stage-III and stage-IV CM patients, either receiving targeted-inhibitors or immune checkpoint blockade. Blood samples were collected at 0, 6 and 12 weeks following treatment. Circulating-free DNA (cfDNA) was obtained from plasma samples and absolute quantification of the prevalent BRAFV600E oncogene was performed by digital PCR. At present, 18 patients were included: 3 stage-III and 15 stage-IV; 5 receiving inhibitors and 13 receiving immunotherapy. With 10/18 BRAFV600E tumor biopsies, no mutation was detected in cfDNA proceeding from WT tumors. cfDNA was detected in all samples analyzed (n= 48), with a sensitivity of 0.1 %. No BRAFV600E cfDNA was detected in stage-III patients, which remained disease-free. Decrease-to-zero BRAFV600E levels were observed in 4 stage-IV patients achieving Stable-Disease or Partial-Response, either receiving inhibitors or immunotherapy (2:2). Patients with high initial BRAFV600E cfDNA levels progressed, including 2 patients with decreasing although

still detectable BRAFV600E cfDNA levels by the end of the follow-up period. Analysis of this initial population showed that cfDNA levels were detected in CM patients from different clinical stages and receiving different treatments. Initial levels of tumor circulating DNA and variations through treatment were in line with patients´ clinical response. These results highlight the potential to monitor tumor burden in CM patients through the study of liquid biopsies.

0234 - ACIDOSIS PROMOTES BREAST CANCER CELL MIGRATION THROUGH THE AHR / C-SRC SIGNALING PATHWAY

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A reduction in extracellular pH (pHe) is a characteristic of most malignant tumors. The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor localized in a cytosolic complex with c-Src kinase, allowing it to trigger non-genomic effects through c-Src. Considering that the slightly acidic tumor microenvironment promotes breast cancer progression in a similar way to the AhR/c-Src axis, we propose that this pathway could be activated by the change in the pHe. We evaluated the effect of pHe 6.5 on AhR/c-Src axis (WB) in two breast cancer cell lines (MDA-MB-231 and LM3) and in a mammary epithelial cell line (NMuMG), as well as its correlation with cell migration (wound healing assay) and metalloproteases (MMP)-2 and 9 activities (zymography). The pHe was adjusted with an isotonic solution of HCl. Results showed that acidosis increased c-Src phosphorylation only in breast cancer cells (MDA-MB-231 at 4-6 h, $p<0.01$; LM3 at 2-4 h, $p<0.05$). The presence of the AhR inhibitor 4,7-o-phenanthroline (PHE, 50 µM) prevented the c-Src activation in MDA-MB-231 ($p<0.05$). Acidosis increased MDA-MB-231 and LM3 cell migration ($p<0.01$) and MMP-9 activity ($p<0.05$). Besides, all these effects were blocked with PHE or the c-Src inhibitor PP2 (0.2 nM) ($p<0.05$). The indicator BCECF-AM was used to measure the cytosolic pH (pHi) in MDA-MB-231, showing a decrease in pHi from 7.6 to 6.9 after treatment with pHe 6.5 ($p<0.001$). To asses if the AhR/c-Src activation is related to pHi reduction, MDA-MB-231 cells were treated with 100 µM amiloride, an inhibitor of the Na+/H+ exchange 1 protein, which is known to reduce the pHi. Amiloride induced c-Src phosphorylation and this was prevented with PHE ($p<0.01$). In conclusion, acidosis stimulates the AhR/c-Src axis only in breast cancer cells, enhancing cell migration and MMPs activity. Although the AhR mechanism of activation still remains elusive, the reduction in pHi could be involved.

0236 - IMMUNIZATION WITH THE CSF-470 VACCINE PLUS BCG AND RHGM-CSF INDUCED IN A CUTANEOUS MELANOMA PATIENT A TCRB REPERTOIRE FOUND AT VACCINATION SITE AND TUMOR INFILTRATING LYMPHOCYTES THAT PERSISTED IN BLOOD

Mariana ARIS (1) | Alicia Inés BRAVO(2) | Heli Magalí GARCIA ALVAREZ(3) | Ibel CARRI(3) | Enrique PODAZA(1) | Paula Alejandra BLANCO(1) | Cecilia ROTONDARO(4) | Sofía BENTIVEGNA(4) | Morten NIELSEN(3) | María Marcela BARRIO(1) | José MORDOH(1)

CENTRO DE INVESTIGACIONES ONCOLÓGICAS-FUCA (1); HOSPITAL INTERZONAL GENERAL DE AGUDOS EVA PERÓN (2); INSTITUTO DE INVESTIGACIONES BIOTECNOLÓGICAS (IIB-UNSAM-CONICET) (3); LELOIR INSTITUTE FOUNDATION - IIBBA CONICET (4)

The CSF-470 cellular vaccine plus BCG and rhGM-CSF increased distant metastases-free survival in Cutaneous Melanoma (CM) patients stages IIB-IIC-III relative to medium dose IFN-a2b (CASVAC-0401 study). Patient-045 developed a mature vaccination site (VAC-SITE) and a regional cutaneous metastasis (C-MTS) which were excised during the protocol, remaining disease-free 36 months following vaccination. CDR3-TCRB repertoire sequencing in PBMC and tissue samples, along with skin-DTH score and IFN-G ELISPOT assay were performed to analyze the T-cell immune response dynamics throughout the immunization protocol. Histopathological analysis of the VAC-SITE revealed a highly-inflamed granulomatous structure encircled by CD11c+nested-clusters, brisk CD8+ and scarce FOXP3+ lymphocytes; with numerous Langhans multinucleated-giant-cells and macrophages. A large tumor-regression area fulfilled the C-MTS with brisk lymphocyte infiltration, mainly composed of CD8+PD1+ T-cells, CD20+ B-cells, and scarce FOXP3+ cells. Increasing DTH score and IFN-G ELISPOT assay against CSF-470 vaccine-lysate was evidenced. TCRB repertoire analysis revealed for the first time the presence of common clonotypes between a VAC-SITE and a C-MTS; most of them persisted in blood by the end of the immunization protocol. In-vitro boost with vaccine-lysate revealed expansion of persistent clones infiltrating the VAC-SITE and/or the C-MTS. Expansion of such persistent clonotypes might derive from two different although complementary mechanisms: proliferation of specific clones as well as expansion of redundant clones, which increased the number of nucleotide rearrangements per clonotype, suggesting a functional antigenic selection. In this patient, immunization with the CSF-470 vaccine plus BCG and rhGM-CSF induced a T-cell repertoire at the VAC-SITE that was able to infiltrate an emerging C-MTS, resulting in the expansion of a T-cell repertoire which persisted in blood by the end of the 2-year treatment.

0240 - INVOLVEMENT OF MICROENVIRONMENT FACTORS IN THE PTHRP EFFECT ON THE AGGRESSIVE BEHAVIOR OF COLORECTAL CANCER CELLS

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PTHrP is a factor from the tumor and its microenvironment that has been associated with the aggressiveness of different types of cancer. Colorectal cancer (CRC) is a heterogeneous disease where microenvironment and tumor factors act together leading to the progression of the tumor towards advanced stages. Previously, we observed that in the cell line HCT116 derived from human CRC, the treatment with exogenous PTHrP stimulates its transcription and also modulates the expression of markers associated with aggressive behavior such as E-Cadherin, FAK, Met and CD44. In addition PTHrP activates mitogenic pathways in HMEC-1 endothelial cells. To understand the PTHrP role in tumor progression through its influence on microenvironment cells, in this work we first evaluated in HMEC cells the effects of PTHrP in the regulation of cytokines expression and we observed by Western blot analysis that the treatment with the hormone for 1 and 16 h increases protein levels of TGF-ß, which is a protumoral factor known to be closely linked to PTHrP. Then we evaluate the effect of the conditioned medium (CM) from the HMEC-1 cells treated with PTHrP on the intestinal tumor cells HCT116. According with our previous results regarding to PTHrP direct action on HCT116 cells, the CM also induces an increase in the protein expression of FAK and CD44 in a time-dependent manner, and decreases the protein level of E-cadherin but at shorter times respect to the direct action of PTHrP. Previously we found that PTHrP increases the protein levels of Met in HCT116 cells and herein we observed by CM treatment the same effect but at longer times of exposure. The results of this work allow us to postulate a mechanism based in the action of PTHrP on tumor niche cells leading to the subsequent release of factors to the environment that could contribute to its protumoral effect.

0246 - BROWNING OF WHITE ADIPOSE CELLS BY INTERMEDIATE METABOLITES: AN ADAPTIVE MECHANISM TO TUMOR ENVIRONMENT

Mariana GANTOV (1) | Priscila Ayelén PAGNOTTA(1) | Gustavo RINDONE(2) | Maria Fernanda RIERA(2) | Silvina Beatriz MERONI(2) | Juan Carlos CALVO(1) | Judith TONEATTO(1)

IBYME-CONICET (1); HOSPITAL DE NIÑOS RICARDO GUTIÉRREZ, CEDIE - CONICET (2)

Interaction between epithelial cells and the adipose environment is a fundamental step in the regulation of tumor behavior in mammary cancer. It has been shown that adipocyte differentiation, browning and thermogenesis, contribute to lactate metabolism, leading to a Warburg effect. We, previously, demonstrated morphologic changes in adipocytes indirectly cocultured with breast cancer cells, suggesting a first step towards browning. We, now, suggest that soluble factors released by breast cancer cells impact adipose lactate metabolism by regulation of MCTs expression. We evaluated the effect of 72 h conditioned media (CMs) from a normal mouse mammary epithelial cells (NMuMG) and three mouse mammary cancer cell lines (LM3, 4T1 and MC4L1), as well as indirect coculture (IC, transwells) on UCP1 uncoupling protein, MCT4 lactate transporter expression (Western blot) and lactate release into the medium by 3T3-L1 adipocytes, compared to control adipocytes. Results obtained (times over control) showed that the cancer cell lines increased UCP1 (CMs: Control= 1, NMuMG= 2.10, LM3= 2.14, 4T1= 5.05, MC4L1= 2.92; IC: Control= 1, NMuMG= 3.82, LM3= 2.12, 4T1= 4.19, MC4L1= 1.28), as well as MCT4 expression (CMs: Control= 1, NMuMG= 1.26, LM3= 1.33, 4T1= 2.95, MC4L1= 2.56; IC: Control= 1, NMuMG= 2.25, LM3= 0.86, 4T1= 2.30, MC4L1= 1.29). When lactate released into the culture medium, an increase was also observed with the cancer cell lines (CMs: Control= 1, LM3= 1.06, 4T1= 1.32; IC: Control= 1, LM3= 2.43, 4T1= 4.08). In conclusion, taken together, these results clearly show the metabolic and phenotypic switch (browning) on 3T3-L1 adipocytes when presented to soluble factors secreted by mammary epithelial cancer cells. This could represent an adaptive mechanism to changes in the microenvironment.

0249 - TRANSCRIPTOMIC ANALYSIS OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA AT DIAGNOSIS AND ITS ASSOCIATION WITH CLINICAL EVOLUTION

Maria Mercedes ABBATE (1) | Maria Cecilia RICCHERI(2) | Laura ORELLANO(3) | Marta ALONSO(2) | Karina GUIÑAZU(4) | Marcela GUTIERREZ(4) | Luis AVERSA(4) | Virginia SCHUTTENBERG(3) | Elba VAZQUEZ(1) | Javier COTIGNOLA(1)

IQUIBICEN (UBA-CONICET). FACULTAD DE CIENCIAS EXACTAS Y NATURALES UBA. (1); HOSPITAL NACIONAL POSADAS (2); 4HOSPITAL SOR MARIA LUDOVICA (3); HOSPITAL DE NIÑOS "RICARDO GUTIERREZ" (4)

The identification of new biomarkers or gene-expression profiles in childhood for acute lymphoblastic leukemia (ALL) could help predicting the disease outcome, improving the response to treatment and reducing therapy-related toxicity. For this purpose, we collected samples from three hospitals (H. Posadas, H. Gutierrez, H. Ludovica) by bone marrow aspiration and isolated total RNA from 37 pediatric patients with de-novo ALL at time of diagnosis to perform paired-end transcriptome analysis (RNAseq). Clinico-pathological characteristics and disease outcome were evaluated and recorded by trained oncohematologists. We performed differential gene expression analysis between early response to prednisone and occurrence of relapse/death (event free survival, EFS). We considered that genes were differentially expressed if the FDR adjusted p-value= 0.05. We performed multivariate analyses including, when necessary, date of transcriptome, gender and risk group as covariates. We found 22 significant differential expressed genes (DEG) for EFS: 12 (54.5 %) were protein-coding genes and 10 (45.5 %) were non-coding RNA genes. Among the protein-coding genes we found MYLK3 (log2FC= 3.9, adj.p= $1.2x10^{-9}$) and PTPRB (log2FC= 3.9, adj.p= $2.2x10^{-5}$). MYLK3 over-expression was associated with poor prognosis in bladder, liver, colon and gastric cancers. PTPs genes are reported as tumor suppressors but it was also associated with increased risk of colorectal metastasis. In the case of response to prednisone we found 40 DEG (75 % protein-coding). Among them we detected the ABCG2 gene (log2FC= 3.1, adj.p= $1.5x10^{-3}$), an ATP Binding protein that functions as a xenobiotic transporter and which may play a major role in multi-drug resistance. In acute myeloid leukemia it was associated with remission failure and shorter disease-free survival. In conclusion, the study of gene expression profiles at diagnosis might help improving risk stratification, therapy efficacy and reducing the occurrence of relapse and toxicity.

0254 - ANALYSIS OF THE CAPABILITY OF DOXORUBICIN DELIVERED FROM MAGNETIC NANOCARRIERS TO INDUCE CHANGES IN CELL DEATH OF COLORECTAL CANCER CELLS: A POTENTIAL ONCOLOGICAL THERAPY

María Julia MARTÍN (1) | Pamela AZCONA(2) | Verónica LASSALLE(2) | Claudia Rosana GENTILI(1)

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Colorectal cancer (CRC) is a disease with a great probability of treatment failure, leading to a high mortality rate. For this reason, science focuses on the development of new therapeutic strategies, including the use of magnetic nanoparticles (MNP), which are being studied for biomedical applications related to CRC. In previous studies, we observed that MNP functionalized with folic acid loaded with Doxorubicin (DOX), named DOX-MAG, internalized into cells derived from human CRC, leading to a decrease of live cell number compared to free DOX treatment even at lower concentrations. This work aims to deepen studies regarding the cell death triggered by DOX-MAG, elucidating the associated molecular mechanisms. By light and fluorescent microscopy, we observed that DOX-MAG induced an increase in the size of the nuclei at 8 hours of exposure, suggesting intensive polyploidization with a dose of 1 µM, being this response absent in free drug conditions at the same dose. In addition, after 24 hours, MNP stimulated the emergence of elongated protrusions, being the drug found in the cytosol and the nucleus, while the free drug is completely located in the nucleus. By Western blot analysis, we observed an increment of cleaved PARP protein and the downregulation of the cell cycle inhibitor p21 after DOX-MAG uptake by the cells respect to free DOX conditions. These findings support the idea of faster cell death, with the apparition of apoptotic morphological features compared to free drug treatment. Summarizing, these results suggest that DOX-MAG markedly increased the effect of doxorubicin on human CRC models probably due to a different mode of action which may involve a dissimilar type of cell death. In this context, this contribution expands the knowledge of the behavior of nanocarriers in contact with in vitro models and proposes the DOX-MAG as potential theranostic agents for the improvement of cancer treatment.

Reproducción / Reproduction II

Chairs: Verónica Bosquiazzo | Vanina Da Ros

0054 - IMPACT OF MATERNAL OVERNUTRITION ON THE SPERM QUALITY OF MALE OFFSPRING IN RATS

Maria Agustina MENEGHINI | Rocío Alejandra GALARZA | **Alicia Graciela FALETTI**

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Maternal overnutrition may induce multiple pathologies in both mother and offspring. The risk of these diseases has a direct relation to the degree of overweight or severity of maternal obesity. The aim of the present work was to study the effect of maternal overnutrition, particularly with high fat content, on the sperm quality of the male offspring. To this end, male offspring from rats fed standard (SD) or cafeteria (CD) diet were used. Considering the overweight of the CD rats when they became pregnant (day 0) and to relate the effects observed in the offspring to the different degree of maternal overweight, offspring from CD (OCD) rats were divided into two groups: offspring from rats with 25 % and 35 % overweight (OCD25 and OCD35, respectively). Offspring were euthanized at 60 days of age. Weight gain, preputial separation, sperm count, sperm motility, sperm capacitation by acrosomal reaction, and the presence of the reactive oxygen species (ROS) by flow cytometry in the germ cells, using a fluorescent probe (2',7'-dichlorofluorescein diacetate), were examined. Compared with OSD and expressed as percentage, both OCD groups showed an increase in the weight gain (13-33, $p<0.001$), decrease in the sperm count (33-50, $p<0.05$) and sperm motility (15-31, $p<0.01$). Likewise, OCD35 exhibited delayed puberty, expressed as days (42.8 ± 0.3, $p<0.01$), lower number of acrosome-reacted sperm, expressed as percentage (47 ± 7, $p<0.01$), and higher fluorescein intensity, expressed as relative units (9 ± 2, $p<0.001$), compared with OSD group (41.0 ± 0.2, 71 ± 3; 2.3 ± 0.3; respectively). These results suggest that the maternal overnutrition, particularly with high fat content, throughout the intrauterine life and lactation, severely affects the quality of sperm, likely leading a subfertility condition.

0062 - MTORC1 REGULATION OF GLYCOLYTIC METABOLISM IN PROLIFERATING SERTOLI CELLS (SC)

Cecilia Lucía CENTOLA | Agostina GORGA | Gustavo Marcelo RINDONE | María Del Carmen CAMBEROS | Eliana Herminia PELLIZZARI | María Fernanda RIERA | Silvina Beatriz MERONI | María Noel GALARDO

CENTRO DE INVESTIGACIONES ENDOCRINOLÓGICAS "DR. CÉSAR BERGADÁ" (CEDIE)-CONICET

The final number of SC reached during the proliferative periods determines sperm production capacity in adulthood. It is well known that FSH is the major SC mitogen exerting its action by activation of PI3K/Akt/mTORC1 dependent pathway. mTORC1 regulates a wide range of cellular functions, including metabolism and cell proliferation. Proliferating cells seem to rely on aerobic glycolysis in order to support anabolic reactions associated with cell cycle progression. Although mature SC metabolism was thoroughly evaluated, the metabolism regulation in proliferating SC has been neglected. The aim of this study was to analyze whether aerobic glycolysis is regulated by FSH through mTORC1 pathway in proliferating SC. SC obtained from 8-day old rats were maintained under basal conditions (B) or stimulated with FSH in the absence or presence of rapamycin (Rap) -specific mTORC1 inhibitor. Lactate (Lac) -glycolytic flux marker- production and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase isoform 3 (PFKFB3) -isoenzyme which catalyzes the synthesis of a positive allosteric modulator for 6-phosphofructo-1-kinase (PFK1)- expression were evaluated. Results are expressed as mean ± SD of three independent experiments (different letters indicate statistically significant differences, $p<0.05$). It was observed that Lac production and PFKFB3 expression were increased by FSH and inhibited by Rap (PFKFB3: FSH: 1.72 ± 0.10b, FSH+Rap: 1.23 ± 0.07c fold variation vs. B). In addition, to evaluate the association of aerobic glycolysis with cell cycle progression in proliferating SC, BrdU incorporation and cyclin (CCN) D1 expression were evaluated in the presence of 3PO -specific inhibitor of PFKFB3. 3PO inhibited BrdU incorporation and FSH-stimulated CCND1 expression (FSH: 1.65 ± 0.15b, FSH+3PO: 1.00 ± 0.10a). These results suggest that mTORC1 pathway is involved in the regulation of aerobic glycolysis by FSH and that glycolysis is necessary to assure cell cycle progression in proliferating SC.

0285 - ASSESSMENT OF TEMPERATURE, PRESSURE, HUMIDITY AND DAYLIGHT IMPACT UPON SEMEN QUALITY

Gustavo Luis VERÓN (1) | Andrea Daniela TISSERA(2) | Fernando BELTRAMONE(3) | Gustavo ESTOFAN(3) | Rosa Isabel MOLINA(2) | Monica VAZQUEZ DE LEVIN(1)

IBYME-CONICET (1); LAR (2); CIGOR (3)

Testicular function is a temperature-dependent process, and high summer temperatures have been linked to lower sperm concentration and count. This study aims to assess the impact of temperature, pressure, humidity and daylight on semen quality. Semen samples were obtained from men subjected to semen evaluation as part of routine andrology workup (n= 11,657; January 2010- November 2016) and computer-assisted sperm analysis (CASA; n= 4,705) at the Laboratorio de Andrología y Reproducción (LAR), following WHO 2010 criteria. Temperature, pressure, humidity and daylight (average, minimum and maximum) readings were obtained from the Servicio Meteorológico Nacional for Córdoba region. Statistical analyses were performed with R (significant differences <0.05). Multiple regression analysis revealed that sperm concentration, count, total-motile spermatozoa and normal-motile spermatozoa were significantly correlated to average humidity, daylight, and minimum temperature and humidity. On the other hand, sperm motility was significantly correlated to average humidity, whereas sperm velocity (VSL, VCL and VAP) was significantly correlated to temperature (daily average, minimum and maximum) and maximum humidity. Moreover, sperm progression (LIN, STR, WOB) was correlated to temperature (average, minimum and maximum) and daylight. Among said climatic variables, temperature explained most kinematic variations, depicting a negative correlation with CASA variables. On the other hand, humidity explained variations in most routine semen parameters variations with a positive correlation. Thus, a deleterious effect of low humidity and high temperatures was found for routine semen parameters and sperm kinematics, respectively, in a large cohort of samples assessed under same laboratory standards subjected to strict quality assurance.

0524 - THE INFLUENCE OF CABERGOLINE ON THE OFFSPRING PHENOTYPE OF HUMAN CHORIONIC GONADOTROPIN (HCG) SECRETING FEMALE MICE: DOES MOTHER'S MILK MAKE THE DIFFERENCE?

Agustina MARCIAL | Ricardo S. CALANDRA | Susana Beatriz RULLI

IBYME-CONICET

Transgenic female mice expressing human chorionic gonadotropin-ß (hCGß+) produce elevated levels of hCG, prolactin and progesterone, show precocious puberty, are infertile and develop pituitary tumors. We have previously demonstrated that a short-term treatment of juvenile hCGß+ females with the dopamine agonist cabergoline normalizes the phenotypic changes of hCGß+ females. Even more, the treatment prevented phenotypic alterations on the transgenic offspring. The aim of this study was to determine if the cabergoline treatment has its effect during pregnancy and/or lactation. Two groups of 2-month-old wild-type (WT) females were mated with hCGß+ males: 1) Six-week-old WT females pretreated with cabergoline (500 µg/kg, i.p.), every other day for one week (WT-CAB mothers); 2) WT females without treatment (WT- mothers). Offspring from each mother was exchanged at birth and analyzed at three weeks of age. Transgenic offspring from WT-CAB mothers that ingested milk from WT mothers showed phenotypic alterations as exhibited in hCGß+

females, in terms of vaginal opening and increased uterus weight, as indicators of precocious puberty. On the other hand, the phenotype of transgenic offspring from WT mothers that received milk from WT-CAB mothers was normalized in terms of vaginal opening, uterus weight and ovarian gene expression of Lhcgr, Cyp11a1, Cyp17a1 and Cyp19a1 (qPCR). To analyze if the milk makes the difference, other group of WT females previously mated with hCGß+ males was treated with cabergoline during lactation from day 1 after birth for one week (0.1 µg/kg, i.p., every other day). Female transgenic offspring also showed a normalized phenotype at 3 weeks of age. These results suggest that cabergoline has an impact on the offspring during the lactating period and protect them from the phenotypic alterations induced by hCG hypersecretion. The molecular mechanisms involved in this phenomenon remains to be further investigated.

0600 - TROLOX ANTIOXIDANT EFFECT ON REFRIGERATED PORCINE SPERM FUNCTIONALITY

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Although porcine spermatozoa have a high proportion of unsaturated fatty acids in their plasma membrane, making them more susceptible to damages caused by temperature descent and oxidative stress, sperm refrigeration is a commonly used biotechnology in this species. Energy is generated mainly in the mitochondria involving the activity of the creatine kinase shuttle. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) is a vitamin E analogue used as antioxidant, which proved to improve sperm quality post-freezing in several species. The aim of our work was to study the changes in functional parameters in porcine sperm refrigerated with or without Trolox. Sperm capacitation was analyzed using the epifluorescent chlorotetracycline technique, plasma membrane functionality by hypo-osmotic test, sperm vitality using Trypan blue stain, acrosome integrity by differential interferential contrast, mitochondrial membrane potential by 5,5',6,6'-Tetrachloro-1,1',3,3'-tetraethyl-benzimidazolylcarbocyanine iodide (JC-1) stain, creatine kinase activity spectrophotometrically and in vitro fertilization (IVF) by the observation of pronuclear formation. Samples were suspended in a commercial diluent in a 1:10 proportion and refrigerated with or without Trolox at 17ºC. Data were analyzed using ANOVA and Tukey test ($p<0.05$, n= 7). Fresh semen presented 74.54 ± 8.79 % progressive motility and a vigor value of 3. The addition of Trolox improved sperm vitality in refrigerated samples ($p<0.05$), but the capacitation and acrosome reaction percentages remained low as in fresh semen. Regarding mitochondrial membrane potential, the lowest level was observed in samples refrigerated without the antioxidant respect to samples treated with Trolox and fresh semen ($p<0.05$), but there were no differences in creatine kinase activity between treatments. Although plasma membrane functionality in refrigerated sperm improved with the addition of Trolox ($p<0.05$), no differences were detected in pronuclear formation after IVF. Therefore, Trolox would have a protective effect on refrigerated sperm by conserving plasma membrane functionality and normal mitochondrial function, which may produce low capacitation and high vitality percentages to allow oocyte fertilization.

0692 - MOUSE SPERM DISPLAYS A CATSPER-INDEPENDENT RAPID INCREASE IN INTRACELLULAR CALCIUM DURING CAPACITATION.

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Freshly ejaculated mammalian sperm do not have the ability to fertilize oocytes. They must undergo a functionally defined process called capacitation, which allows them to develop hyperactivated motility and the ability to undergo acrosomal exocytosis. Sperm become capacitated in vivo by interacting with the female reproductive tract or in vitro in a defined capacitation medium that contains bovine serum albumin (BSA), calcium (Ca^{2+}), and bicarbonate (HCO_3^-;). We previously have shown that only a subpopulation of live sperm respond to incubation in capacitating medium by increasing the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). This increase became rapidly evident, within one minute of incubation, and it remained sustained for the 90 minutes of capacitation. In this work, flow cytometry was used to analyse early changes in $[Ca^{2+}]_i$ during capacitation. For this purpose, sperm were double stained with the Ca^{2+} dye Fluo-4 AM in combination with propidium iodine to analyze these changes in individual live sperm. We observed that the early increase in $[Ca^{2+}]_i$ was dependent on the presence of extracellular Ca^{2+}. In addition, it was determined that, unlike the late capacitation-associated $[Ca^{2+}]_i$ increase, the early rise was independent of CatSper channels, as sperm derived from CatSper knockout (CatSper KO) or incubated in the presence of CatSper inhibitors showed a significant increase in $[Ca^{2+}]_i$. In accordance to these findings, inhibition of sAC/PKA pathway didn't affect this early increase. Our results suggest that Ca^{2+} may be entering into the cell trough a Ca^{2+} channel and not a pump, as sperm incubated in the presence of Ni^{2+} failed to increase $[Ca^{2+}]_i$. We also determined that this rise is mainly promoted by the presence of BSA in the capacitation media. Taken together, our results indicate that there is a rapid entry of Ca^{2+} into a subpopulation of capacitated sperm, through a Ca^{2+} channel sensitive to BSA but not HCO_3^-, and independent of the sAC/PKA pathway.

0800 - CHARACTERIZATION OF THE ENDOCYTIC PATHWAYS INVOLVED IN COMPENSATORY ENDOCYTOSIS IN ACTIVATED EGGS

Matías GOMEZ ELIAS (1) | Lucas Nicolás GONZÁLEZ(1) | Rafael FISSORE(2) | Patricia Sara CUASNICÚ(1) | Debora Juana COHEN(1)

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Exocytosis of egg cortical granules occurs a few minutes after the entry of the fertilizing sperm as part of "egg activation" and it is involved in the block to polyspermy. In this regard, we have recently described, for the first time, the occurrence of compensatory endocytosis (CE) after massive cortical granule exocytosis, probably as a plasma membrane homeostasis mechanism. The aim of this work was to get further insights into the endocytic pathways involved in this internalization process. CE was evaluated through pulse-chase experiments in $SrCl_2$-activated eggs employing rhodamine-coupled Lens Culinaris Agglutinin (LCA), which binds to cortical granule exudate, in the presence of inhibitors of different endocytic pathways. We first observed that after treatment with $SrCl_2$, cortical exudate in the egg membrane decreased significantly over time, consistent with an operating endocytic mechanism. By contrast, Ca^{2+} ionophore-activated eggs, in which CE is impaired, did not exhibit any reduction in cortical exudate after several hours, highlighting once again the mechanistic differences between activation methods. We next evaluated the role of actin filament dynamics in CE through the use of cytoskeleton disruptors (Cytochalasin D (10 µM), Latrunculin A (10 µM)) or a microfilament stabilizer (Jasplakinolide (0.5 µM) after $SrCl_2$ activation. All these compounds produced a remarkable decrease in the relative amount of internalized LCA. Neither 5 µM filipin, which inhibits caveolae-mediated endocytosis, nor PitStop 2 (15 µM), a clathrin N-terminal domain blocker, produced a

reduction in the internalized LCA-labeling compared to control. Finally, the addition of 80 µM dynasore, a dynamin inhibitor, produced a significant decrease in LCA internalization. Altogether, these results indicate that CE in activated mouse eggs is not dependent on clathrin or caveolae but on actin dynamics and dynamin activity.

0975 - RELEVANCE OF CYSTEIN-RICH SECRETORY PROTEINS FOR MALE FERTILITY

Ludmila CURCI | **Valeria SULZYK** | Mariana WEIGEL MUÑOZ | Patricia CUASNICÚ

IBYME-CONICET; CÁTEDRA DE QUÍMICA, CICLO BÁSICO COMÚN, UBA

Cysteine-rich secretory protein (CRISP) 1, 2, 3 and 4 are mainly expressed in the reproductive tract and have key roles in mammalian fertilization. In spite of this, knockout (KO) mice for each individual protein are fertile whereas double KO (DKO) CRISP1/CRISP4 are subfertile, suggesting the existence of compensatory mechanisms between homologous CRISP family members. Recent results from our lab revealed that DKO CRISP1/CRISP3 are also subfertile. Based on this, the aim of the present work was to investigate the mechanisms underlying the lower fertility rates observed in these animals. In order to do this, we first analyzed the percentage of fertilized eggs recovered from the ampulla of superovulated females mated by DKO1/3 or control males. As no significant differences between groups in these in vivo fertilization rates were observed, the recovered fertilized oocytes from both groups were incubated in vitro for additional 5 days to analyze their subsequent development. Results showed that the percentage of oocytes from mutant males that reached the blastocyst stage under these conditions was significantly lower than that corresponding to controls, suggesting that CRISP1 and CRISP3 may be important for early embryo development. To investigate potential functional deficiencies in mutant sperm that could be responsible for these observations, DKO1/3 and control sperm were co-incubated in vitro with eggs (surrounded by both cumulus oophorus and zona pellucida or denuded of these coats) and the percentage of fertilized eggs determined. Results revealed significantly lower fertilization rates for mutant than for control sperm, confirming defects in mutant sperm fertilizing ability. Together, these observations support the role of CRISP1 and CRISP3 for male fertility and fertilization and contribute to a better understanding of how paternal factors could impact on embryo development.

Toxicología / Toxicology II

Chairs: Pablo Evelson | Paola Ingaramo

0086 - EFFECTS OF GLYPHOSATE (G) AND ROUNDUP (R) ON IMMATURE RAT SERTOLI CELL (SC) PROLIFERATION

Agostina GORGA | Gustavo M. RINDONE | Cecilia CENTOLA | Eliana H. PELLIZZARI | María Del Carmen CAMBEROS | María Fernanda RIERA | María Noel GALARDO | Silvina B. MERONI

CENTRO DE INVESTIGACIONES ENDOCRINOLÓGICAS "DR. CÉSAR BERGADÁ" (CEDIE)-CONICET

A declining trend in human fertility has been described and exposure to xenobiotics, such as herbicides, emerges as a potential cause. We have shown that G and its commercial formulation R alter blood-testis barrier between neighboring SC, which would partly explain the decrease in reproductive function observed after herbicide exposure. This observation points the SC as a plausible target for G or R effects. In rats, SC proliferation occurs during fetal and postnatal periods up to 15 days of age. As each SC supports a limited number of germ cells, the number reached during proliferative periods will be decisive for spermatogenic capacity. Thus, disruption of any SC proliferative stage would compromise fertility. The aim of this work is to analyze whether exposure to G or R can alter postnatal SC proliferation. SC cultures from 8-day-old rats were treated with 100 ppm of G or R in the absence or presence of FSH, the main SC mitogen. Proliferation was evaluated by BrdU incorporation. It was observed that R, but not G, decreased FSH-stimulated SC proliferation (FSH: 19.8 ± 2.3; FSH + R: 9.7 ± 1.4*, X ± DS, *p<0.05). Additionally, it was observed that R decreased cyclin D1 and D2 and increased p21Cip expression (p<0.05), evaluated by RT-qPCR. For in vivo studies, male pups were assigned to control and R groups receiving daily sterile saline solution or 50 mg/kg R ip, from postnatal day (pnd) 3 to 7, respectively. At pnd8, pups were injected with BrdU (50 mg/kg) before sacrifice to evaluate cell proliferation. No changes in BrdU incorporation in SC and in testis weight was observed (n= 4/group). In addition, histological analysis showed normal organization of the seminiferous epithelium. The results obtained show that although R could decrease in vitro SC proliferation, these effects could not be observed in vivo. Altogether the results suggest that the harmful effects of R on adult reproductive function would not be mediated by alterations in SC proliferation.

0264 - LENS REDOX IMBALANCE AFTER URBAN AIR POLLUTION EXPOSURE

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UNIVERSIDAD DE BUENOS AIRES, FACULTAD DE FARMACIA Y BIOQUÍMICA, QUÍMICA GENERAL E INORGÁNICA. IBIMOL (1); UNIVERSIDAD DE BUENOS AIRES. FACULTAD DE FARMACIA Y BIOQUÍMICA.TECNOLOGÍA FARMACÉUTICA (2); UNIVERSIDAD DE BUENOS AIRES. FACULTAD DE MEDICINA. DEPARTAMENTO DE PATOLOGÍA (3)

Particulate matter (PM) present in air pollution produces adverse effects on the eye. Oxidative stress has been suggested to play a key role in the toxic mechanism. Lens antioxidant system maintains the redox status of nearby ocular structures. The aim of the study was to evaluate the redox balance in mice lens after the exposure to urban air pollution. 8-week-old Balb/c male mice were exposed to urban air or filtered air (UA-group and FA-group, respectively) in exposure chambers located in highly populated area of Buenos Aires city (average level of PM: 25.6 ± 0.8 µg/m^3). The animals were exposed for 8 h/day, 5 days/week, up to 12 weeks (CICUAL-FFYB, CUDAP 50946/16). Superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) activity, levels of reduced and oxidized glutathione (GSH and GSSG) and protein oxidation (PO) were evaluated in lens lysates. After 1 and 2 weeks of exposure, UA-group presented no significant differences in all measurements compared to the FA-group, except for SOD activity that was increased after 1 week (107 %, p<0.05). After 4 weeks, an increase in GR activity was shown in UA-group (47 %, p<0.05). After 12 weeks, GPx activity was increased in UA-group (63 %, p<0.05), meanwhile GR activity decreased (40%, p<0.05) as well as the GSH/GSSG index (62 %, p<0.05), compared to FA-group. PO increased in UA-group (113 %, p<0.05), and an inverse correlation was found between PO and GSH/GSSG index (r= -0.9114, p<0.001). GPx activity and GSH/GSSG index also presented an inverse correlation (r= -0.7421, p<0.001) in UA-group. These results suggest that urban air pollution exposure alters the redox balance of the lens, which could affect the antioxidant defenses of nearby ocular structures. The correlation between the PO and GSH/GSSG index indicates that lens GSH pool could prevent the protein oxidation, which has been suggested as one of the triggers of cataracts.

0290 - EXPOSURE TO HEXACHLOROBENZENE INDUCES ENDOCRINE ALTERATIONS

ASSOCIATED WITH ENDOMETRIOSIS PROGRESSION

Florencia CHIAPPINI (1) | Leandro Joaquín CEBALLOS(1) | Carla OLIVARES(2) | Juan Ignacio BASTÓN(2) | Carolina Andrea PONTILLO(1) | Noelia V MIRET(2) | Mariana FARINA(3) | José Javier SINGLA(4) | Gabriela MERESMAN(2) | Andrea Silvana RANDI(1)

DEPARTAMENTO DE BIOQUIMICA HUMANA, FACULTAD DE MEDICINA UBA (1); IBYME-CONICET (2); CENTRO DE ESTUDIOS FARMACOLÓGICOS Y BOTÁNICOS (CEFYBO-CONICET), FACULTAD DE MEDICINA, UBA (3); HOSPITAL DE CLINICAS, FAC MEDICINA UBA (4)

Endometriosis is a chronic illness hormone-dependent which is defined by the presence of endometrial tissue outside the uterus. The endocrine disrupting chemicals may be involved in the development and progression of disease. Hexachlorobenzene (HCB) is a pesticide that acts as an endocrine disruptor modulating the hormonal signaling. Aberrant expression of estrogen and progesterone receptors (ER, PR) has been associated with progression of endometriosis, which is referred to as estrogen-dependent and progesterone-resistant. Aromatase is the key enzyme in the estrogen biosynthesis and it is essential for establishment and growth of endometriosis lesions. Previous results showed that HCB induces cell migration and invasion of endometrial cells, increases ERalfa; and reduces PR levels in lesion and eutopic endometrium in rat model. Our aim was to evaluate the dependence of ER on migration (scratch motility assay) and invasion (transwell assay) in endometrial stromal cells (T-HESCs), and to investigate the hormone receptor profile (WB) in T-HESCs and primary cultures of endometrial stromal cells from eutopic endometrium of control (CESC) women. Cells were exposed to HCB (0.005, 0.05, 0.5 and 5 µM) for 24h. Results showed that the pesticide enhanced cell migration (HCB 5 µM, $p<0.001$) and invasion (HCB 0.5 µM, $p<0.001$) in an ER dependent manner in T-HESCs. Moreover, HCB increased expression of Aromatase (0.005, 0.05 and 0.5 µM, $p<0.01$) and ERalfa; (0.5, $p<0.05$), while it reduced PR protein levels (5 µM, $p<0.05$). Instead, the ERß levels were not modified. In CESC, HCB enhanced ERalfa (0.5 µM, $p<0.05$), ERß (0.05, µM $p<0.05$) and Aromatase (0.5 µM, $p<0.05$) protein levels. Also we compared the HCB exposure effects with 17-ß-estradiol, observing that both showed a similar action on REalfa protein expression. In conclusion, our results demonstrated that HCB would act as a xenoestrogen inducing an invasive profile contributing to the development and progression of the disease.

0303 - NEUTRALIZING CAPACITY OF ANTISERA OBTAINED BY IMMUNIZATION WITH BOTHROPS ALTERNATUS VENOM BLOCKED IN THEIR METALLOPROTEINASES

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Specific treatment for snake bite accidents with antivenoms, obtained from immunized animals, is the only recommended therapy. Bothropic intoxication is characterized by proteolytic, coagulant and hemorrhagic effects that induce local tissue damage and systemic alterations that could lead to death. Snake venom metalloproteinases, zinc- dependent, play a relevant role in the pathogenesis of intoxication. They provoke a disruption of the hemostatic system, restricting their use as immunogen in high doses. In previous work, it was demonstrated that is it possible to immunize mice with high doses of Bothrops alternatus venom (BaV) neutralized with disodium salt of ethylenediaminetetraacetic acid (Na2EDTA) avoid of hemorrhagic lesions and then animals reported higher antibodies titer. The present study was performed to test the neutralizing ability of sera obtained from animals treated whit venom-Na2EDTA, and evaluate the effect of this immunogen on pulmonary parenchyma. Groups of 5 BALB/c mice were immunized subcutaneously with BaV, or BaV/Na_2EDTA emulsified with Freund's adjuvant (complete first and incomplete-booster). On day 50 serums were collected for neutralization assays: proteolytic activity on azocasein, phospholipase activity on erythrocytes and thrombine-like activity on citrated plasma. Animals were sacrificed and lungs removed for histological analysis (hematoxylin-eosin). The results showed that the capacity of neutralize each of one of the three enzyme activities assayed was significantly higher ($p<0.05$) by the serum obtained from animals immunized with BaV/Na_2EDTA. Histological analysis showed that the pulmonary parenchyma from immunized mice with BaV was significantly affected (pneumonitis, $p<0.05$), in respect to those were BaV/Na_2EDTA treated. Results demonstrated that the BaV/Na_2EDTA immunogen has a lower organic impact with respect to BaV and, at the same time, providing a serum with high neutralizing capacity.

0322 - PESTICIDES EXPOSURE ENHANCE HIF-1ALPHA, VEGF AND NOS-2 EXPRESSION IN MDA-MB-231 HUMAN BREAST CANCER CELLS

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Organochlorine pesticides such as Hexachlorobenzene (HCB) and organophosphate Chlorpyrifos (CPF) are synthetic compounds used worldwide as insecticides, herbicides and fungicides. Recently, HCB and CPF have become important as a potential risk factor in breast cancer. We demonstrated that these compounds induce proliferation, migration and invasion in human breast cancer cells. Angiogenesis plays a role in local tumor growth and metastasis. It has been observed a correlation between elevated levels of hypoxia inducible factor-1 alpha (HIF-1alpha), tumor metastasis and angiogenesis. HIF-1alpha induces genes like vascular endothelial growth factor (VEGF), nitric oxide synthase-2 (NOS-2) and cyclooxygenase-2 (COX-2). VEGF acts on tumor endothelial cells to increase their proliferation, survival and migration. COX-2 and NOS-2 promote tumor angiogenesis. The aim of our work was to examine the HCB or CPF action on breast cancer angiogenesis. We studied the effects of HCB (0.005, 0.05, 0.5 and 5 µM) or CPF (0.05, 0.5, 5 and 50 µM) exposure in MDA-MB-231 breast cancer cells in dose-response curves on: a) HIF-1alpha expression, b) VEGF secretion, c) COX-2 and d) NOS-2 protein levels (Western blot). Our results showed that MDA-MB-231 exposed for 6 h to CPF increases HIF-1alpha ($p<0.05$) and NOS-2 expression ($p<0.05$) at all assayed doses, as well as VEGF secretion at 0.05, 0.5 and 5 µM ($p<0.05$). Besides, CPF (0.05, 0.5 and 5 µM) stimulates COX-2 levels at 24 h ($p<0.05$). On the other hand, HCB for 6 h enhances HIF-1alpha levels at 0.05, 0.5 and 5 µM ($p<0.05$); NOS-2 expression and VEGF secretion at all assayed doses ($p<0.05$); meanwhile, at 24 h HCB (0.05 and 5 µM) increases COX-2 levels ($p<0.05$). In conclusion, our results demonstrate that HCB and CPF stimulate proangiogenic factors in MDA-MB-231 cells. Altogether, these data highlight that the pesticide exposure could promote the angiogenic processes contributing to mammary carcinogenesis.

0323 - HEXACHLOROBENZENE EXPOSURE INDUCES PRO-ANGIOGENIC MICROENVIRONMENT IN AN IN VITRO MODEL OF ENDOMETRIOSIS

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Endometriosis, characterized by ectopic growth of endometrial tissue, is a common gynecological disease. The symptoms are pelvic pain and infertility, affecting women quality of life. Angiogenesis is critical in the development and maintenance of endometriotic lesions. It is a multistep process of new blood vessel formation that involves secretion of growth factors and extracellular matrix degradation, as well as migration, proliferation, and tube formation by endothelial cells. Vascular endothelial growth factor (VEGF) is an angiogenic factor that plays an important role in endometriosis progression. Exposure to endocrine-disrupting environmental pollutants is associated with the disease etiology. Hexachlorobenzene (HCB) is a pesticide that induces toxic reproductive effects in laboratory animals. Previous results showed that HCB increases the volume of endometriotic like-lesions, microvessel density and VEGF levels in a rat endometriosis model. The present study examined the effect of HCB on endometriosis angiogenesis in vitro. Human endometrial stromal cells (T-HESCs) were exposed to HCB (0.005, 0.05, 0.5 and 5 µM) or vehicle for 48 h, and the conditioned media were then used to stimulate EA.hy926 endothelial cells to evaluate cell proliferation (MTT assay and PCNA expression), migration (scratch motility assay) and tube-like structure formation in a Matrigel assay. The results showed that HCB (0.005-0.05 µM) induced VEGF secretion ($p<0.05$) in T-HESCs cells. Moreover, the conditioned media treatment enhanced cell proliferation (0.005-5 µM, $p<0.05$), migration (0.005-0.5 µM, $p<0.05$), and tube formation increasing total tube length (0.005 µM, $p<0.05$; 0.5 µM $p<0.01$) and branching points (0.5 µM, $p<0.05$) in endothelial cells. Our results demonstrated that HCB exposure induces angiogenic factors secretion in human endometrial cells T-HESC, triggering an increase in the ability of endothelial cells to form new vessels, a critical event for the endometriosis progression.

0483 - SOLUBLE GUANYLYL CYCLASE ALPHA1 SUBUNIT: A NEW MARKER FOR ESTROGENICITY OF ENDOCRINE DISRUPTOR COMPOUNDS

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CENTRO DE ALTOS ESTUDIOS EN CIENCIAS HUMANAS Y DE LA SALUD, CAECIHS (UAI)

Endocrine disruptor compounds (EDCs) comprise naturally occurring and synthetic substances widely spread in the environment that adversely affect human and wildlife. Screening methods and ideal biomarkers to determine EDC potency need to be exhaustively improved. Soluble guanylyl cyclase alpha1 subunit (sGC alpha1) is an abundant cytosolic protein ubiquitously expressed in most tissues. We previously showed that sGC alpha1 is specifically and highly up-regulated by estrogen (E2) in vivo and in vitro, although it lacks estrogen responsive elements. The aim of this work was to evaluate sGC alpha1 protein expression as a potential marker for xenoestrogenic EDC exposure. First, the effect of E2 on sGC alpha1 expression was tested in several E2-dependent cell lines (MCF-7, ECC-1 and GH3). Following experiments were performed using GH3 cells since they are commonly included in in vitro EDCs screening tests. Cells were incubated for 48 h with a wide variety of xenoestrogenic EDCs: Cd, Pb, Cr, Ni, As, ethynylestradiol, diethylstilbestrol, bisphenol A, hexachlorobenzene, and chlorpyrifos at a range of doses from nM to pM. sGC alpha1 protein levels were determined by Western blot. E2 increased sGC alpha1 expression in all cell lines tested: MCF-7 (% of control (C), 130.53 ± 8.06*), ECC-1 (232.26 ± 6.94***), and GH3 (208.7 ±10***), (*$p<0.05$, ***$p<0.001$ vs. respective C). E2 augmented sGC alpha1 through estrogen receptor (ER) activation (sGC alpha1 protein levels, % of C; E2: 208.7 ± 10***, ICI 182,780: 113 ± 9, ICI 182,780+E2: 86 ± 5###, $p<0.001$ vs. respective C). sGC alpha1 expression was strongly up-regulated by all the EDCs tested even by those exhibiting low or null ER binding capacity ($p<0.05$). Natural hormones not binding ER (progesterone, prolactin, and insulin) were unable to modify sGC alpha1 levels. Here we provide evidence that in vitro sGC alpha1 protein assay may be a very sensitive and powerful tool to identify compounds with estrogenic activity, which could improve current mammalian-based screening methods.

0489 - CYTOTOXICITY OF ZINC NANOPARTICLES BIOSYNTHESIZED BY MICROORGANISMS ON HUMAN KERATINOCYTE CELL LINE

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CITAAC - CONICET - UNIVERSIDAD NACIONAL DEL COMAHUE (1); GRUPO VINCULADO AL IMBICE-GBEYB - CONICET - UNIVERSIDAD NACIONAL DE QUILMES (2); CITAAC-CONICET-UNIVERSIDAD NACIONAL DEL COMAHUE; FCQ-UNIVERSIDAD NACIONAL DE CÓRDOBA (3); IMBIV-CONICET-FCEFYN-UNIVERSIDAD NACIONAL DE CORDOBA (4); UNITEFA - CONICET - UNIVERSIDAD NACIONAL DE CÓRDOBA (5); CITAAC-CONICET-UNIVERSIDAD NACIONAL DEL COMAHUE; FACIAS-UNIVERSIDAD NACIONAL DEL COMAHUE (6)

Metal nanoparticles –NPs– (10-100 nm) have an important antimicrobial activity which suggests possible biomedical applications. Zinc NPs (ZnNPs) are widely used for different products. The aim of this work was to investigate the toxicity of ZnNPs biosynthesized by microorganisms, in a human keratinocyte cell line (HaCaT). ZnNPs were synthesized using Pseudomonas aeruginosa (ATCC 27853) and were characterized by UV-Vis spectroscopy and by transmission electron microscopy. HaCaT cells were incubated for 4 h and 24 h at different ZnNPs dilutions (1/2, 1/5 and 1/10). RPMI 1640 culture medium with 5% FBS, a metal precursor salt solution of $ZnSO_4$ (0.1 and 0.25 mM), and a bacterial growth control of biosynthesis (BGC), were used as controls. Cell viability was evaluated by MTT assay, crystal violet and neutral red tests; reactive oxygen species (ROS) were studied by DCF-DA; superoxide dismutase (SOD) activity was determined by riboflavin-NBT method; and reduced glutathione (GSH) by Ellman reactive. ZnNPs cell capture assays were performed by fluorescence microscopy and changes in cell migration were evaluated by wound healing assay. As determined by fluorescence microscopy ZnNPs were able to enter HaCaT cells. The toxicity assays indicated that cell viability was significantly altered by ZnNPs 1/2 and BGC conditions after 4 h and 24 h incubation. ROS levels increased after 4 h incubation with ZnNPs 1/2, 1/5, and BGC, while a 24 h incubation, 1/10 dilution also augmented ROS. SOD activity increases at all ZnNPs dilutions tested, and with BGC. GSH was not modified by any treatment. Finally, the presence of ZnNPs and BGC in the culture media affected cell migration. Altogether these results suggest that ZnNPs are able not only to enter into skin cells but also to modify human keratinocyte viability, oxidant/antioxidant cell balance and cell migration. More studies are needed to unravel the mechanisms underlying these alterations.

Bioinformática, genoma, proteoma y nuevas tecnologías / Bioinformatic III

Chair: Ezequiel Lacunza

0481 - RESPONSE OF MACROPHAGES IN CONTACT WITH SYNTHETIC BIOMATERIALS BASED ON POLY-N-ISOPROPYLACRYLAMIDE AND COPOLYMER HYDROGELS

Virginia CAPELLA (1) | Rebeca E RIVERO(2) | Ana Cecilia LIAUDAT(1) | Cesar A BARBERO(2) | Pablo BOSCH(1) | Claudia Rosana RIVAROLA(2) | Nancy RODRIGUEZ(1)

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Biomaterials are being developed in the last decades, in regenerative medicine field, to create tissue constructs that possess mechanical and physiological similarities with the tissue to be simulated. Hydrogels are one of the most promising materials due to their innate similarity with extracellular matrix (ECM), with mechanical properties adjustable and a good biocompatibility that allow cell adhesion and proliferation. Previously to use the biomaterial in vivo conditions, it is essential to study the macrophage-material interaction and consider it as a variable that influences the biocompatibility and the processes that govern tissue regeneration. The aim of this study was to analyze the response of macrophage RAW 264.7 in contact with biomaterials. Polymeric hydrogels based in poly-N-isopropylacrylamide with positive (3-acrylamidopropyl-trimethylammonium chloride, APTAC), negative (2-acrylamido-2-methylpropanesulfonic acid, AMPS) and neutral (N-acryloyl-tris-hydroximethylaminomethane, HMA) net charges were synthesized. MTT and neutral red uptake, nitric oxide quantification and attachment assays were performed at 1, 4 and 7 days of exposition, in order to assess cell viability and macrophage polarization, respectively. Cells without treatment were included as negative control. The results of cell viability and nitric oxide production did not differ among macrophages exposed to hydrogels and negative control ($p>0.05$). Cell adhesion and morphology varied according to the hydrogel charge net. These preliminary results indicate that PNIPAM based hydrogels could be used in futures applications as cell scaffold for tissue-engineered construct.

0579 - BULL SPERM SELECTION BY ATTACHMENT TO HYALURONIC ACID SEMI-INTERPENETRATED HYDROGLES

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Hyaluronic acid (HA) is a glycosaminoglycan present in oocyte-cumulus cell complexes. A population of motile mature spermatozoa with low incidence of genetic alterations preferentially interact with HA molecules. The aim of this work was to develop polymeric biomaterials containing HA for selection of bull spermatozoa based on their functional characteristics. Polymeric hydrogels composed of poli N-isopropylacrylamide-co-20% N-Tris (hydroxymethyl) methyl acrylamide semi-interpenetrated with HA (PNIPAM-HMA-HA) were synthesized and physical chemically characterized. The interaction and degree of frozen-thawed sperm/hydrogel surface binding was analyzed by phase contrast microscopy. Motility, viability, nuclear morphology, acrosome membrane integrity and plasma membrane functionality of attached/released and non-attached sperm populations were studied. Surfaces of hydrogels interpenetrated with HA were more hydrophilic according to angle of contact study; however presence of HA into the polymeric network was not associated with a change of the volume phase transition temperature. In addition, PNIPAM-HMA-HA hydrogels had higher swelling capacity than PNIPAM-HMA without HA. Fifty percent of frozen-thawed bull spermatozoa attached to PNIPAM-HMA-HA hydrogels and the 47% of them were released upon treatment with medium containing hyaluronidase. Selected sperm have acceptable characteristics of rectilinear motility (70 ± 2.58 %), high viability (58.7 ± 11.7 %), nuclear and cellular morphology and low percentage of acrosome reacted spermatozoa (23.3 ± 4.1 %). The plasma membrane integrity of the released population remained unchanged compared to initial sample. In conclusion, our results indicate that polymeric hydrogels semi-interpenetrated with HA could be useful to select high-quality sperm for use in assisted reproduction techniques.

0624 - USE OF FISH DIGESTIVE EXTRACT FOR CHICKEN FEATHER DEGRADATION

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LABORATORIO DE INVESTIGACIÓN EN PROTEÍNAS/NEA IQUIBA-UNNE

By-products from animal sources are currently used for the benefit of providing added value to production. In the poultry industry, feathers represent up to 8.5 % of chicken weight and constitute an important source of protein, mainly keratin. Due to the high aminoacids content, its processing to obtain hydrolysates becomes a commercial attraction. Besides, in the fishing industry, the viscera constitute 5 % of the total weight of the fish and are a waste for the environment. They are considered an alternative source of enzymes (proteases) of high commercial value by their industrial and scientific applications. In the present work an enzymatic extract from fish pyloric cecae (Pygocentrus nattereri) was assayed on feathers in order to evaluate its capacity of hydrolyzes this keratin source under different medium contiditions. Preparations of extracts were made by mechanical digestion of tissues in buffer pH 7.8, 1:5 g tissue/ml, and then centrifuged. Proteolytic activity of fish enzymatic extract (FEE) was tested using a-Nbenzoyl-DL-arginine-p-nitroanilide. For the hydrolysis, first, feathers were pre-treated with buffer 7.8, 1 % 2-mercaptoethanol (2-ME), for 20 min at 100 °C. Second, 7.0 U/ml FEE was added (1:5) and incubated for 6 days at 37 °C. After finishing hydrolysis stage, feathers were observed at optic microscope (OM) and sobrenadants were analyzed by UV spectra. Feathers were running in parallel under different treatments, such as absence of reducing agent, heat or FEE. OM analysis showed that FEE was capable of attack the feather structure, but the pre-treatment with 2-ME and heat increased the enzymatic action. UV 210-310 nm analyses reveled a major increment of absorbance at 250-280 range in those samples from feathers treated with FEE, 2-ME and heat. Results demonstrate that FEE from P. nattereri is capable of degrade the native structure of feathers, yielding free molecules of hydrolyzed keratin. This property gives FEE a potential industrial use.

0734 - EFFECT OF THE MEDIUM TALP ON THE SELECTION OF PIG SPERMATOZOAS THROUGH THE USE OF HYDROGELS

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In order to select sperm cells with high fertilizer quality, PNIPAM copolymerized with APTA-15 % hydrogel surfaces were used. This biomaterial is becoming very important due to its low cellular toxicity, positive vet charge and its great versatility. The aim of this work was to evaluate the binding and release capacity of pig sperm to surfaces of PNIPAM co-APTA-15% in TALP-Ca^{++} and TALP-without Ca^{++}. Initially, the effect of sperm manipulation on sperm viability of was evaluated by exposure to the TALP-Ca^{++} medium, without the presence of the hydrogel. In order to evaluate the effect of calcium on cell adhesion, pig sperm were exposed to PNIPAM co-APTA-15 % hydrogels surfaces, in TALP-Ca^{++} and TALP-medium without Ca^{++} for 30. Subsequently the medium was replaced by TALP-Ca^{++} and TALP-without Ca^{++} in order to analyze whether the cation affects sperm cells relase from hydrogel. Results

were statistically analyzed by one way ANOVA and Bonferroni as a post-hoc test ($p<0.05$). The results suggested that the presence of Ca^{++} in the manipulation medium increased sperm cells adhesion to hydrogel surfaces and the absence of this cation released the cells of the PNIPAM co-APTA 15 % surfaces. In addition, it was found that the hydrogel did not cause any alteration on the sperm viability, compared to the initial semen sample. These results suggest that the presence of calcium in sperm manipulation medium affect pig spermatozoa binding and release to PNIPAM co-APTA 15 % hydrogel surfaces.

0791 - MOLECULAR DETECTION OF BEE PATHOGENS IN HONEY

Martín EGUARAS | **Gregorio FERNÁNDEZ DE LANDA** | Giselle FUENTES | Sandra FUSELLI | Carolina GARCÍA IZA | Sandra MEDICCI | Eugenia OLIVERA | Santiago PLISCHUK | Silvina QUINTANA | Pablo REVAINERA | Sergio RUFFINENGO

CONICET

Honey bees have a wide variety of parasites and pathogens associated with their nests. One of them, the causative agent of the American foulbrood, Paenibacillus larvae, was previously found in bee honey. Considering that other spore-forming microorganisms are expected to remain latent in honey, the presence of, at least, microsporidia, spore- forming bacteria, and viruses protected by peptide structures might represent a threat. Parasites and pathogens that affect honey bees health seem to play a major role in the worldwide decline of pollinators, therefore their detection in honey could be used to prevent the spread of diseases among colonies. Honey from 57 apiaries located in Buenos Aires, Córdoba, Corrientes, Entre Ríos, Formosa, La Rioja, Neuquén, Río Negro and Santa Fe was collected between March and October, 2012. DNA was extracted from pollen obtained by centrifugation of 10 g. of honey samples and amplified by qPCR. PCR products were purified, sequenced and analysed using BLAST software. Honey from every apiary contained DNA of at least one pathogen, with a high occurrence of Apis mellifera Filamentous Virus (96.5 %) and the neogregarine Apicystis bombi (75.5 %). A lower proportion of samples were positive for Nosema ceranae (51 %), P. larvae (44 %), and Ascosphaera apis (28 %). Here, we report the presence of DNA of several bee pathogens in honey from commercial apiaries, and provide a fast and efficient screening method that could be useful to estimate pathogen presence in apiaries.

0802 - VALIDATION OF A FAST AND SIMPLE DIAGNOSTIC KIT FOR HLB CAUSAL AGENT DETECTION

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INSTITUTO DE CIENCIA Y TECNOLOGÍA "DR. CESAR MILSTEIN" (1); ESTACIÓN EXPERIMENTAL AGROPECUARIA - INTA MONTECARLO (2)

The Huanglongbing (HLB), is the most devastating disease in citrus worldwide, due to the damage it causes, the difficulty of diagnosis and the speed of its expansion. The causal pathogen, Candidatus Liberibacter spp (Ca. L), is transmitted by the insect Diaphorina citri. The species Ca. L asiaticus has been detected in Argentina, and the vector distributed at least in nine provinces. Since 2010, the Argentinian National Service for Health and Agro-Food Quality (SENASA), has implemented a National HLB Prevention Program to safeguard productivity in this important sector. Nowadays, HLB diagnosis is performed by PCR, nested PCR, real time PCR or some combination of them, requiring purified genomic DNA, sophisticated equipment and qualified human resources. The aim of this study was to evaluate the performance of a sensible, fast and simple diagnosis test based on specific DNA isothermal amplification of Ca. L asiaticus by comparison with PCR and qPCR, considered the Gold standard methods to HLB diagnosis. We applied the test in a group of samples whose true disease status was defined by the mentioned gold standard techniques. Analyzing the results by a 2 x 2 contingency table, we determined Sensitivity and Specificity of the test, and the positive and negative predictive values (PPV and NPV). In a first test, 30 DNA samples were analyzed and compared with qPCR technique with a concordance in 28 samples (PPV and NPV of 100 and 88.88 %, respectively). Adjusting test parameters of reading out, 23 new samples consisting on midribs and purified genomic DNA from uninfected or Ca. L. asiaticus infected plant lines were analyzed in a blind assay comparing with PCR/nested PCR applied by the Molecular Laboratory of the EEA Montecarlo (INTA), obtaining 100% of concordance (PPV and NPV, both 100%). The results obtained in the present study demonstrated a high quality of our diagnostic test, with low cost, making it a valid and useful tool to support the diagnosis of HLB disease.

*The authors contributed equally to this work.

0924 - EFFECTS OF IONIC DISSOLUTION PRODUCTS FROM BIOACTIVE GLASS-CERAMIC SCAFFOLDS ON THE CELLULAR AND MOLECULAR RESPONSE OF ENDOTHELIAL CELLS AND FIBROBLASTS UNDER HYPERGLYCEMIA

Luis Alberto HARO DURAND (1) | Maria Alejandra FANOVICH(2) | Aldo Rubén BOCCACCINI(3) | Alejandro GORUSTOVICH(4)

LABORATORIO DE PATOLOGÍA Y FARMACOLOGÍA MOLECULAR, IBYME-CONICET (1); INSTITUTO DE INVESTIGACIONES EN CIENCIA Y TECNOLOGÍA DE MATERIALES (2); INSTITUTE OF BIOMATERIALS, UNIVERSITY OF ERLANGEN-NUREMBERG (3); GRUPO INTERDISCIPLINARIO EN MATERIALES IESIING-UCASAL INTECIN UBA-CONICET (4)

Several aspects of tissue repair are altered in diabetes mellitus (DM) i.e., endothelial cells and fibroblasts are affected by cellular dysfunction in a hyperglycemic (HG) environment. Thus, it is of biomedical interest to study different therapeutic strategies to optimize the repair and/or regeneration of tissues under HG conditions. The aim of this work was to study the cellular and molecular response of ionic dissolution products (IDPs) released from 3D porous bioactive glass-ceramic scaffolds manufactured from a 45S5 glass (% w/w composition: 45 % SiO_2, 24.5 % Na_2O, 24.5 % CaO, and 6 % P_2O_5) added with 2 % of B_2O_3 (45S5.2B) in primary cultures of dermal fibroblasts (DFs) and endothelial cells (ECs) grown in HG (30 mM D-glucose). The results showed that IDPs from the 45S5.2B scaffolds positively modulate the in vitro proliferative and migratory response in both ECs and DFs grown under HG in comparison with controls. Further, IDPs improve the ability of ECs to form tubules in vitro. The supernatant from DFs grown in HG during 7 d and post-stimulated with IDPs for 2 d, showed significantly higher levels of secretory VEGF and it was able to increase the proliferative response of ECs. On the other hand, the IDPs from 45S5.2B were able to modulate key cellular signaling pathways altered in HG conditions. This was corroborated by changes in the phosphorylation status of MEK/ERK1/2, JNK/p38, PI3K/AKT, and a significant increase of relative levels of SIRT-1 and Nrf-2. Additionally, IDPs modulated the expression levels of procaspase 3/caspase 3, Bax and Bcl-2. These findings may be relevant in regenerative medicine since 45S5.2B scaffolds could act as inorganic agents that positively modulate the cellular and molecular response thus promoting processes of tissue repair and/or regeneration in patients with DM.

Gastroenterología / Gastroenterology

Chairs: Cristina Carrillo | María Laura Ruiz

0314 - SYNERGISTIC ANTITUMORAL EFFECT OF COMBINED GEMCITABINE WITH CATECHOL-RUTINOSIDE BY INHIBITING DCLK1 EXPRESSION IN PANCREATIC CANCER

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Doublecortin-like kinase1 (DCLK1) is upregulated in various cancers including pancreatic ductal adenocarcinoma (PDAC). Although the standard first-line treatment for pancreatic cancer is the use of gemcitabine alone, the resistance to this compound is a common cause of therapeutic failure in this type of cancer. Combined chemotherapy with a second drug constitutes an interesting strategy to inhibit malign cells and/or prevent the emergence of resistance. Owing to the difunctional benzene, catechol, has antitumor activity against many cancers while its effect on PDAC remains unknown. We explored the anti-cancer activity of an enzymatically glycosylated variant of the aromatic compound (catechol-rutinoside) combined with gemcitabine on the human pancreatic cancer cells (PANC-1). Interestedly, the addition of the glycosidic moiety, rutinose, significantly chemo-sensitized gemcitabine-resistant cells. We also evaluated the expression of DCLK1 in primary pancreatic tumoral cells, and found that DCLK1 expression y highly associated with gemcitabine resistance. Moreover, the effect of gemcitabine treatment combined with conjugated catechol exerted an interesting synergistic inhibitory effect on PDAC cells associated to the inhibition of the expression of DCLK1.

0315 - ANTITUMOR EFFECTS OF GLYCOSYLATED 4-METHYLUMBELLIFERONE IN A MURINE MODEL OF FIBROSIS ASSOCIATED- HEPATOCELLULAR CANCER

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INCITAP - CONICET – FCEN, UNIVERSIDAD NACIONAL DE LA PAMPA. (1); INSTITUTO DE INVESTIGACIONES EN MEDICINA TRASLACIONAL, CONICET-UNIVERSIDAD AUSTRAL (2)

Most of drugs lack of systems that direct them to the target tissues. In this way, a large part of drugs that remains in the bloodstream increases the secondaries effects in patients. The glycosylation of molecules is an interesting strategy for drug delivery to the specific target. The compound 4-methylumbelliferone (4MU) was described as inhibitor of the synthesis of hyaluronic acid and anticancer agent for hepatocellular carcinoma (HCC) associated with advanced fibrosis. In addition, treatment with 4MU remodel tumor microenvironment resulting in a potent inhibition of tumor growth. The aim of this work was to evaluate the antitumoral effect of the glycosylated 4MU. We modified 4MU, by the addition of a glycosidic moiety to obtain the product denominated 4-methylumbelliferilrutinose (4MUR). Half maximal inhibitory concentration (IC50) of 4MU and 4MUR were determined on both, human and murine liver tumor cell lines (HUH 7 and Hepa 1.6, respectively) and compared the toxicity on normal human fibroblasts. The tumor cell lines were significantly more sensitive to the glycosylated compound 4MUR in a dose-dependent manner ($p<0.05$). We also evaluated in vivo toxicity of both compounds in an orthotopic HCC established in fibrotic livers via intrahepatic inoculation of Hepa 129 cells. Five days after tumor implantation, a group of mice received 4MU and 4MUR treatments by i.p at a dose of 20 mg/kg per day, for 14 days. Tumor volume (mm3) was calculated and we found that 4MUR showed higher inhibition of tumor growth than 4MU. The 4MUR treatment did not cause any sign of tissue damage and liver histology was normal. Our results suggest that the glycosylated compound result safely and more effective, probably due to the specific targeting of hepatic tumor cells.

0518 - INHIBITION OF NITRIC OXIDE AGGRAVATES PANCREATIC MITOCHONDRIAL DYSFUNCTION DURING ENDOTOXEMIA

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Pancreas has been described to be early affected in endotoxemia. As mitochondria are the main source of ATP, it is essential to maintain mitochondrial bioenergetics during recovery from pancreatic cellular, tissue and organic damage in this syndrome. Moreover, high systemic NO levels observed during endotoxemia could be involved in pancreatic tissue damage, as well as initiating cellular recovery processes such as autophagy. The aim of this project was to analyze the relationship between the severity of the systemic inflammatory insult and pancreatic mitochondrial dysfunction during endotoxemia, focusing on NO as a possible damage modulator. Female Sprague Dawley rats (45 days) were i.p. injected with: vehicle (control); LPS 0.5 mg/kg (LPS 0.5) and LPS 8 mg/kg (LPS 8). Co-treatment with i.p. L-NAME 20 mg/kg was performed in all experimental groups. Blood NO levels (by EPR), as well as plasma nitrate/nitrite content increased 4-fold (LPS 0.5) and 7-fold (LPS 8) compared to control group ($p<0.05$). Plasma TNF-alpha; and IL-6 levels increase was related to the severity of endotoxemia. Pancreatic mitochondrial function was evaluated by mitochondrial oxygen consumption, ATP production and mitochondrial complex activities. ATP production was found significantly decreased (30 %) in both LPS-treatments (control value: 70.4 ± 5 nmol ATP/min mg protein, $p<0.05$). L-NAME co-treatment showed decreased NO markers without influencing the levels of TNF-alpha; and IL-6. However, L-NAME co-treatment significantly worsened mitochondrial function in all parameters studied. Our results show that the severity of the endotoxemic process is correlated to both blood NO levels and pancreatic mitochondrial dysfunction. In addition, they also suggest that NO is necessary to partially preserve pancreatic mitochondrial function in these pathologies.

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0529 - SPARC (SECRETED PROTEIN ACIDIC AND RICH IN CYSTEINE) PROMOTES LIPID DEPOSITION AND HEPATOCARCINOGENESIS IN NON-ALCOHOLIC FATTY LIVER DISEASE MODELS IN MICE

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Non-alcoholic fatty liver (NAFLD) is characterized by excessive lipid accumulation in >5% of hepatocytes. It encompasses a set of clinical conditions ranging from steatosis alone (NAFL) to NASH (non-alcoholic steatohepatitis), which is characterized by the presence of hepatocellular ballooning, inflammation and fibrosis. NAFLD can progress to cirrhosis and hepatocellular carcinoma (HCC). SPARC is a matricellular protein associated with inflammatory processes, tissue remodeling, regulation of fibrillar collagen deposits, among other biological functions. The aim of this study was to evaluate the role of SPARC in the development of HCC in NAFLD models in mice. Two days born SPARC knockout (SPARC-/-) or wild type (SPARC+/+) mice were subcutaneously injected with 200 µg of streptozotocin and fed with high fat (HF) or chow diet for 8 or 16 weeks. SPARC

and proinflammatory cytokines expression were measured by qPCR. Hepatic damage, fibrosis and tumor development were evaluated. Liver differential gene expression in 16 weeks mice HF-fed was analyzed by RNAseq. Primary hepatocyte cultures and SPARC knockdown HepG2 cell were used in free fatty acids presence to study SPARC effect on lipid droplets and expression of lipogenic genes. Results: HF diet induced SPARC overexpression after 8 weeks. Proinflammatory factors and collagen deposits were decreased in SPARC-/- HF despite increased in steatosis. After 16 weeks, all SPARC-/- HF mice (n= 10) developed spontaneous HCC while in SPARC+/+ HF mice (n= 14) incidence was about 60%. Pathways related to lipid metabolism and cellular detoxification were upregulated in SPARC-/- HF mice. SPARC inhibition in HepG2 showed an increase in triglyceride deposits. In SPARC-/- primary hepatocyte cultures we observed that genes involved in lipid transport and lipogenesis were overexpressed, coupled with an increase triglyceride synthesis. Conclusion: The absence of SPARC is associated with a low degree of inflammation and fibrosis in NASH, but exacerbated hepatocarcinogenesis.

0660 - GLYCOGEN SYNTHASE KINASE 3 ß (GSK3ß) PARTICIPATES IN ESTRADIOL-17ß-D-GLUCURONIDE (E17G) INDUCED IMPAIRMENT OF MRP2 FUNCTION IN ISOLATED RAT HEPATOCYTE COUPLETS (IRHC).

Ismael Ricardo BAROSSO | Romina Belén ANDERMATTEN | Virginia Soledad SCHUCK | Nadia CIRIACI | Diego TABORDA | Fernando Ariel CROCENZI | Enrique SANCHEZ POZZI

INSTITUTO DE FISIOLOGÍA EXPERIMENTAL (IFISE-CONICET). FAC. DE CS. BIOQUÍMICAS Y FARMACÉUTICAS. UNR

E17G induces acute cholestasis in rat with endocytic internalization of the canalicular transporter Mrp2, activating two different pathways, each one involving an estrogen receptor: ERalpha or GPR30. cPKC activation precedes that of ERalpha. cPKC probably uses intermediaries to phosphorylate ERalpha. Several kinases can be phosphorylated by cPKC and are able to phosphorylate the ERalpha, among them we find GSK3ß. The aim of this study was to evaluate the role of GSK3ß in the E17G-induced alteration of Mrp2 activity. IRHC were treated with GSK3ß inhibitors Li (3 mM) or BIO (1 µM) and then exposed to E17G (100 µM). To investigate in which pathway GSK3ß participates, IRHC were exposed to BIO and inhibitors of ERalpha (ICI182,780, ICI, 1 µM), cPKC (Gö6976, Gö, 1 µM) or PI3K (Wortmannin, W, 100 nM) before exposure to E17G. All preparations were incubated with CMFDA (intracellularly converted in gluthation-methylfluorescein [GMF], substrate of Mrp2). IHRC accumulating GMF in their canalicular vacuoles (cVA) were counted and compared to control IHRC. Results (% Control): GSK3ß inhibition (Li+E17G: 71 ± 7b; BIO+E17G: 70 ± 5b) partially prevented the effect of E17G (48 ± 4a) on cVA of GMF. The preventive effects of W (W+E17G: 75 ± 5b) and BIO on the decrease in cVA induced by E17G were additive (BIO+W+E17G: 91 ± 1a,c). Contrarily, the preventive effects of ICI (ICI+E17G: 69 ± 3b) or Gö (Gö+E17G: 77 ± 3b) did not modified BIO protective effects (BIO+ICI+E17G: 72 ± 2b) and (BIO+Gö+E17G: 77 ± 10b). a: significantly different from Control; b: significantly different from E17G and Control; c: significantly different from E17G+BIO and E17G+W. BIO, Li, W, Gö, and ICI did not affect % cVA. ($p<0.05$, n= 3). GSK3ß inhibition protects against E17G-induced impairment of Mrp2 transport, indicating a role of the kinase in estrogen cholestasis. Co-inhibition studies suggest that GSK3ß participates in the same pathway of ERalpha and cPKC and in different pathway of PI3K (downstream of GPR30).

0875 - ATRIAL NATRIURETIC PEPTIDE (ANP) ENHACES ANTIOXIDANT CAPACITY IN EXPERIMENTAL ACUTE PANCREATITIS

Ana Paula COURREGES (1) | Guadalupe ALVAREZ(1) | Mario CONTIN(2) | Federico OCHOA(3) | Fabiana LAIRION(4) | Marisa REPETTO(4) | Marcelo VATTA(5) | Liliana G. BIANCIOTTI(1)

INSTITUTO DE INMUNOLOGÍA, GENÉTICA Y METABOLISMO (INIGEM-UBA-CONICET), FFYB, UBA (1); DEPARTAMENTO DE TECNOLOGÍA FARMACÉUTICA, FFYB, UBA (2); INSTITUTO DE FISIOLOGÍA Y BIOFÍSICA B HOUSSAY, FAC. MEDICINA, UBA (3); INSTITUTO DE BIOQUÍMICA Y MEDICINA MOLECULAR (IBIMOL-UBA-CONICET), FFYB, UBA (4); INSTITUTO DE QUÍMICA Y METABOLISMO DEL FÁRMACO (IQUIMEFA-UBA-CONICET), FFYB, UBA (5)

We previously reported ANP attenuates the severity of acute pancreatitis by reducing trypsinogen activation and the inflammatory response. Recent studies support that endoplasmic reticulum (ER) stress and oxidative stress (OS) precede these events. Indeed, we showed that ANP attenuates ER stress and stimulates ER-dependent apoptosis. ER stress is intimately related to OS in the pathophysiology of numerous diseases. Given that the exocrine pancreas is rather susceptible to OS due to the extremely weak expression of antioxidant enzymes, in the present we sought to establish whether ANP affected OS in experimental AP by studding the main antioxidant (enzymatic and non-enzymatic) defense. AP was induced in Sprague-Dawley strain rats (200-220 g) by four repetitive cerulein injections (40 µg/Kg). Thirty minutes before the first cerulein injection animals were infused with either saline (control) or ANP (1 µg/Kg/h) for 60 min. Following euthanasia (60 min after the last cerulein injection) pancreatic samples were harvested for further assays (CICUAL-FFYB #4107/18). ANOVA followed by a Student's t test modified by Bonferroni was used for statistical analysis. Results are expressed as the means±S.E.M. and p values of 0.05 or less were considered statistically significant. AP induces OS as previously reported. ANP stimulated Nrf-2 nuclear translocation (assessed by immunohistochemistry) which is a transcription factor that induces the expression of antioxidant enzymes ($p<0.001$). ANP also enhanced the activity of superoxide dismutase (SOD) ($p<0.05$), catalase ($p<0.01$) and glutathione transferase. Furthermore it also restored reduced glutathione and total glutathione levels (assessed by HPLC- tandem mass spectrometry) to control values ($p<0.05$). Present findings show that ANP enhances the antioxidant defense capacity of the exocrine pancreas in AP, further supporting its beneficial role in the disease.

Neurociencias / Neurosciences III

Chairs: Claudia Bregonzio | Analía Reinés

0324 - EFFECT OF LITHIUM IN PYRAMIDAL NEURONS OF CORNUS AMMONIS

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UBA, FAC MED, DEPTO PATOLOGÍA, CPEA; HOSPITAL ALEMÁN; CONICET (1); UBA, FAC MED, DEPTO PATOLOGÍA, CPEA; HOSPITAL ALEMÁN (2); UBA, FACULTAD DE MEDICINA, DEPARTAMENTO DE FISIOLOGÍA, LABORATORIO DE ENDOCRINOLOGÍA (3); UBA, FACULTAD DE MEDICINA, DEPTO DE PATOLOGÍA, CENTRO DE PATOLOGÍA EXPERIMENTAL Y APLICADA (4); FLENI, DEPTO NEUROPATOLOGÍA; CORTE SUPREMA DE JUSTICIA, MORGUE JUDICIAL, LAB HISTOPATOLOGÍA (5); UNIVERSIDAD FAVALORO, FUNDACIÓN INECO, INCYT; CONICET (6)

Lithium (Li) is a first-line drug for long-term prophylactic treatment of bipolar disorder (BD). However, mechanisms by which lithium exerts its mood-stabilizing effects are not very clear. A decrease in the overall volume of the hippocampus (H) by imaging studies has been described in patients with BD, it has also been reported that treatment with Li would reverse this effect, highlighting its neuroprotective effect. The aim of this work was to evaluate the effect of Li on pyramidal neurons within Cornu Ammonis (CA) subregions of the H. Wistar male rats (n= 16) were randomized into two groups: control group (CG) fed ad libitum powered standard

diet and experimental group (EG) fed ad libitum the same diet supplemented with 60 mmol of lithium/kg diet for 1 month. Lithium serum levels were measured and reached therapeutic values in EG (0.57 ± 0.18 mmol/L). The brains were removed for histopathological analysis, fixed, and cut coronally. From each brain we selected a section (Bregma -2.8 mm) and stained with cresyl violet. First, we took serial pictures of the entire CA region with a 60x objective starting at the midline (CA1-2-3). Serial photos were divided into 4 groups, and the first 5 photos from each of them were selected for the analysis. Then, using the Image J Software we measured the area of the cell body and nucleus of CA pyramidal neurons on each selected picture. The criteria for selecting neurons to be measured included a well-defined nucleus and nucleolus. All assessments were performed blinded to Li treatment. We observed that the mean size (μm^2) of the neuronal soma and nucleus of pyramidal neurons in the third group were significantly larger: CG= 140 ± 24 vs. EG= 174 ± 36, t= -2.15, p= 0.049 for cytoplasm; and CG= 75 ± 12 vs. EG= 92 ± 16; t= -2.28, p= 0.038 for nuclear size. This sub-region could correspond to CA2 subfield. Our results support the theory that lithium acts at the H level producing an increase of the cell and nuclear area of the pyramidal neurons in a specific sub-region of the CA.

0337 - EVIDENCE OF CANNABINOID MODULATION IN NUCLEAR SIGNALING

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INIBIBB-CONICET, DEPTO. BIOLOGÍA, BIOQUÍMICA Y FARMACIA-UNS

The endocannabinoid system (ECS) is a signaling mechanism involved in many pathophysiological processes, especially in the nervous system. Former studies from our lab demonstrated the presence of the different components of the ECS in nuclei from rat cerebellum and cerebral cortex (CC). We detected diacylglycerol lipase and monoacylglycerol lipase activities which are involved in maintaining the levels of the endocannabinoid 2-arachidonoyl-glycerol. In addition, we demonstrated a nuclear CB1 protein expression by Western Blot and immunocytochemistry. CB1 is a GPCR receptor that triggers different signaling cascades, modulating intracellular Ca^{2+} levels, ERK1/2 phosphorylation, and other second messengers at the plasma membrane. Nevertheless, we also observed an increase in ERK phosphorylation in isolated nuclei from rat cerebral cortex (CCN) incubated with a CB1 synthetic agonist (WIN 55-212-2). Phosphorylated ERK could be involved in activating MSK1/2, a nuclear kinase that phosphorylates histone 3 (H3). The aim of this work was therefore to study how a cannabinoid agonist modulates ERK1/2 and H3 phosphorylation in CCN. To this end, CC from Wistar rats were dissected and homogenized, and highly purified nuclei (CCN) were isolated on a sucrose-density ultracentrifugation. CCN were subsequently incubated at 37 °C with WIN and ERK1/2 and H3 signaling cascades were studied by Western Blot. Interestingly, it was observed that ERK1/2 and H3 phosphorylation increased in nuclei treated with WIN 5 µM for 30 min with respect to controls ($p<0.05$). As expected, this was reversed by pre-incubating for 10 min with a MEK inhibitor (U0126, 10 µM), however, no changes were observed when a CB1 antagonist (SR141716, 1 µM) was used ($p<0.05$). Taken together, these results demonstrate that cannabinoids at nuclear level could modulate H3 phosphorylation by ERK1/2 signaling. This indicates a potential role of these lipids in chromatin regulation and gene expression in cerebral cortex.

0339 - SILDENAFIL EFFECTS ON MEMORY AND FUNCTIONAL AND STRUCTURAL PLASTICITY IN THE HIPPOCAMPUS

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Sildenafil (SILD) is a drug widely used in clinical practice for its inhibitory effects on phosphodiesterase type 5 (PDE-5), that generate increases in cGMP levels, indirectly enhancing the signaling pathway activated by nitric oxide (NO/ GC / GMP). SILD crosses the blood brain barrier, and PDE-5 is expressed in the brain. In the hippocampus (HP), NO increases glutamate release, which is essential for long-term potentiation (LTP) maintenance, a phenomenon of synaptic plasticity that underlies the formation of learning and memory. An acute exposure to SILD improves memory consolidation in mice and previous results from our laboratory showed a facilitation in the generation of LTP in HP 2 hours later, however little is known about the persistence of these changes in that structure and its correlation with learning and memory processes. The objective of the work is to evaluate the effect of SILD on the acquisition of HP-dependent memories and characterize the persistence of the functional and anatomical changes produced in this structure 24 hours, 7 and 30 days after the administration of SILD. For this purpose, male Wistar rats were administered with SILD or saline before training in the "step-down", object recognition test and Y maze, and 4 or 24 hours later the memory acquisition was evaluated. Immediately after the test, 7 or 30 days later, the animals were sacrificed for electrophysiological and neuroanatomic experiments of dendritic spine density. Our results showed that animals administered with SILD have a longer latency time in the step-down test compared to the control group, but a lower rate of exploration of the new object in the object recognition test, while preliminary data on Y maze showed no changes arm discrimination rate compared to control group. On the other hand, SILD improves the synaptic plasticity of HP, reducing the threshold to induce LTP at all times measured, and increases the density of total spines in the HP. These results indicate that SILD would have selective effects on different types of memories, and would induce persistent changes in the functional and structural plasticity of the HP, which temporarily coincide with the effects on memory. It is necessary to carry out new studies on the impact of acute or chronic use of SILD on different types of memories to justify the use of this drug in pathologies related to cognitive deficits.

0350 - ADMINISTRATION OF MGLU2/3R AGONIST IN A MODEL OF CHRONIC CEREBRAL HYPOPERFUSION

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INBIOMED- INSTITUTO DE INVESTIGACIONES BIOMÉDICAS. UBA-CONICET (1); DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS - STATE UNIVERSITY OF MARINGÁ (2)

Chronic cerebral hypoperfusion (CCH) resembles central changes in aging-related vascular dementias and Alzheimer´s disease (AD). Our group has demonstrated, in vitro, that astroglial subtype 3 metabotropic glutamate receptors (mGlu3R) present protective actions against neurotoxic agents including Aß. However, contradictory results were reported when mGlu3R ligands were administered in vivo. We examined the effect of the mGlu2/3R agonist, LY376298 (LY) 1 mg/kg i.p., in middle aged rats with CCH. The effect of mGlu2/3R agonist in neuron cell death and glial activation of the hippocampus were determined by immunohistochemistry. Moreover, the expression of GRM2, GRM3 (mGlu2/3R genes), GFAP and BDNF was studied by RT-qPCR technique. NeuN expression presents a decreased in CCH animals ($p<0.05$), that was reversed with the LY ($p<0.05$), only when the CA1 hippocampus subregion was studied. GFAP mRNA levels remained unchanged, but GFAP immunolabeling decreased in CCH rats ($p<0.05$) and increased in CCH+LY animals ($p<0.05$). We observed that the expression of GRM3 increased in CCH+LY ($p<0.05$), whereas GRM2 decreased ($p<0.05$) with the surgery compared to control animals. BDNF mRNA levels also increased in CCH animals

(p<0.05). To conclude, our results suggest that the in vivo administration of an mGlu2/3R agonist increased neuron viability produced by CCH, which could be linked to increased mGlu3 receptor levels.

0439 - EXPERIMENTAL FEBRILE SEIZURES IN YOUNG POSTNATAL RATS: GENDER DIFFERENCES IN LONG-LASTING EFFECT ON THE EPILEPTIC THRESHOLD AND GLIAL RESPONSE.

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Febrile seizures occurs in 3–5 % of children between 6 months and 5 years of age. Retrospective studies in adult epilepsy patients show an initial precipitating injury, usually febrile seizures, during childhood. Using an animal model of hyperthermic seizures (HS), we have previously shown that male HS-exposed animals exhibit a significative reduction in the convulsive threshold compared with controls and moderate reactive gliosis with an atypical astrocyte distribution in the pyriform cortex and other brain structures. Here we investigate consequences of early HS exposure in adolescent female rats compared to males. Rat pups (10-11 postnatal days old, PND) were placed in a glass chamber, and their core temperature was raised by a regulated stream of moderately heated air (39-42 °C). Body temperature was measure at baseline, seizure onset and every 2 min during the seizures. Hyperthermic temperatures (39.5–42.5 °C) were maintain for 30 min. The seizures onset was monitored behaviourally, and consisted of an acute sudden arrest of hyperthermia-induced tonic freeze postures and occasional oral automatism (biting and chewing) and often body flexion. Rats were then placed on a cool surface, monitored for 5 min before being returned to their mothers. At PND37-39 rats were exposed to repeated pilocarpine subconvulsive doses (10 mg/kg every thirty minutes). Another group of animals (PND35) was deeply anesthetized, fixed and brains processed for immunohistochemistry. We observed that, contrary to the males, the females did not develop SE after four repeated doses of pilocarpine and histological analysis of their brains exhibited lower reactive gliosis compared to males. Our results suggest that HS exposure early in the postnatal brain development produce long-lasting effects in males, which could be related to their future susceptibility to develop epilepsy.

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0441 - NITRIC OXIDE AND APOPTOSIS DUE TO AFTER-EFFECTS OF ACUTE ETHANOL EXPOSURE IN BRAIN CORTEX

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Alcohol hangover (AH) is defined as a combination of mental and physical symptoms experienced the day after a single episode of heavy drinking, starting when blood alcohol concentration approaches to zero. We previously evidenced bioenergetics alterations and oxidative stress in brain cortex synaptosomes from AH mice. The aim of the present work was to study the after-effects of acute ethanol administration on nitric oxide (NO) metabolism, mitochondrial calcium uptake and induction of apoptosis. Mice received an i.p. injection of ethanol (3.8 g/kg body weight, AH group) or saline (control group) and were sacrificed 6 h afterwards. Synaptosomal NOS activity and total NO levels were determined, as well as the expression of nNOS, iNOS, PSD-95 and the NR2B-subunit of NMDA receptor. Mitochondrial calcium uptake, permeability transition (MPT) and the expression of apoptotic markers were analyzed in mitochondrial fractions. Results showed a 35-37 % decrease in NOS activity and total NO content in AH mice (p<0.05), both in the absence and presence of glutamate and calcium. Protein expression of nNOS and PSD-95 were 19 and 15 % decreased, respectively (p<0.05) while no changes were observed in iNOS protein expression. Furthermore, a 60 % decrease in NMDA receptor protein expression (p<0.01) was found in AH synaptosomal membranes. Impairment of calcium handling and MPT induction were observed in AH mitochondria (p<0.05) together with a 21 % increase and 18 % decrease in Bax and Bcl-2 protein expression (p<0.05), respectively. Moreover, a 4-fold decrease in cytochrome c mitochondria/cytosol ratio was found due to AH (p<0.01). In conclusion, alcohol after-effects include changes in NO synthesis probably related to the observed decrement in NMDAR and PSD-95 protein expression at synaptic membranes. Impairment of mitochondrial capacity to accumulate calcium due to mitochondrial dysfunction and oxidative stress can lead to cell death by the activation of apoptotic signalling pathways.

0452 - MODULATION OF METABOTROPIC AND IONOTROPIC FUNCTIONS OF THE NICOTINIC α7 RECEPTOR BY THE INTRACELLULAR DOMAIN

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INIBIBB-CONICET, DEPTO. BIOLOGÍA, BIOQUÍMICA Y FARMACIA-UNS (1); INIBIBB CONICET- INSTITUTO DE INVESTIGACIONES BIOQUÍMICAS DE BAHÍA BLANCA (2)

The α7 receptor is a nicotinic receptor present in the nervous system and in non-neuronal cells. It has been demonstrated that α7 not only mediates fast synaptic transmission in neurons, but also regulates inflammatory responses in immune cell, neurite growth and neuronal protection, as well as cancer cell proliferation. The concept of α7 as a dual metabotropic/ionotropic receptor is attracting increasing attention. A key role in this dual nature is played by the receptor intracellular domain (ICD), which contains sites for phosphorylation and intracellular signaling. To explore the relationship between metabotropic and ionotropic activities we expressed α7 in mammalian cells and performed single-channel recordings to determine the channel properties and western blot to determine signaling pathways triggered by α7 activation. Single-channel recordings of human α7 from cells exposed to inhibitors of Src family kinases showed increased open durations and frequency of opening events. The effects were recapitulated using a receptor carrying mutations of the two ICD tyrosine residues, thus indicating that phosphorylation modulates receptor ionotropic activity. Cells exposed to the specific α7 agonist, PNU-282987, showed an increase of ERK1/2 phosphorylation, which was abolished by exposure to a tyrosine kinase inhibitor. PNU-282987 was not able to trigger ERK phosphorylation neither from cells expressing the double mutant receptor lacking tyrosine residues nor from cells co-expressing α7 and the ICD domain. Finally, the exposure of cells co-expressing α7 and ß2 adrenergic receptors to nicotine (α7 agonist) and isoproterenol (ß2 agonist) decreased phosphorylation of CREB, a known effector of the ß2 adrenergic receptor. This study indicates that the phosphorylated state of α7-ICD plays a role in the dual metabotropic/ionotropic receptor responses. It also opens doors for future studies exploring the role of the ICD as a modulator of the crosstalk between α7 and G-protein coupled receptors.

0752 - EXTRACELLULAR PROTEOLYSIS OF THE HORMONE GHRELIN GENERATES A SPECIFIC SUBSET OF GHRELIN-DERIVED PEPTIDES WITH DIFFERENTIAL BIOACTIVITIES

Antonela FITTIPALDI (1) | Daniela LUFRANO(1) | Gimena FERNANDEZ(1) | Daniel CASTROGIOVANNI(1) | Pablo N. DE FRANCESCO(1) | Leonard LUYT(2) | Sebastián TREJO(3) | Mario PERELLO(1)

IMBICE (CICPBA-CONICET-UNLP) (1); DEPARTMENTS OF CHEMISTRY, ONCOLOGY, AND MEDICAL IMAGING,

UNIVERSITY OF WESTERN ONTARIO (2); YPF TECNOLOGIA (3)

The stomach-derived hormone ghrelin is a peptide of 28 residues acylated with an octanoic acid at Ser3. The N-terminal sequence of ghrelin along with the octanoyl group are essential to act on the ghrelin receptor. Here, we tested the hypothesis that ghrelin can be extracellularly cleaved in order to generate ghrelin-derived peptides with differential bioactivities. Initially, we incubated ghrelin with plasma and then performed MALDI-TOF MS analysis. We found that ghrelin is mainly cleaved in the region extended from residue 11 to 16. Then, we incubated ghrelin with liver carcinoma HepG2 cells or with extracellular medium derived from these cells, and also found that ghrelin cleavage occurs in the same "hot cleavage region" of its sequence. Since ghrelin1-14 was derived from ghrelin proteolysis, we then tested the ability of this shorter version of ghrelin to act in the brain and stimulate appetite in mice. We found that ghrelin increases food intake (0.29 vs. 0.07 g in vehicle-injected mice, p-value 0.0008) while ghrelin1-14 failed to do it (0.005 g). Similarly, ghrelin increases the levels of the marker of neuronal activation c-Fos in the ARC (48.0 vs. 12.0 cells/side/section in vehicle-injected mice, p-value 0.0036) while ghrelin1-14 was unable to induce neuronal activation (16.1 cells/side/section). In addition, ghrelin1-14 failed to impair the orexigenic effect of full-length ghrelin. Thus, these data support the existence of a proteolytic extracellular mechanism that generates ghrelin-derived peptides with different bioactivity than ghrelin. Moreover, the liver may be involved in this mechanism during the passage of ghrelin through the hepatic portal circulation.

Oncología / Oncology IV

Chairs: Ezequiel Lacunza | Gabriela Martin

0155 - LIQUID BIOPSIES: A NEW EMERGING STRATEGY FOR THE EARLY DETECTION OF BREAST CANCER

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INSTITUTO DE BIOLOGÍA Y MEDICINA EXPERIMENTAL (IBYME-CONICET) (1); HOSPITAL MILITAR CENTRAL (2); HOSPITAL MUNICIPAL DE ONCOLOGÍA MARÍA CURIE (3); HOSPITAL INTERZONAL GENERAL DE AGUDOS "PROF. DR. LUIS GUEMES" (4); INSTITUTO QUIRÚRGICO DEL CALLAO (5)

Breast cancer (BCa) is the leading cause of death by cancer in women worldwide. Even though early diagnosis is improving the survival of BCa patients, more sensitive and specific tools are still necessary. Here, we propose the use of miRNAs as a biomarker alternative in BCa diagnosis. miRNAs are small non-coding RNA molecules that can be found in body fluids, such as urine or blood. Additionally, miRNAs can be release by tumors in early stages of BCa, even when they are undetectable by other diagnostic methods, which makes these molecules useful tools for early BCa detection. The aim of this work was to identify circulating miRNAs (liquid biopsies) in plasma of BCa patients at different stages using microarrays and mice model validation. For this purpose, plasma from 30 BCa patients was distributed into 5 clusters, according with their BCa stage: i) stages 0 and IA, ii) stage IIA, iii) stage IIB, iv) stage IIIA, and v) stages IIIB, IIIC and IV. As control, plasma from 32 healthy donors was distributed into 4 clusters. We hybridized miRNAs of each group using GeneChip® miRNA 4.0 Array (Affymetrix). Data analysis showed that miR-93-5p, -150-5p, -3178, -4459, -4467, -4486, -4730, -6514-3p, -6716-3p, -7107-5p and -7110-5p were upregulated in the plasma from BCa patients compared to healthy donors. Analytical validation of these results was performed in NOD scid gamma (NSG) mice. We inoculated female mice with BCa cell line MDA-MB-231 (BCa mice). After tumor growth, we sacrificed the mice to collect blood and tumor samples. Plasma from healthy animals was also obtained (control mice). We tested all miRNAs obtained from microarrays analysis from patients. Xenografts expressed miR-93-5p, -150-5p, -3178, -4467 and -6716-3p, being miR-3178 the most expressed. We detected several circulating miRNAs in the plasma of these animals. Interestingly, miR-93-5p was found significantly enriched in the plasma from BCa mice compared to control mice.

0272 - EXPLORING THE ROLE OF GAMMA-SYNUCLEIN IN MELANOMA

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Cancer is one of the leading causes of morbidity and mortality worldwide. In particular, malignant melanoma is the most lethal form of skin cancer, with incidence indexes increasing over the years. Synucleins are small proteins expressed primarily in neural tissue and in certain tumors. Gamma-synuclein (gS) is detected in a wide range of cancer types, including breast and ovarian cancer, but to date there are no rigorous studies to address the role this protein could have in progression of melanoma. Our goal was to analyze if gS was related to melanoma development. First, by bioinformatics we observed that gS was expressed in melanoma samples, and we confirmed that result by Western blot (WB) and immunocytochemistry (ICC) studies in melanoma cell lines. WB analysis revealed that gS was expressed as different molecular weight species, and by sequential lysis with detergents we could detect that high molecular weight species were present at the cytoplasm of melanoma cells, while monomeric gS was mostly present at the nucleus. By shRNA techniques and the use of expression vectors, we were able to modulate gS expression in B16-F0 (mouse) and A375 (human) melanoma cells. MTT-based proliferation studies revealed that gS expression was not significantly affecting melanoma cells growth ($p>0.05$). Nevertheless, by fluorescent F-actin staining we observed that increased expression of gS was associated with major cytoskeletal changes. Indeed, by wound healing assays we noted that reduced expression of gS promoted a migratory defect of B16 melanoma cells ($p<0.01$), while overexpression lead to a more migratory phenotype ($p<0.01$). Moreover, increased expression of gS was associated to an increment in focal adhesions ($p<0.05$) observed by ICC for the focal adhesion kinase (FAK). Altogether our data indicate a putative role for gS in events associated to melanoma progression. Further experiments are required to address the molecular function of gS in melanoma cells.

0275 - HEME OXYGENASE 1 (HO-1) MODULATES AEROBIC GLYCOLYSIS THROUGH REGULATION OF LACTATE DEHYDROGENASE (LDH) IN PROSTATE CANCER CELLS

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Neoplastic proliferation requires tumour cells to reprogram their metabolic pathways in order to support the higher proliferation rate (known as the "Warburg effect"). As a result, even under normal oxygen concentrations, transformed cells predominantly generate ATP through glycolysis followed by lactic acid fermentation, converting most incoming glucose to lactate rather than metabolizing it in the mitochondria through oxidative phosphorylation. We previously demonstrated that heme oxygenase 1 (HO-1), a cellular homeostatic regulator, has an antitumoral activity in prostate cancer (PCa) cells. In addition, after treatment with hemin, an inducer of HO-1 expression and activity, PC3 cells showed significantly lower glucose uptake, ATP production and oxygen consumption rate. In this context, we aimed to study whether HO-1 is involved in the regulation of aerobic glycolysis through modulation of lactate dehydrogenase (LDH) in PC3, C4-2B and MDA PCa 2b cell lines under HO-1 induction with hemin (80 μM, 24h). We found a significant reduction in LDHA expression by RTqPCR ($p<0.05$), total LDH enzymatic activity ($p<0.05$) and extracellular lactate levels ($p<0.05$). The analysis of the TCGA-PRAD public database revealed higher LDHA mRNA levels in tumor samples compared to non-tumoral prostate tissues ($FC=1.2$; $P=2.75x10^{-6}$), with increased expression as Gleason score is higher, and poorer overall survival for patients with high LDHA tumour levels ($p=0.03$). On the other hand, we observed decreased levels of LDHB (isoform with higher affinity for lactate, preferentially converting lactate to pyruvate) mRNA ($FC=0.5$; $P=3.33x10^{-15}$), with lower expression in patients with higher Gleason score. Altogether, our findings indicate that HO-1 induction alters both transcriptional and enzymatic activity of LDH and, in turn, lactate production, confirming its relevance as a key modulator of the energetic metabolism in PCa cells.

0276 - TARGETING ANDROGEN RECEPTOR AND WNT PATHWAY IN ENDOCRINE-RESISTANT BREAST CANCER.

Virginia FIGUEROA (1) | Gabriela PATACCINI(1) | Martín ABBA(2) | Ana SAHORES(1) | Claudia LANARI(1) | Caroline LAMB(1)

IBYME-CONICET (1); UNIVERSIDAD NACIONAL DE LA PLATA (2)

Endocrine therapy is the standard treatment for patients with luminal breast cancer. However, after treatment most patients develop hormone resistance, by mechanisms that may include deregulation of growth factor signaling pathways. Fibroblast growth factor 2 (FGF2) consists of a secreted low molecular weight form (LMW-FGF2) and several nuclear high molecular weight forms (HMW-FGF2). We previously demonstrated that FGF2-overexpression in endocrine responsive T47D cell lines, induced hormone resistance. The aim of this study was to explore the mechanisms underlying endocrine resistance. By RNAseq, we compared LMW- and HMW-FGF2-T47D cells, with T47D cells transfected with an empty vector (T47D-ctrl) and found that FGF2 overexpressing cells had a deregulated WNT signaling pathway with the upregulation of several WNT ligands. We also detected decreased estrogen receptor α and progesterone receptors (PR) along with an increase in androgen receptors (AR), both at the mRNA and protein levels. We found a more pronounced decrease of PR isoform A (PRA) than isoform B (PRB) resulting in a low PRA/PRB ratio, which is consistent with an endocrine resistant phenotype, according to previous results from our lab. To explore the role of AR and WNT signaling pathways in FGF-triggered endocrine resistance, we evaluated the effect of dihydrotestosterone (DHT, AR agonist), enzalutamide (E, AR antagonist) and LGK974 (WNT inhibitor) in LMW- and HMW-FGF2-T47D cells compared with T47D-ctrl cells. In endocrine resistant cells, DHT induced cell proliferation while blocking AR and WNT pathways inhibited cell proliferation and tumor growth. Conversely, DHT inhibited T47D-ctrl cell proliferation and blocking the AR had no significant effect on tumor growth. Our results suggest that targeting AR and/or WNT pathways may be an alternative therapy for endocrine-resistant breast carcinomas with low PR and high AR levels.

0284 - INITIAL CHARACTERIZATION OF FOXP3 BLOCKADE IN BRAIN TUMOR MODEL USING GENE THERAPY VECTORS

Alejandro J. NICOLA CANDIA (1) | Antonela S ASAD(1) | Sofia SAGRIPANTI(1) | Araceli ABT(1) | Matias GARCIA FALLIT(1) | Mercedes IMSEN(1) | Camila Florencia ZUCCATO(1) | Adriana SEILICOVICH(1) | Flavia ZANETTI(2) | Marianela CANDOLFI(1)

INBIOMED-UBA-CONICET (1); INSTITUTO MILSTEIN (2)

Our previous results indicate that systemic administration of a cell-penetrating peptide (P60) that inhibits Foxp3, a transcription factor required for Treg function, improves the efficacy of antitumor vaccines in experimental breast cancer models. In order to develop a gene therapeutic strategy to deliver P60 in vivo, we have generated an adenovector that encodes the P60 sequence as well as a fluorescent reporter gene dTomato (Ad.P60.dTomato), which successfully transduced breast tumor cells in vitro and in vivo. Here we aimed to perform an initial characterization of Ad.P60.dTomato in experimental glioblastoma. This vector successfully transduced glioblastoma cells in vitro and in vivo in mice bearing intracranial GL26 syngeneic tumors that received intratumor injections of Ad.P60.dTomato or its control vector (6x10^7 pfus) 21 d post-tumor inoculation. Expression of d-Tomato was also detected in the normal mouse brain 3 and 5 days post-injection of Ad.P60.dTomato. We next injected Ad.P60.dTomato in intracranial GL26 tumors growing in transgenic C57BL/6 mice that express Foxp3 fused to fluorescent GFP protein, in order to easily detect Tregs by flow cytometry. Seven days after adenovector injection we observed a significant decrease in the number of tumor-infiltrating Tregs in mice treated with Ad.P60.dTomato ($p<0.05$), an effect that was not detected in the spleen. Our findings suggest that local administration of Ad.P60.dTomato may improve the response to immunotherapeutic strategies that are inhibited by Treg function, such as antitumor vaccines.

0291 - DEVELOPMENT AND IN VITRO EVALUATION OF MAGNETIC/HYBRID NANOSTRUCTURED LIPID CARRIERS AS A TOOL FOR TARGETED DELIVERY OF ANTICANCER DRUGS

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INIBIOLP (1); CINDEFI (2); CINDECA (3); CEQUINOR (4)

Cancer is the second cause of death in the world and many of the current therapies are still ineffective or present highly toxic side effects. Nanostructured lipid carriers (NLC) are nanosized colloidal drug-delivery systems composed of a binary solid/liquid lipids core and functionalizable surface. NLC were developed to improve the encapsulation, stability, bioavailability, controlled release and selective targeting of lipophilic therapeutic drugs. Here, we designed biocompatible hybrid chitosan (Chi) coated NLC containing 1,8-cineole (CN), acting as both bioactive monoterpene and nanostructuring liquid lipid, and magnetic nanoparticles (MNP), as a smart system for drug delivery to cancer cells. NLC, NLC/Chi and NLC/Chi/MNP nanoparticles (NPs) were prepared by ultrasonication. NPs were characterized by determining particle size, surface charge (SC), magnetic behavior, encapsulation efficiency (EE) and kinetic release of CN. Cell viability and cellular uptake of NPs were evaluated in human liver (HepG2) and human lung (A549) cancer cells, and non-tumoral lung (WI-38) cells. NPs presented spherical shape, sizes in the range of 190-270 nm with narrow distribution, and SC of -2.0 mV (NLC), +7.0 mV (NLC/Chi) y +10.0 mV (NLC/Chi/MNP). MNP and NLC/Chi/MNP showed

magnetic response with saturation moments of 79 and 60 emu/g Fe, respectively. The EE of CN in all NPs was greater than 77 % and they showed biphasic CN release profile. CN-loaded NPs inhibited up to 73.6 % (A549) and 77.2 % (HepG2) cancer cells viability ($p<0.001$) at concentrations not cytotoxic for WI-38 cells. NLC showed stronger cell growth inhibition than free CN (8 mM, 48h) on HepG2 (77.2 vs. 39.2 %, $p<0.001$) and A549 (72.7 vs. 50.7 %, $p<0.001$) cells, respectively. NPs were time-dependently incorporated into cells being Chi-coated NPs highly incorporated by cancer cells. Our results suggest that the developed NPs present a promising potential as innovative systems for targeted delivery of anticancer drugs.

0295 - EFFECTS OF FLAVONOIDS WITH ANTI-TUMORAL ACTIVITY ON THE EXPRESSION OF RECEPTORS ASSOCIATED WITH EGFR ACTIVITY IN BREAST CANCER CELLS.

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Flavonoids have been associated with a reduced incidence of cancer. For that reason, they were proposed as chemopreventive and chemotherapeutic agents per se or in combination with traditional antitumoral drugs. Epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor associated with tumorigenesis of several tissues. It was proposed to be involved in the molecular mechanism of action of several flavonoids. With the aim of investigating if a combinatory therapy involving flavones and EGFR inhibitors would be a possible effective treatment for breast cancer, the present study analyzes if flavones with breast cancer antitumoral action -2´-nitroflavone and apigenin- modulate EGFR expression or the expression of receptors that interact or modulate EGFR activity. For that purpose, MDA-MB-231 breast cancer cells were stimulated with 2´-nitroflavone or apigenin at two different concentrations (a concentration close to the IC50 and a higher one) for 48 h. Assays were performed in absence or presence of fetal bovine serum. Afterwards, the protein content of EGFR, ErbB2, Met and IGF-IR were assessed by immunoblotting. Besides, PARP cleavage and phosphorylation of p38 were determined. Results showed that incubation with apigenin induced a reduction in EGFR and ErbB2 protein expression ($p<0.05$); while incubation with 2´-nitroflavone resulted in a diminution of ErbB2 ($p<0.05$) depending on the experimental condition, but no conclusive effects on EGFR content were observed. 2´-nitroflavone caused a reduction in the expression of IGF-IR and Met ($p<0.05$). Apigenin also induced a decline in Met content but only when cells were incubated in absence of serum ($p<0.05$). In addition, an increment in p38 phosphorylation and PARP cleavage was observed in both treatments ($p<0.05$). In conclusion, the flavones demonstrated to have effects on the expression of receptors associated with EGFR activity which could justify a possible combinatory therapy involving flavones and EGFR inhibitors.

0300 - BACILLUS CALMETTE-GUERIN (BCG) DOWN-REGULATES FGFR3 IN THE HUMAN BLADDER CANCER CELL LINE UMUC14 ASSOCIATED WITH AN INCREASE OF PRO-APOPTOTIC AND A DECREASE IN PRO-SURVIVAL PATHWAYS

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INSTITUTO DE ONCOLOGÍA ANGEL H. ROFFO

Bacillus Calmette-Guerin (BCG) is the standard treatment for high-grade non-muscle invasive (NMI) bladder cancer (BC). Using a murine orthotopic BC model we demonstrated that BCG induces tumor growth inhibition, associated with FGFR3 down-regulation. The objective was to study the effect of BCG treatment on the human BC cell line UMUC14, which overexpress an active constitutively FGFR3 by a) cell viability and FGFR3 expression and b) pro-survival pathway PI3K and pro-apoptotic pathway mediated by Cathepsin B and Caspase 3. Human BC cell line UMUC14 was treated with +/-BCG (2mg/ml) for 24 and 48hs. Viability was assessed by MTS and FGFR3 expression by qPCR and western blot. AKT, pAKT, Cathepsin B, Caspase 3 and PARP were analyzed by western blot. a) BCG reduces 50% of cell viability ($p<0.001$) and FGFR3 expression ($p= 0.016$) in UMUC14. Transfection with an expression plasmid carrying the wild type form or the activating mutation (K650E) of FGFR3 increases UMUC14 viability about 50 % ($p<0.01$) and 100 % ($p<0.001$) respectively compared to control cells. On the other hand, treatment with BGJ398 (a pharmacological inhibitor of FGFR3) reduces UMUC14 viability, in higher concentrations than 100 nM ($p<0.001$). b) The reduction in cell viability in response to BCG was associated with a down-regulation of 40 % of total AKT after 24 h post treatment (without differences in pAKT). The increase of apoptotic pathway was demonstrated by the cleavage of PARP as consequence of the activation (cleavage) of Cathepsin B and Caspase 3. Our results show that BCG was able to reduce viability of the human BC cell line UMUC14 associated with a down-regulation of FGFR3. Cell viability inhibition was, at least in part, mediated by the reduction of PI3K, as well as an increase in pro-apoptotic pathways.

0316 - CHARACTERIZATION OF NOVEL MURINE MAMMARY CELL LINES WITH DIFFERENT AGGRESSIVE PHENOTYPE AND IDENTIFICATION OF POTENTIAL TARGETS BY MASS SPECTROMETRY

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Breast cancer is the first cause of death from female cancer. After the treatments against the primary tumour, a few cells can remain in a state of quiescence for a long period, becoming undetectable. These cells can resume growth and establish as metastasis, which are responsible for 90 % of deaths from cancer. In a previous work, we isolated and characterized two cell lines, F3II-TP and F3II-NM. F3II-TP represents tumours with high proliferation rate whereas F3II-NM represents a quiescent cell phenotype. The comparison between these cell lines is expected to yield new molecular targets for both the design of antiproliferative drugs as well as drugs that point quiescent cells. The aim of this work was to continue with the characterization of these cell lines and identify possible targets. First, we analysed the expression of EMT markers such as E-cadherin, N-cadherin, Vimentin, Pan-Cytoqueratin and β-catenin by immunofluorescence. We determinated that F3II NM has a mesenchymal phenotype and F3II TP an undifferentiated one. In addition, the expression of the dormancy related gene NR2F1 was analysed by qPCR and we found that F3II-NM showed higher levels of transcript than F3II-TP. Furthermore, in an orthotopic model of BALB/c mice, F3II NM presented a longer latency time and lower tumour growth rate in comparison to F3II-TP. Finally, analysis of the cell lines using LFQ-Mass spectrometry revealed 256 differentially expressed genes between the three lines studied. Overexpressed genes of survival paths were identified in F3II-NM, such as DDX42, COX-2, Aldh3a2 involved in the inhibition of apoptosis, promotion of tumour recurrence via SOX-2 and therapy resistance, respectively; and in F3II-TP, proliferation related genes were identify such as Flnb, Aldoa, G6pdx, involved in actin cytoskeleton reorganization and in cellular metabolism. To conclude, these results contribute to the characterization of both new cell lines and to the identification of new molecular targets.

0317 - GLYCAN PROFILE OF HUMAN GLIOMA CELL LINES AND ITS IMPACT IN CELL BIOLOGY

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Gliomas are the most prevalent primary tumors of the brain and spinal cord with high morbidity and mortality. Little is known about glycosylation and its participation in glioma. Our objective is to describe the glycan profile in human glioma and its participation in cell biology. On a panel of human high- and low-grade glioma cell lines we evaluated expression of cancer associated glycans (Lewis and truncated), where SLeX was the major glycan expressed particularly in high grade gliomas. LeY and LeA were also associated with the high grade condition. N-glycans participation on glioma cell biology was evaluated by inhibiting their synthesis using Tunicamycin and Swainsonine and by silencing of MGAT5 transcription, the glycosyltransferase responsible for ß1-6 branching, using siRNA. Participation of core 2 O-glycans on cell behavior was also evaluated by inhibiting the glycosyltransferase C2GNT1. SLeX expression was significantly reduced by Tunicamycin and Swainsonine treatment on LN229 cells (more than 90 %) while C2GNT1 silencing reduced its expression by about 50 %. Also, inhibition of N-glycosylation decreased cell adhesion and cell migration to a greater extent than O-glycosylation inhibition in all the cell lines evaluated. High performance anion exchange chromatography analysis of high grade glioma cell lines showed a broad expression of N- glycans with a high abundance of branched tri-antennary structures. Owing to the impact of targeted therapies in glioma has been modest, the knowledge of glycan structures and their participation in cell biology can enable us the identification of new targets for glioma treatments.

0326 - SIMULTANEOUS STIMULATORY AND INHIBITORY EFFECTS OF AN ANTI-TUMOR VACCINE DEPENDING ON THE SIZE OF THE TUMOR TARGET

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CEDIE

Immune-checkpoint inhibitors and antitumor vaccines may produce both tumor inhibitory and tumor-stimulatory effects on growing tumors depending on the stage of tumor growth. These paradoxical results might be explained assuming the hypothesis of tumor immunostimulation according to which the inhibition or stimulation of tumor growth would be dependent on the ratio between the number of immune reactants and the number of tumor cells. To test this claim, we studied the effect of an anti-tumor vaccine administered to mice bearing both a relatively large primary tumor and a small secondary tumor implant. The tumor used was the strongly immunogenic methylcholanthrene-induced MC-C fibrosarcoma and the anti-tumor vaccine was prepared with lethally irradiated MC-C tumor cells. Mice bearing a primary tumor exceeding 500 mm^3, received simultaneously the vaccine and the secondary tumor implant. Tumor volumes of both the primary and secondary tumors was determined at different times after the vaccine. The vaccine produced a significant enhancement of the primary tumor (mean of six independent experiments; $p<0.01$, paired sample test) while, simultaneously, it induced a striking inhibition of the secondary tumor growth (mean of six independent experiments; $p<0.01$, paired sample test). Our results seem to support the immunostimulation theory on the basis that the very immune response induced by an anti-tumor vaccine produced both tumor stimulatory and inhibitory effects depending on the size of the tumor target.

0335 - HO-1 INDUCES MX1 EXPRESSION TILTING THE BALANCE OF ENDOPLASMIC RETICULUM STRESS TOWARDS PRO-DEATH EVENTS IN PROSTATE CANCER.

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Prostate cancer (PCa) is one of the most common cancers in men worldwide. We previously reported that heme-oxygenase 1 (HO-1), had a strong anti-tumoral effect in PCa, interacted with MX1 (myxovirus resistance protein) and upregulated its mRNA levels in PCa cell lines. In this work we assessed the cellular implications for this modulation and the clinical relevance and correlation of MX1 and HO-1 in PCa. RT-qPCR and immunofluorescence analyses in PCa cells showed significant MX1 increased expression under HO-1 induction. Next, considering that HO-1 is inducible by inflammatory and stress conditions and that is anchored to the endoplasmic reticulum (ER), we analyzed the expression levels of MX1 and HO-1 in response to ER stress (ERS), using thapsigargin. Results showed significant increase of mRNA expression levels for both genes under ERS (6-fold induction, $p<0.05$ and 50-fold induction, $p<0.05$; respectively). Confirmation of ERS was seen by up-regulation of known markers of ERS: HSPA5, DDIT3 and XBP1. Further, we assessed ERS effect on apoptosis and autophagy. Results showed that under ERS, apoptosis increased by 20 % ($p<0.05$) and Western blot detected a significant increase in LC3I/II conversion, depicting an augmented autophagic process. Conversely, these effects were reversed by siMX1 under the same conditions. Efficiency of MX1 depletion was confirmed by qPCR. Additionally, we undertook a bioinformatics approach to assess the clinical relevance of MX1 and HO-1 in PCa. MX1 was one of the most consistently down-regulated gene in PCa vs. normal prostate and Kaplan-Meier analyses showed that its loss was associated with decreased overall and disease-free survival ($p<0.05$). Of note, there was a significant positive correlation between MX1 and HO-1 (Pearson $r= 0.23$, $p<0.0001$). In summary, we propose that HO-1 induces MX1 expression and MX1 in turn, tilts the balance of ERS towards pro-death events in PCa.

0336 - CIRCULATING MIRNAS AS POTENTIAL BIOMARKERS FOR THE EARLY DIAGNOSIS OF PROSTATE CANCER

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Prostate cancer (PCa) is the most common type of cancer and the third cause of death by cancer in Argentinian men. miRNAs are small non-coding RNA molecules that regulate gene expression. miRNAs can be secreted by tumor cells and circulate in the bloodstream. Our aim was to identify circulating miRNAs as candidate biomarkers for the diagnosis of PCa. GeneChip® miRNA 4.0 Arrays (Affymetrix) were hybridized with circulating RNA obtained from serum of PCa patients or healthy donors. Diagnosed PCa patients, free of treatment, were divided into subcategories according to Gleason grade. After data normalization, we identified a list of miRNAs (miR-4668-5p, miR-2277-5p, miR-3613-3p, miR-101-3p, miR-320e-5p, miR-6750-5p, miR-548x-3p, miR-320a, miR-4532, miR-21-5p) that were increased in PCa patients serum compared to healthy donors. To validate these results, NSG mice were inoculated s.c. with PC3 or 22Rv1 PCa cell lines. After tumor growth, mice with tumors and a non-tumor mice group (control) were sacrificed. Blood and tumor samples were collected for RNA

isolation. miRNA expression levels were assessed by stem-loop RT-qPCR. miR-4668-5p, miR-2277-5p, miR-3613-3p and miR-21-5p were significantly increased in the circulation of mice inoculated with PC3 cells compared to control. Also, miR-101-3p and miR-3613-3p were significantly upregulated in the plasma of mice that were inoculated with 22Rv1 compared to control. miR-2277-5p was not detected in the plasma of 22Rv1 injected mice. Interestingly, miR-101-3p was increased in circulation of 22Rv1 compared to PC3 injected mice, while miR-4668-5p was increased in plasma of PC3 compared to 22Rv1 injected mice. Additionally, miR-101-3p was upregulated in 22Rv1 compared to PC3 xenografts. In summary, our work defines novel candidate biomarkers for PCa diagnosis based on circulating miRNAs from human serum samples. These biomarkers were also detected in xenografts and plasma from mice.

0338 - "EFFECTS OF CO-CULTURE CONDITIONS ON THE METABOLIC TRANSCRIPTOMIC PROFILE OF PROSTATE CANCER CELLS".

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Prostate cancer (PCa) is the most common type of cancer diagnosed in men being bone the main site of progression. Tumor cells interact with the bone microenvironment and disrupt the tissue balance. Alterations in energy metabolism have emerged as one of the hallmarks of cancer, evidenced by the numerous connections between signaling pathways that include oncoproteins and key enzymes in energy metabolism. Variations in a single gene could orchestrate a change in the metabolic fate of the cell and thus confer an adaptative advantage. In this work, we aimed at identifying cellular energetic deregulations of metastatic PCa cells using an in vitro co-culture transwell system, in which PCa cells (PC3) were grown alone or co-cultured with osteoblasts precursors (MC3T3 cells). RNAseq was performed on tumoral cells and differential gene expression analysis was performed. The gene ontology (GO) analysis revealed an enrichment of ketone and lipid metabolism in PC3 co-cultured with MC3T3 compared to PC3 grown alone ($p<0.05$). We also identified the specific KEGG pathways enriched among the differentially expressed gene lists. We found that oxidative phosphorylation (NES* -4.27), glycolysis (NES* -2.01) and tricarboxylic acid cycle (NES* -1.98) were significantly downregulated ($p<0.05$) when PC3 cells were co-cultured with MC3T3 compared with PC3 grown alone. We conclude that communication between PCa and bone cells might shift the glycolytic metabolism of tumoral cells to a lipid dependent metabolic profile. Further validation of these results would lead to discover a metabolic-associated molecular signature in PCa progression.

*Normalized Enrichment Score.

0342 - KANSL2 REGULATES RRNA SYNTHESIS AND PROLIFERATION

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KANSL2/NSL2 is a subunit of the non-specific lethal (NSL) complex which is important for epigenetic regulation of embryonic stem cells. Previously, we reported KANSL2 is enriched in glioblastoma (GBM) tumors and regulates the stem cell subpopulation. Here, we further characterize KANSL2 role in GBM as a way of understanding the mechanisms behind its tumor-promoting activity. We determined that KANSL2 has a heterogeneous subcellular localization with enriched accumulation in nucleoli. The nucleolus hypertrophy has long been considered a marker of tumor malignancy in the clinic, but only recently has been shown to be an active regulator of tumorigenesis and cellular plasticity. We show here KANSL2 regulates ribosomal RNA (rRNA) synthesis in nucleoli. KANSL2-RFP overexpression positively regulated an rDNA-Luc reporter construct in GBM cells whereas KANSL2 knockdown diminished luciferase activity. A similar expression profile was observed for the polymerase I subunit POLR1E and the Upstream binding factor (UBF), both vital for rRNA synthesis. As anticipated from these findings, cellular proliferation was enhanced in KANSL2-RFP transfected cells while KANSL2 knockdown reduced the proliferation. Statistical significance was obtained with a cut-off p-value of 0.05. Our findings indicate KANSL2 is a nucleolar protein that plays role in rRNA biogenesis and cellular proliferation.

0346 - HEMIMETHYLATION OF TUMOR SUPRESSOR GENES IN LEUKEMIA PATIENTS

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IMBICE

Methylation and hemimethylation are important epigenetic modification related to the regulation of the genetic expression. In many types of cancer occur epigenetic aberrations, among which is the hypermethylation of tumor suppressor genes. DNA methylation increases, mainly in promoter genes regions, generates a reduction or transcription blockage in patients with different types of cancer. Hypermethylated tumor supresor genes are inactive. This could be a factor leading to the progress or malignancy of this disease. In this work we study the methylation profile of 3 tumor suppressor genes APAF-1, DBC-1 and ER in 30 patients with acute myeloid leukemia and chronic myeloid leukemia (16 AML and 14 CML) and in 30 controls samples by the methylation-specific PCR technique (MSP) with previous treatment of DNA with sodium bisulfite technique, for the conversion of unmethylated Cytosines into Uracil bases. We analyzed 22/30 patient samples and 25/30 controls. Significant correlations in patient methylation status ($p<0.05$) were found in APAF-1 and there is a tendency in DBC-1 gene's promoters by Fisher's test analysis. We found 41 % methylated samples in APAf-1 (5 % methylated + 36 % hemimethylated) and 78 % methylated in DBC-1 (39 % methylated + 39 % hemimethylated). ER % were no significative (data not shown). Finally, near 40 % of the patients analyzed, contain partial methylation in their promoters, which could mean a decrease in their expression. These results suggest that methylation in the promoters of the analyzed genes could be related to the events that generate the onset or progression of the disease. The silencing by hemimethylation in the promoter of the APAF-1 and DBC-1 genes, and the high percentage of methylation in both types of DBC-1, would lead to a decrease in its expression, which could generate effects that favor the process of leucemogenesis. We have to analyze deeply DBC and increase much more the number of samples to make these results more effective.

0347 - AHCYL1 AS A REGULATOR OF CELLULAR PLASTICITY IN LUNG CANCER

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Lung cancer, like many other solid tumor types, presents deregulation of gene expression involved in cellular plasticity. Even though the molecular mechanism involved is not fully characterized, it is considered that the acquisition of stem-like properties would contribute to the maintenance of the heterogeneous cell population seen in tumors. Previously, AHCYL1 was identified by using a bioinformatic tool INSECT as a potential regulator of stem properties of cancer cells. In AHCYL1-depleted cells (i.e. AHCYL1 shRNA) genes associated with pluripotency such as OCT4/POU5F1 were increased, with a decrease of Mucin 5B expression linked to pulmonary differentiation. Also, these cells showed a higher spheres formation capacity in vitro and tumorigenic capacity in vivo (Nod/Scid mice). In AHCYL1-overexpressing cells, the expression of OCT4 was reduced. When OCT4 was overexpressed, AHCYL1 expression was decreased suggesting a mutual regulation. Statistical significance was obtained with a cut-off p-value of 0.05. We propose a better understanding of AHCYL1 roles would contribute to new strategies for both diagnosis and therapy of cancer.

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0367 - METABOLIC SYNDROME INDUCES EPIGENETIC CHANGES IN PROSTATE TUMORS

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DNA methylation and histone modifications are important epigenetic mechanisms of gene regulation that can be detected before prostate cancer (PCa) becomes invasive, suggesting they are pivotal events in tumor initiation and progression. Metabolic syndrome (MeS) increases PCa's risk and aggressiveness. Our hypothesis is that MeS induces aberrant epigenetic changes in PCa favoring tumor development and progression. To address this hypothesis, MeS was induced in NSG or C57BL/6J male mice by chronically feeding them with high fat diet (HFD). After 12 weeks of diet, PC3 (NSG) or TRAMP-C1 (C57BL/6J) PCa cells were injected s.c. on MeS and control diet fed mice. After tumor growth, mice were sacrificed and tumors were collected. DNMT1 and SUV39H1 expression levels were repressed while RIZ1 and GADD45A were increased in MeS mice. Also, mRNA levels of CHD1 and ZEB1, two DNMT1 targets, were repressed and induced, respectively, in these tumors. Additionally, we evaluated multiple microarray datasets from patients using Oncomine. We found that DNMT1 expression was significantly downregulated, whereas RIZ1 was upregulated, in prostate tumors compared to normal prostate gland. Although SUV39H1 expression showed no changes comparing prostate tumors vs normal tissue, we found a deep deletion in its expression compared to other tumor types, such as breast or lung cancer. We also analyzed whole exome data for these genes (cBioportal) revealing amplification or deletion as the most frequent genetic alterations in PCa. Finally, PCa patients with DNMT1 and SUV39H1 alterations showed a decreased in overall survival while no differences in disease/progression were observed. In summary, MeS induces epigenetic modifications that result in global alterations in chromatin packaging, regulating the access of the transcriptional machinery to target genes and thereby modulating gene expression profiles.

0372 - EPIGENETIC LATERALITY DIFFERENCES IN BREAST CANCER

Sofía MASUELLI | María ROQUÉ

IHEM-UNCUYO

Breast Cancer is a heterogeneous disease. By previous studies we determined that mammary tumors of left-right sides (L-R) differ in their behavior as inferred from their methylation profiles. Normal breast L-R tissues have not identical environments; they differ in size, irrigation and fat composition. It is also known that epigenetics functions as a bridge between environment and gene expression. Our hypothesis sustains that L-R tumors differ in epigenetically regulated pathways, provoked by the diverse L-R microenvironment. To study this, we performed in-silico and in-vivo analyses. From database c-BioPortal Provisional Breast Cancer, 708 tumors with information for 16.000 genes were included and L-R methylation medias were compared for each gene. The top 169 genes with significant L-R difference above 3% were selected (T test, $p<0.0001$) and filtered by cancer related search terms in Metascape. Fifty three genes were associated with the terms "inflammation", "proliferation negative regulation", "immune response", "DNA damage response", "P53 pathway", "angiogenesis", "migration", "cell death regulation", "survival" and "apoptosis". Then, the methylation profiles were converted into the 7 cancer terms. Interestingly, the cancer term profiles clustered into 2 groups associated with L-R laterality (Hierarchical cluster analysis, bootstrap 90-100 %), suggesting the existence of different L-R methylation profiles associated with functional terms. In the in-vivo studies, the methylome of 6 L-R xenografts generated by inoculation of MDA-MB231 cells in NSG mice were analyzed by RRBS. Preliminary analyses reveal 197 gene promoters, with significant L-R difference (FDR corrected $p<0.01$). Further functional and expression studies will allow to evaluate the impact of these methylation differences on the tumor behavior. So far, our studies support an interesting epigenetic related laterality hypothesis for breast cancer, which could serve as proof of principle for other bilateral tumors.

0377 - CYTOSTATIC AND ANTIMIGRATORY ACTIVITY OF REPURPOSED HEMOSTATIC DRUG DESMOPRESSIN AGAINST AVPR2-EXPRESSING HUMAN OSTEOSARCOMA CELLS

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Osteosarcoma (OS) is the most common malignant primary bone tumor in children and young adults, with alarmingly elevated mortality rates. OS patients bear highly invasive and vascularized tumors, and are in urgent need of novel therapeutic strategies. Desmopressin (dDAVP) is a repurposed hemostatic drug in oncology that acts as a selective agonist for the AVPR2 receptor present in blood microvessels and several tumor types. dDAVP displayed cytostatic effects through canonical adenylate cyclase-cAMP-PKA axis activation in a wide variety of preclinical cancer models including breast and colorectal cancer, as well as a potent antiangiogenic and antimetastatic activity. The aim of this work was to evaluate in vitro dDAVP antitumor activity in OS cells. The human OS cell lines MG-63 and U2-OS were used. AVPR2 expression was assessed by qPCR, and sensitivity to dDAVP was evaluated by in vitro proliferation and Transwell chemotaxis assays. AVPR2 expression was detected in MG-63 cells but not in U2-OS. The presence of AVPR2 in MG-63 cells was confirmed by Western blot using MCF-7 breast cancer cells as a positive control. dDAVP showed significant cytostatic effects against exponentially growing MG-63 cell cultures after a 72-h exposure to the compound at 1 μM or higher (~30 % inhibition; $p<0.01$), while no direct cytotoxic effects were detected in semiconfluent, quiescent cell monolayers at the same concentrations (24-h incubation). A potent inhibitory effect on MG-63 cell chemotaxis was observed at concentrations of 100 nM or higher, reducing migratory capacity by up to 57% in comparison to vehicle-treated cells ($p<0.01$). dDAVP exerted cytostatic and antimigratory activity on AVPR2-expressing human OS cells. The compound could represent an interesting repurposing

drug for the management of OS that deserves further investigation in preclinical in vivo models.

0383 - EXPERIMENTAL VALIDATION OF THEORETICAL SURVIVAL MODELS FOR HIGH RADIATION DOSES IN CELL LINES OF DIFFERENT TYPES OF CANCER

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COMISIÓN NACIONAL DE ENERGÍA ATÓMICA (CNEA) (1); UNIVERSIDAD FAVALORO (2)

Technological advances in radiotherapy enable radiation dose to be delivered in a highly conformal manner, protecting healthy tissues. Clinical interest in hypofractionation requires new models to predict tumor response to higher dose per fraction than that used in conventional radiotherapy (2Gy). The linear-quadratic model e-(aD+bD2) (LQ) is a useful tool to compare conventional fractionations. However, LQ curve bends continuously on the log-linear plot resulting in the underestimation of survival for high doses. It has been demonstrated that LQ fits well for doses lower than 7Gy. Theoretical models have been described to introduce corrections to LQ for high doses, such as the linear-quadratic-cubic (LQC), the linear-quadratic-linear (LQL) and the Universal (USC) models. The aim of this work was to experimentally validate these models by fitting survival curves up to high doses in cell lines of different types of cancer. Survival curves were obtained by clonogenic assay after irradiating thyroid (TPC-1 y FRTL-5), breast (T47D) and lung (A549) cancer cells with a gamma source (^{137}Cs). Survival curves were fitted to the LQ model by weighted least squares in the low dose region, obtaining a and b parameters. These values were compared with those obtained for high doses using the LQL, LQC and USC models. Results showed that for curves fitted to LQL, parameters values were a= (0.50 ± 0.07) Gy-1, b= (0.020 ± 0.013) Gy-2 and g=(0.71 ± 0.15) Gy-1 (for TPC-1 cells); and a= (0.17 ± 0.04) Gy-1, b= (0.040 ± 0.009) Gy-2 and g= (0.84 ± 0.16) Gy-1 (for T47-D cells). For curves fitted to LQC the values were a= (0.19 ± 0.03) Gy-1, b= (0.029 ± 0.007) Gy-2 and g= (0.0005 ± 0.0001) Gy-1 (for FRTL-5 cells). A549 fitted parameters are in process. We conclude that for the evaluated cell lines, the quadratic linear model fits well up to doses of 8 Gy and for higher doses, the theoretical model that best fits varies according to the different cell lines, demonstrating a different behavior between different tumor cells.

0388 - STUDY OF AKT1, AKT2 AND PHOSPHO-S6 AS POTENTIAL BIOMARKERS OF BREAST CANCER PROGNOSIS

Maria Cecilia PERRONE (1) | María Jimena RODRIGUEZ(1) | Marina RIGGIO(1) | Marina BENES(2) | Diego ENRICO(2) | Mora AMAT(2) | Pablo MANDO(3) | Estrella LEVY(3) | Virginia NOVARO(1)

INSTITUTO DE BIOLOGÍA Y MEDICINA EXPERIMENTAL (IBYME-CONICET) (1); INSTITUTO ALEXANDER FLEMING (2); FUNDACIÓN CÁNCER FUCA, INSTITUTO ALEXANDER FLEMING (3)

PI3K/AKT/mTOR pathway has been shown to be altered in over 70% of breast tumors, and has consequently become a promising therapeutic target. However, PIK3CA and PTEN mutations do not always correlate with pathway activation or breast cancer prognosis. Therefore, there is a need for defining other biomarkers of PI3K/AKT/mTOR activation in order to correctly assess which patients would benefit from using specific inhibitors. We have focused our study on isoforms AKT1 and AKT2 as well as ribosomal protein S6, which have been shown to be upregulated in luminal breast cancer. Previously, in luminal breast cancer cell lines we found that AKT1 promotes cell proliferation through S6 phosphorylation (pS6), while AKT2 favors cell migration and invasion. Moreover, in 46 stage IV luminal breast cancer samples, AKT1 correlates with ki67 staining, whereas AKT2 expression is higher in tumors from patients with earlier relapse. In the present work, we extended our analysis to 62 luminal breast cancer samples (stages I, II and III) in order to further understand the role of PI3K/AKT/mTOR pathway in breast cancer progression. We performed immunohistochemistry for AKT1, AKT2 and pS6 and assessed tumor histology and protein quantification. We found that AKT1 protein levels decrease with greater tumor stage and Nottingham differentiation score ($p<0.05$). Moreover, primary tumors from patients that relapsed expressed markedly low levels of AKT1. On the other hand, higher AKT2 and pS6 levels were found to be associated with greater nuclear grade and Nottingham score ($p<0.05$), which correlate with worse prognosis. In summary, assessment of AKT1, AKT2 and pS6 protein level by immunohistochemistry could have a prognostic value in breast cancer. Whereas AKT1 expression seems to correlate with better prognosis, AKT2 and pS6 appear to be associated with unfavorable outcome.

0976 - IRGD FUNCTIONALIZED PEG-PCL POLYMERSOMES: CHARACTERIZATION OF CELLULAR UPTAKE IN ER ALPHA; POSITIVE BREAST CANCER CELLS

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INSTITUTO DE NANOSISTEMAS - UNSAM (1); VRIJE UNIVERSITEIT AMSTERDAM (2)

Our group studies endocrine resistance mechanisms with the aim of developing new therapeutic strategies for breast cancer treatment. We hypothesize that a therapeutic strategy based in the use of Tamoxifen loaded NPs coated with the tumor penetrating peptide iRGD would be more effective than the current free Tamoxifen treatment, and would reduce recurrence. In earlier studies, we found that functionalization of PS with iRGD increased PS uptake in breast cancer cells. However, it was not yet clear the fate of the PS carrier after cellular uptake. The objective of this study was to further understand the mechanisms of iRGD functionalized polymersome uptake and its cargo intracellular delivery in ERα positive breast cancer cells. To accomplish this, we synthetized polymersomes (PS) carrying Rohodamine (Rho), functionalized or not with FAM labeled iRGD. T47D cells were treated for 1h with the PSs and dyes were detected by confocal microscopy. We confirmed that iRGD functionalization increased PS uptake. Merged pictures of iRGD-PS-Rho showed some overlap of FAM and Rho dyes, although a linear correlation intensity of both dyes, a strong indicator of colocalization, was not found. However, a similar behavior of Rho and the nuclear dye, and Rho and FAM was observed in multichannel intensity plot profiles. Surprisingly, confocal pictures showed an accumulation of Rho in the nucleoli and nuclear membrane. To further understand the degradational pathway of iRGD-PS-Rho after cellular uptake, we evaluated PS colocalization with EEA1 (early endosomal marker) and LAMP-1 (lysosomal marker). To conclude, we demonstrate that the uptake of Rho increases in T47D ERα positive human breast cancer cells after iRGD functionalization of PS-Rho. Additionally, accumulation of PS cargo inside the nucleus is a promising feature for a drug nanoparticle-based delivery system.

0977 - POTENTIAL OF HOMING PEPTIDES TARGETING PRIMARY TUMOR-ASSOCIATED MACROPHAGES FOR NANOTECHNOLOGY SOLID TUMOR TREATMENT

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INSTITUTO DE NANOSISTEMAS - UNSAM (1); INSTITUTO DE QUÍMICA BIOLÓGICA DE LA FACULTAD DE CIENCIAS EXACTAS Y NATURALES (IQUIBICEN) (2)

The tumor microenvironment plays a key role in solid tumor growth and progression. A plethora of interactions between the stroma and different types of immune cells are established in a dynamic equilibrium defining patient prognosis. Among these cells we are interested in tumor associated macrophages (TAMs). TAMs originated from blood monocytes display a variety of phenotypes depending on the tumor generated microenvironment. In general, they acquire a M1 antitumoral phenotype during the first stages of cancer progression but as the disease proceeds, they develop a protumoral M2 phenotype, enhancing angiogenesis and contributing to chemotherapy resistance. Our group is focused in developing new strategies for solid tumor treatment using TAM-targeted nanoparticles. To do so, we are developing nanoparticles coated with peptides that specifically target M2 macrophages. We established a M2 macrophage mice model to test the effectiveness of multifunctional nanoparticles. We developed M2 macrophages by growing C57BL/C mice bone marrow precursors in different mediums. The control group (CG) grown in DMEM 20 % FCS (D20F), group one (G1) grown in D20F with GMCSF and IL4 and group 2 (G2) grown in D20F and conditioned medium of C6 tumoral cells. Flow citometry of these cells at day 5 reveal that G1 and G2 conditions enhance the expression of CD206, a M2A specific receptor while CG did not. Furthermore, we tested the binding of two different peptides that target TAMs. The CSPGAKVRC peptide (codenamed "UNO") that binds specifically to CD206 receptor and the CKRGARSTC peptide (known as "TT1") that has affinity for the mitochondrial p32 protein also membrane located in TAMs. Peptides showed specificity in after 30 min incubation at 4º C setting the bases for a potential nanotechnology based strategy for solid tumor treatment.

Inmunología e Inflamación / Immunology and Inflammation II

Chairs: Ana María Eijan | Mónica Galleano

0844 - CLONED OF SAG IN VIRUS LIKE PARTICLES OF THE JUNIN Z PROTEIN (Z-VLP) AS POTENTIAL BIOLOGICAL VEHICLE THERAPEUTIC TOOL.

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In different species including man, many T lymphomas have a mono or oligoclonal character, in terms of the expression of a given Vβ region. Superantigens (Sags) are proteins that bind to molecules of the major histocompatibility class II complex (MHC II) and interact with a specific Vβ chains in T cell receptors. We have previously described that Sags are able to induce apoptosis of murine lymphoma cells in vitro and in vivo. Besides, the advance in genetic engineering allowed the generation of virus-like particles (VLP), which maintain the same structural properties of virions without genome so, they are not infective. In this study we designed a VLP platform carrying Sag Vβ14 fused to JUNV Z protein, as these constructions are very efficient platforms for vaccines and transport systems of therapeutic molecules. In this way, arenavirus matrix protein Z plays an important role in virus budding allowing generating enveloped Z-VLP in absence of any other viral proteins. First, we observed that Z-VLP induces maturation of bone marrow-derived dendritic cells from BALB/c which is a prerequisite for Sag-TCR interaction. Then we determined the interaction between Sag sequences and the specific Vβ14 region in TCR. Finally, the sequence was amplified and cloned in the Z vector using the NotI and BamHI restriction sites. Four positive clones were sequenced and analyzed by MEGA Software. Thereafter, positive plasmid was transfected on 293T mammalian cells, and VLPs were purified from the supernatant. Next step will be to evaluate the capability to induce apoptosis of reactive lymphocytes by the construct to be considered as a new tool to eliminate specific T-cell.

0851 - VIRUS LIKE PARTICLES OF THE JUNIN Z PROTEIN (Z-VLP) BEARING EGFP AS A MODEL ANTIGEN.

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Virus-like particles (VLPs) are multiproteic nanostructures that mimic the organization and conformation of authentic viruses. Due to their lack of genome, these particles are incapable of infection or self-replication, yet maintain the efficient antigenicity required for the development of an immune response. Thus, VLPs are often included into vaccine formulations and at present, several licensed vaccines based on VLPs are already being used clinically against pathogens. The matrix protein Z of arenaviruses plays an important role in the process of virus budding and it is able to induce the generation of VLPs in a cell culture, even in absence of viral infection. We have previously demonstrated that Z-eGFP-VLPs induce dendritic cell (DCs) maturation in vitro. The objective was to evaluate VLP ability to induce lymphocyte activation in vivo. To achieve that, Balb/C mice were immunized or not with Z-eGFP-VLPs intraperitoneally. After 5 days, mononuclear cells were fractioned from the spleens, labeled with CFSE and later they were incubated with DCs previously pulsed with Z-eGFP-VLPs or PBS. Fluorescence of CFSE (FL1) was evaluated by FACS over 50,000 events collected. Results showed that Z-eGFP-VLPs stimulate a specific proliferative response in Balb/C vaccinated mice compared to control mice ($p<0.05$). Besides, contribution of CD4+ and CD8+ cells to Z-eGFP-VLPs induced proliferation seems to be equitable ($p<0.05$ and 0.04 respectively). As a consequence, this VLP would be a good vaccine candidate, given their safety and immunogenicity. Taking into account the worldwide situation about the lack of vaccines for multiple diseases, these nanoparticles could be used as platforms for vaccine production, through the cloning of different antigens into the plasmid vector, replacing the ORF of eGFP.

0852 - POLYMORPHISM IN DECTIN-1 AND TUBERCULOSIS DISEASE

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Tuberculosis (TB) infection is determined by the complex interaction between Mycobacterium tuberculosis (Mtb) and host genetic factors. We have demonstrated that the simultaneous binding of Mtb with Dectin-1 and TLR2 induces the generation of ROS in neutrophils. Until today, those studies of the implication of SNP mutations in Mtb receptors have been variable except for TLR2 which effect on TB susceptibility is indisputable. The relationship of polymorphism in Dectin-1 with tuberculous disease has not been studied until today. A single nucleotide functional polymorphism

(SNP) in Dectin-1 (Y238X, rs16910526) generates an early stop codon, resulting in the loss of the last 10 amino acids of the carbohydrate recognition domain which was associated with recurrent cutaneous mycoses, and reflected in a lower membrane expression. The aim of this work was to evaluate the frequency of polymorphism in the population, the possible association with TB and tests in vitro. The polymorphism Y238X, rs16910526 was not present in the total population studied (healthy subject, HS, n= 25 and TB, n= 25). On the other hand, TB patients showed a significant increase in the total GB cell count ($p<0.01$) and also the percentage of neutrophils ($p<0.002$). TB showed a lower expression of dectin-1 in neutrophils compared to HS, who could be both susceptible or not to the disease. In turn, we observe that TB generated lower levels of ROS in response to the Mtb stimulus ($p<0.002$) that correlated with the lower expression of dectin-1 ($p<0.02$). Until now we cannot attribute these results to the presence of this polymorphism although a larger population number could be needed or some other mutation not studied might be involved.

0856 - NITRIC OXIDE AND CYTOKINES LEVELS AFTER LPS CHALLENGE IN RATS: EFFECT OF DIETARY (−)-EPICATECHIN

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CATEDRA DE FISICOQUIMICA, FACULTAD DE FARMACIA Y BIOQUIMICA, UBA-IBIMOL (UBA-CONICET) (1); UNIVERSIDAD DE BUENOS AIRES, FACULTAD DE FARMACIA Y BIOQUÍMICA, INMUNOLOGÍA, IDEHU (UBA-CONICET) (2)

Endotoxemia is responsible for numerous effects both at tissue and systemic level which can be related with the activation of inflammation signaling pathways. Those mechanisms are triggered by complex interactions and involve multiple cell types, which produce a variety of signaling molecules such as nitric oxide (NO) and cytokines, driving to cell and tissue damage and disfunction. Some polyphenols from diet have known anti-inflammatory effects and this work has the aim to investigate the role of a flavanol, (−)-epicatechin (EC), in modulating inflammatory response at different molecular and tissue levels. Sprague-Dawley male rats, pretreated for 4 days with control diet or with diets supplemented with 20 (low-level, LLE) or 80 (high-level, HLE) mg (−)-epicatechin (EC)/kg of body weight/day, were challenged with bacterial lipopolysaccharide (LPS) (i.p. 4 mg/kg BW) and euthanized 6 h post LPS administration. In LPS-treated endotoxemic animals, plasma GPT, GOT and LDH levels were slightly or non-affected. However, NO levels in blood, measured by electronic paramagnetic resonance, increased significantly, and HLE avoided 41 % ($p<0.05$) of that increase. Plasma TNF-alpha levels correlated with blood NO changes ($r= 0.52$, $p= 0.02$). RAW 264.7 macrophages in culture were challenged with LPS (0 - 50 nM) for 24 h, and TNF-alpha, IL-6 and NO2- and NO3- levels were analyzed in the supernatants showing dose-dependent responses for the three parameters. Pre- and co-incubation with EC (0.25 - 5 µM) showed a statistically significative effect only preventing NO_2^- and NO_3^- production. These results suggest that EC was effective preventing NO production induced by LPS in vivo and in vitro, with a slight or not effect on pro-inflammatory cytokines. Further experiments are necessary to dilucidated the mechanisms involved in this effect, as well as the main NO sources under these experimental conditions.

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0881 - BLS AS AN IMMUNOMODULATOR AND CARRIER OF TUMOR ASSOCIATED-ANTIGENS FOR THE TREATMENT OF B16 MELANOMA

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FUNDACIÓN INSTITUTO LELOIR - IIBBA

Brucella lumazine synthase (BLS) is a homodecameric protein that activates dendritic cells (DC) via TLR4 inducing a proinflammatory response. Due to its structure, proteins can be fused to the 10 N-termini, constituting an already proven platform for the development of vaccines. The chimera BLS-OVA (containing ovalbumin peptide 257-264) induces the cross presentation of the peptide and a specific CTL response through TLR4. We have shown that treatment of B16-OVA melanoma-bearing mice with BLS or BLS-OVA have similar therapeutic outcomes, inducing significant but equal tumor growth delay and increased survival. It has been previously reported that the use of synthetic long peptides (SLP) improve the efficacy of immunotherapies. Hence, in order to increase the specific immune response, we designed two new chimeras: BLS-gp100, containing a long peptide from melanoma antigen gp100 with increased affinity to MHCI, and BLS-OVAXL, containing a longer peptide of the ovalbumin protein. To evaluate if the fusion of SLP increases the immune response in vitro, BMDC were stimulated with BLS-OVA or BLS-OVAXL and co-cultured with splenocytes from OT-I mice. Results show that the secretion of IFN-γ is similar in T cells activated with BMDC stimulated with BLS-OVAXL and with BLS-OVA. Interestingly, CD40 levels are higher in BMDC stimulated with BLS-OVAXL, showing that the use of SLP induces a greater DC activation. To evaluate the therapeutic effect of the different chimeras, mice were inoculated sc with B16-OVA cells and 2 days p.i., BLS-OVA, BLS-OVAXL or BLS-gp100 were sc administered. All treatments delay tumor development and improve mice survival. Moreover, both chimeras generated in this work increase the previously described therapeutic effect of BLS-OVA. In conclusion, we demonstrate that BLS can be used as a platform for immunotherapy and that the carrier effect can be enhanced by fusion of SLP, increasing the anti-tumor response.

0929 - CROSSTALK BETWEEN ANGIOGENESIS AND IMMUNOSUPPRESSION MEDIATED BY DYSFUNCTIONAL CD8 T CELLS

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IHEM-CONICET. UNCUYO. MENDOZA, ARGENTINA (1); IBYME-CONICET, BUENOS AIRES, ARGENTINA (2)

Aberrant angiogenesis and immune evasion stand out as hallmarks of cancer since they are essential processes for the development of most solid tumors. T cell exhaustion is one of the mechanisms that accounts for immune escape in the tumor microenvironment restraining effective antitumor response. The aim of this study was to investigate the crosstalk between angiogenesis and immunosuppression, focusing on exhausted CD8 T cells. To address this, we purified splenic CD8 T cells from C57/BL6 mice and exposed them to an activation/reactivation cycle using anti CD3/CD28, under normoxic or hypoxic conditions, and characterized them as activated or exhausted (ex) T cells according to expression of inhibitory receptors and cytokines. CD8ex showed higher expression of PD-1 and Tim-3 and lower levels of IL-2, TNF, IFN-g; and grzB ($p<0.05$). Moreover, degranulation of CD8ex was impaired as shown by low levels of CD107a expression ($p<0.05$). Interestingly, hypoxia and VEGF augmented the dysfunctional phenotype of CD8 T cells ($p<0.05$), reinforcing the idea that vascular and immune compartments are closely interconnected. To evaluate the impact of CD8ex on vascularization, we carried out an angiogenesis array, and in vitro angiogenesis assays using conditioned media derived from these cells. We found that CD8 T cells secrete proangiogenic factors, that are modulated during the activation process towards a proangiogenic profile. Importantly, CD8ex showed higher pro-angiogenic capacity, as shown by their ability to induce endothelial cell tubulogenesis and migration. Of note, hypoxia significantly enhanced the pro-angiogenic capacity of CD8ex ($p<0.05$). Taken together our results suggest a crosstalk between angiogenesis and immunosuppression, executed by CD8 T cells. Our results may contribute to generate a more integrated picture of the tumor microenvironment with critical implications in

clinical settings involving combination of immunotherapeutic and anti-angiogenic modalities.

Nanomedicina / Nanomedicine I

Chairs: Mariela Agotegaray | Marisa Taverna Porro

0028 - HYBRID LIPOSOMES FOR SENTINEL NODE

Mirel CABRERA(1) | Camila GONZALEZ(1) | Romina GLISONI(2) | Marcos TASSANO(1) | Marcelo FERNANDEZ(1) | Juan Pablo GAMBINI(1) | **Pablo CABRAL** (1)

CENTRO DE INVESTIGACIONES NUCLEARES, FACULTAD DE CIENCIAS, UNIVERSIDAD DE LA REPUBLICA (1); INSTITUTO DE NANOBIOTECNOLOGÍA (NANOBIOTEC UBA-CONICET), FACULTAD DE FARMACIA Y BIOQUÍMICA, UBA. (2)

Intraoperative lymphatic mapping and sentinel node biopsy (BGC) followed by selective complete lymphadenectomy, is a strategy for the treatment of patients with solid tumors that determines a greater sensitivity in histopathological evaluation and lymph node staging and a lower surgical morbidity. The nanoparticles are absorbed by the lymphatic system and be retained in the regional lymph nodes at the injection site. Among its advantages are high stability, high load capacity, possibility of incorporating hydrophilic and hydrophobic substances. The objective of this work was to develop a new hybrid radiopharmaceutical using 99mTc-labeled nanoparticles and fluorescein. Liposomes were prepared by "hand-shaken" method, obtaining DTPA-liposome film combining phosphatidylcholine, cholesterol and stearylamide-DTPA in chloroform mixture: methanol (2:1 v/v), rotated to dryness in a water bath at 60 °C. It was hydrated with fluorescein, DMSO and double distilled water, obtaining multilamellar vesicles extruded by 400 nm membranes. Nanoparticle size, size distribution and polidispersity indexes (PDI) was analyze by dynamic light scattering (DLS). These were labeled with 99mTc, chemical controls were performed by ascending chromatography and molecular exclusion. In vitro stability was assessed by confronting both complexes at different concentrations of cysteine. As a preclinical evaluation, normal C57 black mice injected into a hind leg were used subcutaneously. Images were taken at 5, 10, 15, 30 and 60 minutes post-injection. The size of the liposomes was 410 ± 40 nm. The labeling yield was >90 %. Biodistribution in normal mice at 1 h post-injection for 99mTc-DTPA-Liposomes-fluorescein demonstrates statistically significant uptake in the lymph nodes of the injected member compared to the lymph nodes of the non-injected member (0.40 ± 0.04 %, expressed as the average of % Act/gr ± DS vs. 0.04 ± 0.01 %, p= 0.02. The hybrid tracers tested in this work could be a radiopharmaceutical potential for lymph node evaluation in the sentinel node technique. Acknowledgement ANII, CSIC, PEDECIBA for financial support.

0084 - CYTOTOXICITY OF IRON OXIDE NANOPARTICLES WITH NATURAL COMPOUNDS AS COATING. COMPARATIVE STUDY ON MC3T3-E1 AND L929 CELLS

Mariángeles CARRÁ | **Ariel GONZALEZ** | Claudia Alejandra GRILLO | Natalia FAGALI | Mónica Alicia FERNÁNDEZ LORENZO

INSTITUTO DE INVESTIGACIONES FISICOQUÍMICAS TEÓRICAS Y APLICADAS (INIFTA); CONICET-UNLP

Magnetite nanoparticles (NP) are proposed for application in diagnostic techniques as RMI, treatments for hyperthermia, tissue ablation or as drug carriers. In this work, NPs were synthesized by electrochemical methods (1). Once prepared and dried, the NPs were weighed and mixed with alkaline gallic acid (GA) or green tea extract (GT) solutions, to achieve 1 mg/ml. In order to obtain stabilizing coatings NPs were sonicated, and then dialyzed and filtered to ensure sterility. The coated NPs were characterized by DLS, TEM and ATR-FTIR. Its hydrodynamic diameters were 26.01 ± 2.95nm (GA coating) and 52.25 ±7.43 nm (GT coating). The green tea coating NPs also formed agglomerates with sizes up to 185.0 ± 13.5 nm. Viability assays of pre-osteoblast (MC3T3-E1) and fibroblast (L929) cells were made after 24h of incubation with 1/10 - 1/1000 dilutions of the 1 mg/mL suspensions. The cells were stained with acridine orange fluorescent dye and the images were obtained with an epifluorescence microscope. The pictures were analyzed by the ImageJ software and the statistical analysis was performed (99.9 % confidence). These assays showed that GT coated NPs didn´t decrease the viability in L929 cells in the whole concentration range analyzed. However, MC3T3-E1 cells showed a significant decrease in viability from 10 µg/mL (85.4 ± 10.8 %). For GA coated NPs, the viability in L929 cells decrease from 50 µg/mL (81.0 ± 11.6 %); while MC3T3-E1 cells showed a viability reduction from 20 µg/mL (86.9 ± 5.3 %). Previous reports suggested that iron oxide NP may induce toxicity in cells via ROS production (2). From this study and according to other authors (3), it could be concluded that the toxic effects of magnetic iron oxide NP are highly dependent on the type of cell encountered with nanoparticles. Consequently, an appropriate cytotoxicity study requires assays with more than one cell line.

References: 1. doi: 10.1016/j.jmmm.2017.02.048 2. doi: 10.1155/2012/614094 3. doi: 10.1039/c5tb02007g Supported by ANPCyT-CONICET-UNLP grants.

0255 - SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES AS AN HYBRID (RADIOACTIVE-FLUORESCENCENT) IMAGING AGENT

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In the last decades, nanotechnology has radically changed the diagnosis of many human pathologies. Particularly, silver nanoparticles (AgNPs) have provided powerful biomedical applications in different areas and effectively contributed to the diagnosis of oncological diseases. The aim of this work was to obtain AgNPs with appropriate properties for hybrid (radioactive-fluorescent) (99mTc-AgNPs-ICG) imaging. For the preparation of AgNPs, three different solutions were prepared: (i) silver nitrate $2.5x10^{-3}$ M solution, (ii) ascorbic acid $1.7x10^{-4}$ M solution and (iii) citric acid $8.0x10^{-4}$ M solution. Briefly, 2 mL of (ii) were mixed with 5 mCi of 99mTcO4-, subsequently 2 mL of (iii) and 0.5 mL of (i), permanently stirring for 20 minutes at 37 °C. Preparation final pH was 5. Finally, 2 µL of green indocyanine (ICG, 1.3 mM) was added to obtained 99mTc-AgNPs-ICG. A TOSOHAAS 6300SWXL HPLC column (7.8 mm x 30 cm), double-distilled water as mobile phase (flow of 1 mL/min) and an UV detector (λ1 = 420 nm and λ2 = 254 nm) and gamma detector were used to characterize 99mTc-AgNPs-ICG. Fluorescence imaging was performed using BRUKER MS FX PRO equipment. Nanoparticle size, size distribution and polidispersity indexes (PDI) was analyze by dynamic light scattering (DLS) and FT-IR spectrum, were determined. AgNPs was obtain in aqueous solution and remained stable for at least one week. HPLC profile shown a good correlation between both the UV and GAMA chromatogram, with matching retention times. Main peak spectrum presented an absorption band close to 420 nm, corresponding to plasmon absorption band for AgNPs. The hydrodynamic diameter was 46 ± 1.5 nm and the FT-IR spectrum displayed characteristic bands at 3,193, 2,624, 1,596 and 1,212 cm^{-}

[1], confirming the synthesis. Stable silver nanoparticles with hybrid (radioactive-fluorescent) dual labeling (99mTc-AgNPs-ICG) were obtain with a simple and reproducible method. These silver nanoparticles could potentially be used as scintigraphic and fluorescent imaging agents for different biomedical applications.
Acknowledgement ANII, CSIC, PEDECIBA for financial support.

0571 - DEVELOPMENT AND CHARACTERIZATION OF METAL AND POLYMER NANOPARTICLES FOR CLINICAL DIAGNOSIS METHODS

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The impact of advances of nanotechnology are particularly relevant in diagnostics, in which nanoparticle-based assays have been developed for specific detection of analytes of clinical interest. In particular, the use of magnetic nanoparticles attracts more attention because of their ability to be recovered and reused. Besides, the large surface area of the material allows adsorbing molecules of interest such as peptides, enzymes or antibodies needed to diagnose. The aim of this study is to develop a new method for the preparation of polymeric-nanoparticles with magnetic core to be used in a diagnostic device that detects target molecules in a biological sample. In order to achieve this goal, we synthesized nanoparticle magnetic covered with a polymeric matrix reactive to pH. Briefly, magnetic nanoparticles of cobalt ferrite ($CoFe_2O_4$) have been synthesized by the method of chemical coprecipitation with $CoCl_2$ and $FeCl_3$ solution. Later, a lax polymeric matrix was synthesized around the metal core using methacrylic acid solution. Then, the incorporation of a fluorescent dye was allowed in those nanoparticles. Finally, another denser polymeric matrix thin layer was added to the nanoparticles and antibodies of interest were adsorbed by them on their surface. We obtain spherical and monodisperse nanoparticles with diameter between 150 - 500 nm and a core of 65- 200 nm by DLS and TEM. FTIR was used to confirm links formation correspondent of each stage. The magnetic properties were investigated using a vibrating sample magnetometer. Here, the sample presented a superparamagnetic behavior, determined by the hysteresis cycle. Besides, we study the fluorescent dye release profile when the nanoparticles were exposed to different acidic pH. The polymeric matrix changes its conformation in 5.5 - 6.0 pH which allows to release the dye. This study shows a new synthesis of nanoparticles to use in a diagnostic test that presents a magnetic behavior and a polymer that can response a pH to release the dye.

0636 - TOPICAL CO-DELIVERY OF VITAMIN D3 (VD3) AND BACTERIORUBERIN FOR PSORIASIS TREATMENT: FORMULATION AND IN VITRO STUDIES

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Psoriasis, a chronic immune-mediated disease, is characterized by excessive growth and abnormal differentiation of keratinocytes, angiogenesis, infiltration of neutrophils and inflammatory cells and cytokine production. Calcipotriol (vitamin D3 analogue) inhibits dendritic cells and IL-2 and IL-6 production by epidermal T cells, suppressing epidermal proliferation and differentiation. On the other hand, bacterioruberin (BR), the major C50 carotenoid found in halophilic Archaea, is one of the highest efficient natural antioxidants. However, the high hydrophobicity of VD3 and BR, and their susceptibility to chemical degradation impair its topical administration. The objective of this work was to combine the high antioxidant activity of BR with VD3 in one nanoparticle (VD3-BR-NP) to be administered by the topical route. The BR extract (350 µg BR/ g dry Halorubrum tebenquichense culture) showed the typical three-fingered profile (460, 490 and 525 nm) spectrum and an inhibitory concentration providing 50 % reduction of the DPPH radical (IC50) of 21 µg/ml. NP made of a core of compritol and BR extract (50, 75 and 100% of BR) covered by a shell of polar archaeolipids from H. tebenquichense and Tween 80 (2; 2; 1.2; 3% w/w) were prepared by homogenization-ultrasonication. The best formulation (50 % BR) in terms of smaller size (67 nm), high colloidal stability (6 months), lower cytotoxicity (5 % at 0.02 mg/ml LN) and high uptake by macrophages and keratinocytes was loaded with VD3. Nanosized (75 ± 2 nm), monodisperse (polydispersity index 0.496 ± 0.053), negative potential (-36 ± 0.3 mV), 6.5 mg/ml VD3 and IC50 to 4,8 µg/ml (activity was potentiated by co-encapsulation) VD3-BR-NP were obtained. Effectiveness of VD3-BR-NP was assessed on an in vitro psoriatic model and compared with conventional NP (lacking BR). VD3-BR-NP, and no conventional NP, showed anti-inflammatory and antioxidant activity. Concluding, VD3-BR-NP deserves further studies as promising topical option for treatment of psoriasis.

0652 - ADSORPTION OF IMMUNOMODULATORY PROTEINS ON SILICA NANOPARTICLES

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Nanoparticles (NPs) are excellent platforms for protein immobilization due to their high surface/volume ratio and they are under study as immunomodulation tools. Our objective is to analyze the adsorption of different proteins on silica NPs and evaluate their physico-chemical properties and stability. Silica NPs (SiNPs) were synthesized according to the Stöber method. A portion of these NPs were grafted with APTES to add amino groups to their surface, generating $SiNPsNH_2$. Size and shape of the NPs were analyzed by TEM and microimages were processed by ImageJ software. NPs showed a spherical form, with a mean diameter of 111 ± 11 nm. FTIR analysis revealed the SiO_2 characteristic spectrum. Adsorption isotherms of different proteins to both NPs were analyzed according to Langmuir and Freundlinch models. For TGF-ß, the maximum adsorption capacity (MAC) was 0.0175 mg/mg SiNPs with an adsorption efficiency (AE) of 60 % that fits Langmuir isotherm model (R^2= 0.9022) better than Freundlich isotherm model (R^2= 0.8767). For IL-1ß, MAC was 0.0526 mg/mg SiNPs with an efficiency of 86 %. Both proteins showed lower adsorption capacity and efficiency over $SiNPsNH_2$ than over SiNPs. IL-1ß adsorption on NPsSiO2 fit only Freundlich model (R^2= 0.9350). BSA immobilization on NPsSiO2 has a MAC of 0.2 mg/mg SiNPs with an AE of 76 %. Same results were obtained with $SiNPsNH_2$. All NPs-protein complexes were stable at 4°C and pH 7.4 with a slight protein release after a wash with PBS. Human monocytic leukemia cells (THP-1) were cultured with SiNPs and SiNPs/TGF-ß for 24 - 96 h. A decrease on the cell metabolic activity in presence of TGF-ß alone was observed ($p<0.05$). While the SiNPs and SiNPs/TGF-ß were fully biocompatible without affecting cell viability. As conclusion, different immunological relevant proteins were successfully immobilized on SiNPs and characterized. One of these nano-complex was tested in cell culture demonstrating to be biocompatible and a potential immunomodulatory tool.

0697 - LIPID NANOPARTICLES FOR THE TREATMENT OF REFRACTORY EPILEPSY: PK/PD EVALUATION.

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Epilepsy is the most common neurological disorder affecting around 50 million people worldwide, most of them refractory to traditional antiepileptic drugs (AED) therapy. Due to the restrictions imposed by the blood brain barrier (BBB) to the entrance of foreign substances into the central nervous system (CNS), generally, high doses of AED are needed in order to achieve a therapeutic effect. A hypothesis that would explain refractoriness is the overexpression at the BBB of efflux transporters such as P-gp, that would decrease the CNS bioavailability of the AEDs. Our goal was to develop lipid nanoparticles (LN) loaded with the AED Carbamazepine (CBZ), with enhanced distribution and brain bioavailability. LN were synthesized using the emulsification by ultrasonication technique. All formulation prepared were characterized in terms of their entrapment efficiency (% EE), particle size (PS), polydispersity index (PDI), Z-potential (Z-pot), thermal and structural properties, stability, in vitro release kinetic, permeability through cells monolayer and anticonvulsant activity. Finally, the amount of CBZ in brain was quantified at different times after administration. All formulation showed high % EE (89 - 96 %) and spherical shape, with an average PS of 163 nm, a low PDI and z-pot (between -2.4 and -6.3 mV). Sustained release was obtained during the time of the assay. Thermal and structural tests proved that CBZ was molecularly dispersed within the lipid matrix. Some of the LP formulations showed an enhance permeability ($p<0.05$) through MDCK cells monolayer. Pharmacodynamic (PD) studies showed protection against seizures for up to 4 h after administration, which was in agreement with the pharmacokinetic (PK) measures in brain. In conclusion, CBZ loaded SLN, with suitable size, shape and permeability characteristics were developed, able to achieve a sustained release over 24 h and with prolonged anticonvulsant activity.

0739 - ANTI-INFLAMMATORY ACTIVITY OF NEBULIZED CURCUMIN IN NANOVESICLES

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Curcumin (CUR) is a polyphenolic compound obtained from the roots of Curcuma Longa with a wide range of therapeutic properties such as antioxidant and anti-inflammatory activity. However, due to its hydrophobicity and instability in aqueous solution, it has a poor oral absorption and a rapid systemic elimination, which impede its clinical use. Archaeosomes (ARC) are nanovesicles made of archaeolipids that resist chemical and enzymatic hydrolysis as well as oxidation. Moreover, archaeolipids are ligands of scavenger receptors so they facilitate active targeting to cells that express these receptors in their surface. The aim of this work is to develop novel nanoformulations of CUR for the treatment of inflammatory lung diseases that can be nebulized and improved the activity of the drug. CUR was incorporated in lipidic bilayers of ARC (ARCUR) and of conventional liposomes (LCUR). Both formulations of nanovesicles had sizes of nearly 150 nm that were successfully nebulized and lipid and CUR recovery up to 85 %. To evaluate safety and efficacy aspects of nebulized nanoformulations we used a model of alveolar epithelium, which consisted on A549 cell line growing in transwells membranes in an air-liquid interface that were activated with LPS to release pro-inflammatory cytokines. ARCUR could completely inhibit the release of IL-8 and IL-6 while nanoLCUR could only completely inhibit IL-6 release. Transepithelial electrical resistance (TEER) and lucifer yellow permeability across the monolayer (LYP) were monitored because these are important parameters of the epithelial tightness. Monolayers activated with LPS decreased TEER values and increased LYP, with co-incubation of ARCUR these values were restored to the control values, while LCUR did not produced an inhibitory effect against LPS. In conclusion, ARCUR has potential for being used as therapeutic agent in lung inflammatory diseases.

0756 - ARCHAEOSOMES AS BACTERIORUBERINE VEHICLES: STABILITY, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY

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Reactive oxygen species (ROS) are responsible for the development of certain diseases such as cancer and atherosclerosis through the oxidation of DNA, proteins and lipids. Antioxidant compounds decrease the rate of mutagenesis and carcinogenesis by inhibiting oxidative damage to cells. The extreme halophilic archaebacteria produce a unique group of polar (PA) and neutral membrane archaeolipids (NA). PAs are composed of saturated isoprenoid chains linked by ether bonds to the glycerol carbons in the sn 2, 3 position and are highly resistant to hydrolytic, oxidative and enzymatic attack. NA are mainly composed of bacterioruberin (BR), a carotenoid with high antioxidant activity, able to reduce the rate of mutagenesis and carcinogenesis by inhibiting oxidative damage to cells. Its hydrophobicity and susceptibility to oxidation, however, makes its application difficult. The encapsulation of BR in nanovesicles could protect its antioxidant activity. In this work, we prepared archaeosomes made of PA and BR (ARQ-BR) and studied its antioxidant activity compared with the one of nanovesicles made of soybean phosphatidylcholine and BR (LIPO-BR). LIPO-BR (164 ± 10 nm, -16 ± 2 mV Z-potential and 1.3 µg/mg phospholipids/BR) showed a higher IC50% (concentration of BR which scavenge 50 % free radicals of DPPH) than ARQ-BR (222 ± 53 nm, -36 ± 3 mV Z-potential and 4.7 µg/mg phospholipids/BR): 43.26 µg/mg in the first case against 82,47 µg/mg in the second. However, the antioxidant activity of ARQ-BR remained unchanged after incubation at high temperatures (30' at 80°C) while it was completely lost in LIPO-BR. ARQ was able to protect the antioxidant activity of BR, even in hostile conditions, allowing future application and enabling its antioxidant and anti-inflammatory activity

0757 - DENDRITIC CELL TARGETING IN CULTURED FISH IN ARGENTINA FOR VACCINE DEVELOPMENT

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Aquaculture is a fast-developing sector in the food industry worldwide. In Argentina two main species present an important economic value, these are Pacú (Piaractus mesopotamicus, Pm) and Rainbow Trout (Oncorhynchus mykiss, Om). Due to stress and changing environmental conditions cultured fish are exposed to, the use of antibiotics has become a common solution for treatment and avoidance of disease. This practice presents several problems such as overdose, contamination and resistance generation. The development of effective and affordable vaccines is necessary for aquaculture in order to produce safe products for consumption and the environment. This work focuses on the evaluation of a species unspecific nanovaccine platform in Pm and Om, composed of liposomes decorated with a1,2-mannobiose, a specific disaccharide that targets DC-SIGN receptor, mainly expressed on dendritic cells (DC). We cultured DC obtained from head kidney (HK), of Pm and Om in complete D-MEM (10% FBS) for 1, 7 and 14 days at room temperature (RT) in order to obtain non-adherent cells, enriched in DC. These cells where later incubated for 30 m or 12h at RT in D-MEM without FBS with undecorated liposomes for unspecific cell

targeting (plain-L), a1,2-mannobiose decorated (Mana-L) and DOTAP (DOTAP-L) liposomes as a positive control, all marked with rhodamine. Prior liposome formulation and characterization with Z sizer was done. Incubation was stopped adding complete D-MEM. Cells were washed and fixed with PFA at 0.02 % w/v final concentration and then analyzed by flow cytometry. Results were statistically analyzed with two-way ANOVA followed by Bonferroni's test. Results demonstrate that HK cultures at day 7 and 14 are enriched in DC-SIGN expressing cells, and Mana-L targets specifically these cells (***p<0.0001). These preliminary results indicate that the nanovaccine platform would be efficient in targeting DC, therefore could be an important tool in aquaculture vaccine development.

0798 - SYNTHESIS OF PACLITAXEL POLYMERIC MICELLES BY MICROFLUIDIC TECHNOLOGY

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Polymeric micelles (MPs) are nanoparticulates systems usually used to optimize drug delivery. MPs are generally composed by hydrophobic polymers and surfactants or amphiphilic co-polymers that form a hydrophobic core, where it is possible to include lipophilic drugs. Generally, MPs are obtained by nanoprecipitation, where the bioactive molecules and the polymer are dissolved in organic solvent, such us acetone, and then are added in an aqueous solution containing surfactants. Then, the acetone is evaporated and the nanoparticles remain in an aqueous suspension. This process is substantially dependent on fluids dynamic, and the control of this, is critical to obtain homogeneous and reproducible results. In recent years, microfluidic technology-assisted nanomedicines synthesis (MT), where fluid are subtly controlled, significantly improve these processes and allow reproducibility between different batches. The objective of this project was to optimize the synthesis of MP and encapsulation of paclitaxel (PTX), a cancer drug used as a first-line treatment of breast and ovarian cancer through MT. To perform MPs microfluidic-assisted synthesis we obtain a micromixer chip with T design by the 3D printing technique. In addition, we synthetized MPs and PTX encapsulation in batch in traditional way in order to compare. In both cases, the quantification of PTX was performed by high performance liquid chromatography (HPLC). The linearity of the last method was evaluated, not observing differences between the retention times of the peaks corresponding to the MPs containing PTX and the standard solutions. Size of MPs was obtained by dynamic light scattering (DLS). The co-polymer of polycaprolactorna (PCL) and polyethylene glycol (PEG) in diblock arrangement (PEG-PCL) and different PTX concentrations were evaluated. Size, polydispersity and levels of PTX encapsulation were strongly optimized employing MT respect traditional methods.

0850 - INCORPORATION OF CARBAMAZEPINE, A NON-IONIC DRUG, IN LAYERED DOUBLE HYDROXIDES

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Layered double hydroxides (LDHs) are inorganic solids composed of cationic layers and inter-lamellar spaces filled with negative ions. In the last years, LDHs have gain interest as carriers of anionic drugs, which replace the inter-layers ions. Most anti-tumor drugs are systemically administered and this produces many side effects. LDHs could protect and target them to the tumor; however, most anti-tumor drugs have no charge and thus, they are not able to enter into the inter-lamellar space. Aiming to overcome this problem, a surfactant was used in this work. Carbamazepine (CBZ), an anticonvulsant drug with a formal charge of 0, have shown efficacy against some solid cancer cell lines; it also produces alterations in red blood cells. Here, this drug was used as a model drug to be load into the system. CBZ was incorporated into HDLs, composed of Mg_2-Al-NO_3, in micelles of sodium cholate (a surfactant with negative charge) by using different methods: ionic exchange, co-precipitation and reconstruction. Different amounts of surfactant were assessed, at a critical micelle concentration. X ray powder diffractograms showed that the drug incorporated in the system when synthetized by ionic exchange and reconstruction, but not by co-precipitation. The hydrodynamic sizes of the HDLs loaded with the drug in the micelles were measured by dynamic light scattering. The amount of CBZ loaded in the system was determined by UV and found to be ~2 %, which represents almost the 100 % of the original amount of drug. Assays of drug released were done in Franz Cells with a Simulated Body Fluid (pH 7.4) and an Acetate buffer (pH 4.8) in order to analyze the protector roll of the system in the blood and the drug release inside the tumor cells respectively. Results suggest that LDHs are promising drug delivery carriers by allowing the incorporation of all kind of drugs and not only of that with anionic charge.

0887 - MULTIFUNCTIONAL NANOSYSTEMS BASED ON GOLD NANOPARTICLES AND MITOCHONDRIA-TARGETED ANTIBIOTICS FOR THE TREATMENT OF MELANOMA

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Malignant melanoma is a tumor characterized by a very high level of heterogeneity, responsible for its malignant behavior and resistance to conventional therapies. Thus, new treatment strategies are urgently needed to help remedy this unmet clinical need. Treatment failure and disease progression has been attributed to the existence of cancer stem cells (CSCs). These have been implicated in tumor initiation, metastatic spread and poor survival in multiple tumor types, melanoma included. CSCs selectively overexpress key mitochondrial-related proteins and inhibition of mitochondrial function may represent a new potential approach for their eradication. Moreover, the combined use of radio and chemotherapy is being commonly used in cancer treatment. The side effects of the combined treatment can be minimized through the use of nanotechnology. In this regard, gold nanoparticles (AuNPs) can play a significant role since they can be used as radiation dose enhancers and anticancer drug carriers. In this context, we propose the evaluation of the radiosensitizer capacity of AuNPs functionalized with doxycycline (DOXY) and modified 9-aminoDOXY, both inhibitors of mitochondrial biogenesis, in melanoma cells. First, the in vitro metabolic activity and cytotoxic effect of the free DOXY and 9-aminoDOXY in human melanoma cell lines A375, A375-G10 and Mel-J was studied by MTT and trypan blue dye exclusion, respectively. Both compounds reduced the metabolic activity of all cell lines and diminished their viability. Then, melanoma cells pre-treated with both antibiotics for 48 h were irradiated with a 137Cs gamma source (0-5 Gy). A significant increase in radiation effect in DOXY and 9-aminoDOXY

treated cells was found. These results showed that both antibiotics can be used as radiosensitizers in radiotherapy of melanoma. Finally, for their conjugation to AuNPS, polyethyleneglycol-DOXY conjugates were obtained by two different approaches: (i) a direct route of synthesis by DCC/DMAP catalyzed reaction and (ii) a two-step synthesis through the introduction of a -NO_2 group in the structure of DOXY followed by a reduction and the final amidation. These polymers will be conjugated to AuNPs synthesized by the Turkevich method. Based on our preliminary results we believe in the possibility of treating melanoma resistant cells through a mitochondria-targeted therapy and the development of these nanosystems as a multifunctional theranostic platform.

0939 - GREEN EXTRACTION AND ENCAPSULATION OF CAROTENOIDS FROM HALOPHILIC ARCHAEA IN NANOPARTICLES AND NANOEMULSIONS

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Carotenoids are highly demanded by pharmaceutical, cosmetic and food markets. Halophilic archaea are an important source of C50 carotenoids, including bacterioruberin (BR) and its precursors. Due to their high antioxidant properties (free radical scavenger, quencher of reactive oxygen species (ROS) and nitrogen oxidative species (NOS), and chain-breaking antioxidant), BR is an interesting candidate in the development of new therapies for preventing and treating oxidative stress-related pathologies. However, its high lipophilicity and photolability often limits its use in human applications. On the other hand, conventional extraction methods of carotenoids include the use of organic solvents with negative impact on health, safety, and environment, hence there is a growing demand for using greener, bio-based and renewable solvents for extraction of natural products. The objective of this work was to develop a new process for carotenoid extraction using miglyol as alternative liquid lipid and formulated it into nanoparticles (NP-ME) or nanoemulsion to protect BR's antioxidant activity. Two successive extractions at 10: 0.5 v/v archaeabacteria cell paste: miglyol caused the highest BR concentration (21 µg/ml), although lower than that obtained in acetone/hexane (56 µg/ml). Miglyol extract (ME) resulted in the red characteristic colour of BR with the typical UV-vis spectrum and a concentration which scavenged 50 % of free radicals of 10 µg BR/ml. NP (compritol, ME, archaeolipids and Tween 80 (1.8; 1.8; 1.1; 2.8 % w/w) (82 nm; -31mV Z potential) and emulsions (ME, archaeolipids and Tween 80 (26; 0.8; 2.1 % w/w) (350 nm; -13 mV Z potential) retained antioxidant activity after two days of daylight exposure, while the ME lost it. ME and NP-ME inhibited the hemolysis of human erythrocytes induced by the toxicity of peroxyl radicals (ROO•). We considered these formulations could be useful for treatment of new therapies for preventing and treating oxidative stress-related pathologies.

Metabolismo y Nutrición / Metabolism and Nutrition II

Chairs: Adriana Burgueño | Susana Feliú

0029 - DIETARY IRON BIOAVAILABILITY, SERUM HEPCIDIN AND IRON OVERLOAD RISK IN A GROUP OF ADULT MALES.

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Iron (Fe) occurs as non-heme Fe and highly bioavailable heme Fe in the human diet. Feeding habits in Argentina are characterized by high bovine meat intake (55.5 kg/per capita/y, FAO 2013). Modulation of dietary Fe absorption is regulated by hepcidin. To study relationship between serum hepcidin and dietary Fe intake, 92 male blood donors (18-62 y) - clinically healthy- attending Hospital de Clínicas, UBA (2017-2018) were enrolled. Daily Fe intake (FeI), non-hem FeI, and hem FeI were estimated (Food Consumption Frequency questionnaire) (ARGENFOODS and USDA Database). Serum hepcidin (sHep) (DRG Hepcidin 25 (bioactive) HS ELISA Kit) was measured in blood samples negative for infectious diseases and C-reactive protein (PCR-latex, Wiener lab). C282Y, H63D and S65C genotypes were studied in whole blood by DNA extraction (Accupred Genomics DNA Kits) and PCR-RFLP (Bcl-I, Hinf-I and Rsa-I). The study was approved by Comité de Ética, Hospital de Cínicas (UBA); informed written consent was obtained. FeI was over 6 mg Fe/d in all participants, and 3.0% surpassed the upper level (45 mg Fe/d) (NAS, 2001). Males were divided into wild type (WT) (71 %), and those carrying mutations in the HFE gene (non-WT) (29 %). No significant differences were observed between Fe intake in both groups: FeI (mg/d): 23.5 ± 8.9 vs. 20.5 ± 7.6 (p= 0.2); non-hem FeI (mg/d): 21.3 ± 8.1 vs. 18.8 ± 7.3 (p=0.3); hem FeI (mg/d): 2.1 ± 1.2 vs. 1.65 ± 0.76 (p=0.3); as well as in sHep (ng/ml): 31.4 ± 20.3 vs. 37.0 ± 21.5 (p=0.3), WT vs. non-WT, respectively. Moreover, in WT and in non-WT, sHep did not correlate significantly with: FeI (p=0.8 and p=0.4); non-hem FeI (p=0.9 and p=0.4), nor hem FeI (p=0.4 and p=0.4), respectively. These results show no increase in serum hepcidin level with Fe intake, even with high hem Fe intake. However, risk of Fe accumulation in subjects carrying mutations in the HFE gene should not be ruled out. Support: UBACyT 20720150100004BA

0073 - A REDUCED LACTOSE YOGURT CONTAINING GALACTOOLIGOSACCHARIDES (GOS) AS A TOOL FOR LACTOSE INTOLERANT COVER CALCIUM DAILY INTAKE THAT ENSURES BONE HEALTH

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GOS, natural prebiotics of human milk could be incorporated in dairy products, such as yogurt, by enzymatic action on milk lactose. The functional characteristics of such reduced-lactose yogurt containing GOS were previously demonstrated in normal growing rats. The aim of this study was to evaluate the beneficial effects of this reduced-lactose yogurt containing GOS in Ca and P absorption and bone retention. Male weaning Wistar rats (n= 10 per group) received during 30 days AIN´93-G control diet (CD) or the yogurt containing GOS diet (ED). Ca and P Abs were evaluated during the last 3 days of the experience; depth of intestinal crypts, femur Ca and P content, bone mineral content (BMC) and bone mineral density (BMD) at the end of the study. BMD of lumbar spine (LS), of total (TT) and proximal (PrT) tibia, and TT BMC were also evaluated. The results (ED vs. CD) were expressed as mean ± SD. Food consumption and PC were similar in both groups. The % AbsP was significantly higher in group ED (86.6 ± 6.6 vs. 78.0 ± 7.1 %; p<0.05),

and % AbsCa showed a non-significant higher level in group ED (84.9 ± 2.2 vs. 80.0 ± 5.4 %; p= 0.062). TT BMD (0.25 ± 0.02 vs. 0.25 ± 0.02 g/cm^2), TT BMC (0.029 ± 0.004 vs. 0.030 ± 0.014 g), and CL BMD (0.25 ± 0.02 vs. 0.25 ± 0.02 g/cm^2) no showed significant differences, while BMD TPr (0.30 ± 0.06 g/cm^2 vs. 0.27 ± 0.02; p<0.05) was significantly higher in group ED. The depth of the intestinal crypts was non-significant greater value in group ED (212.6 ± 12.4 vs. 205.1 ± 21.3 µm; p = 0.28). Femur Ca and P content showed no significant differences between groups. These results suggest that the assayed here functional product would be an optimal tool to achieve the bone mass peak, preventing future bone alterations, in lactose intolerant individuals.
Supported by PIP and UBACyT grants.

0108 - ACTIVATION OF NRF2 PATHWAY BY COMPOUND A PROTECTS AGAINST OXIDATIVE STRESS AND ß-CELL DYSFUNCTION

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Pancreatic ß-cells are specialized to secrete insulin in response to circulating nutrients, mainly glucose. Type 1 diabetes is a T cell-mediated autoimmune disease that selectively destroys ß-cells; both ER stress and subsequent insulin secretory deficiency precede the onset of disease. Hyperglycemia triggers excess production of mitochondrial reactive oxygen species (ROS) that overwhelm the anti-oxidative capacity of ß-cells, leading to oxidative stress. The crosstalk between the ER and oxidative stress further contributes to ß-cell dysfunction. The inflammatory microenvironment of the islet during the autoimmune attack leads to activation of ER stress, oxidative stress, ß-cell dysfunction and death. We reported that Compound A (CpdA), a dissociative glucocorticoid receptor-ligand, is an effective modulator of T and dendritic cells and ameliorates cytokine (IL-1ß;+IFN-g; CYT)-induced ER stress in ß-cells. The aim of this study was to explore the protective effects of CpdA on CYT-induced ß-cell oxidative stress. CpdA enhanced Nrf2 transcriptional activity (antioxidant defense pathways) and the expression of Nrf2 target genes (NQO1, HMOX-1 and Txnrd1) in the rat insulinoma INS-1E (p<0.05). CYT-induced ROS generation was reduced by CpdA in INS-1E cells (p<0.05). CpdA diminished the CYT-induced upregulation of Bax/Bcl-2 ratio (p<0.05) and DP-5 mRNA expression (p<0.05) suggesting a protective effect against CYT-induced apoptosis in INS-1E cells. CpdA protected against the reduction of viability and enhanced basal insulin secretion in CYT-challenged INS-1E cells (p<0.05). In summary, we demonstrated that CpdA attenuates oxidative stress and improves viability and function in CYT-challenged ß-cells. These results, together with our previous reports on immune cells modulation and the reduction of CYT-triggered ß-cell ER stress encourage further research on CpdA as an agent with potent therapeutic activity on autoimmune diabetes.

0248 - GLUTATHIONE IN THE CONTROL OF THE INTRACELLULAR REDOX HOMEOSTASIS OF RATS WITH COPPER OVERLOAD

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Copper (Cu(II)) toxicity is associated with oxidative stress (OS) and the oxidation of biomolecules mediated by the generation of reactive oxygen species, mainly hydroxyl radical and organic hydroperoxides (ROOH). The objective of this work is to assess whether oxidative damage (OD) due to acute toxicity of Cu(II) in rats involves changes in the multiorganic redox homeostasis. Male Sprague Dawley rats (200 g) received 0.9 % w/v sodium chloride (controls) or three different doses of Cu(II): 5.0, 6.5 and 7.5mg/kg (i.p.) as copper sulfate. In homogenates of heart (H), kidney (K), lung (L), liver (Li) and brain (B), OS markers were determined by spectrophotometry (6 h): phospholipid oxidation, measured as the content of thiobarbituric acid reactive substances (TBARS); reduced glutathione (GSH) content; enzymatic activity: superoxide dismutase (SOD), catalase and glutathione transferase (GT). Results indicated that TBARS were increased in all the organs and dose, mainly in H, B and Li with increases higher than 100 % in H (p<0.05) and B (p<0.01), and Li with dose 6.5 and 7.5 mg/kg (p<0.01). Antioxidant defenses showed a dual behavior: GSH content decreased in B (all dose, p<0.05), H and Li (5 mg/kg, p<0.05); but increased with the highest dose in L (p<0.05), K (p<0.01) and Li (p<0.05). SOD decreased in H and B with all dose (p<0.01), in K with 6.5 and 7.5 mg/kg (p<0.01) and in Li with highest dose (p<0.05). Catalase decreased in K (6.5 mg/kg, p<0.01) and L (5 mg/kg, p<0.05), and increased in H and L (p<0.05), Li (p<0.01) with dose 6.5 mg/kg, and L with 5 mg/kg (p<0.05). GT only was affected in K (decreased with 5 and 6.5 mg/kg, p<0.05 and 0.01 respectively) and Li (decreased with 6.5 mg/kg and increased with 5 and 7.5mg/kg, p<0.05). Cellular antioxidant protection involves GSH consumption in response to the generation of oxidant species and phospholipid peroxidation, and redox regulation of antioxidant enzyme activities that responds to GSH content and GT activity.

0562 - STUDY OF THE GLYCEMIC AND LIPID PROFILE IN AN ARGENTINE POPULATION WITH ACUTE INTERMITTENT PORPHYRIA UNDER A HIGH-CARBOHYDRATE DIET

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Acute intermittent porphyria (AIP) is a hereditary disorder of heme biosynthesis, characterized by a decrease in the activity of porphobilinogen deaminase enzyme, associated with an increase in the expression of δ-aminolevulinic acid synthetase 1, first and regulatory enzyme of this pathway, and with the accumulation of the neurotoxic precursor δ-aminolevulinic acid (ALA). In the event of an acute attack, patients diagnosed at CIPYP with AIP receive an overload of glucose (400/500 g/day) intravenously, folic acid and vitamin B complex, then the treatment is continued for life, with a high-carbohydrate diet (300g/day), folic acid and B complex. According to clinical follow-up, this treatment has proven effective in preventing attacks and / or decreasing their intensity. According to numerous studies, type 1 insulin-like growth factor (IGF1) interacts with insulin to modulate carbohydrate metabolism and its decrease alters lipid metabolism. In our laboratory, 40 % of patients with AIP have low IGF1 values, so the aim was to study the influence of a high-carbohydrate diet on the glycemic and lipid profile in a population of AIP patients with low and normal IGF1 levels. The study was done in AIP individuals (n= 33) aged between 19 to 68 years old. IGF1, glucose, insulin, cholesterol, triglycerides, low- and high-density lipoproteins and total lipids level were measured in blood samples. In addition, the levels of ALA, porphobilinogen and total porphyrins in 24-h urine were quantified. Respect to the lipid and glycemic analysis performed in the subpopulation of AIP patients who had normal or low IGF1, no alterations were detected in any of the studied parameters. In the cohort of AIP patients analyzed, no alterations in glycemic or lipid profiles were observed in AIP patients that receive a high and continuous diet of carbohydrates, independently of IGF1 levels.

0618 - EGG PROTEIN OVOTRANSFERRIN DERIVED PEPTIDE IRW ON HUMAN

ENDOTHELIAL INFLAMMATORY RESPONSE DURING HYPOXIA.

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Due to the unavoidable side effects of synthetic drugs, there is an increasing interest in the search for novel bioactive food components for the prevention and treatment of cardiovascular diseases. The tripeptide IRW, from egg white protein ovotransferrin, was found to exhibit an anti-inflammatory effect blocking the nuclear translocation of the transcriptional factor NFkB. The objective of the present study was to examine the molecular mechanisms of its anti-inflammatory activities during hypoxia. IRW is produced by solid phase peptide synthesis. Human endothelial cells (EA.hy926 line) were incubated in the presence of 5 or 50 µM IRW during 20 h, before adding cobalt chloride (250 µM) for 6 h to induce chemical hypoxia. Endothelial sterile inflammatory response was evaluated by NFkB subcellular distribution through immunofluorescence analysis and zymography studies. Endothelial induced nitric oxide synthase (iNOS) expression was determined by RT-PCR. After hypoxia, NFkB was localized both at the cytoplasmic and nuclear compartment. However, the transcriptional factor is restricted to the cytoplasm in the presence of IRW (5 or 50 µM). An increased activity of metalloproteinase-9 during hypoxia was registered (quantification was not possible at equal protein concentration evaluated, by zymography studies, among different treatment). Nevertheless, a significative decrease was recorded in the presence of IRW ($p<0.05$). Remarkedly, hypoxia induced a significative increase in iNOS expression ($p<0.05$, control vs. hypoxia), which was only decreased near to control levels after 5 µM IRW treatment. Our results suggest the IRW potential as functional food ingredient or nutraceutical for the prevention of both endothelial dysfunction and endothelial inflammatory response, key factors for the development of cardiovascular diseases.

0898 - EFFECTS OF HEMIN TREATMENT ON HEPATIC PARAMETERS IN A RAT MODEL OF NON-ALCOHOLIC FATTY LIVER DISEASE

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Non-Alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease. It has been associated with cardiometabolic risk factors. Previous results from our laboratory showed an increase in oxidative stress parameters in the liver of rats fed a sucrose rich diet (SRD) for 12 weeks. A normalization of these parameters was observed after the administration of hemin (15 mg/kg ip, every 48 h) for the last two weeks of the dietary modification. Since oxidative stress has been associated with the induction of tissue damage and/or with metabolic changes in the tissue, in the present study we proposed to evaluate the effects of this pharmacological treatment on liver histology and metabolic parameters. Male Wistar rats were randomly distributed into control (C) and SRD groups (30 % sucrose in the drinking water). Hemin treatment was administered as described (H and SRD+H groups). Animals fed a SRD showed lower insulin sensitivity than controls, as assessed by an insulin tolerance test (KITT, $p<0.0001$ vs. C) and the KITT was not affected by hemin treatment. Similar results were obtained by analyzing the TAG/HDL-c index, a secondary marker for IR ($p<0.0001$ vs. C). Hepatic insulin resistance was assessed by a pyruvate tolerance test (PTT). An increased glucose output was observed in SRD-treated animals ($p<0.05$ vs. C). In agreement, protein levels of PEPCK, a gluconeogenic enzyme, were also increased in this group ($p<0.05$, vs. C). Hemin treatment corrected both parameters ($p<0.05$ vs. SRD) The histological analysis showed a higher ballooning score in the hepatic tissue of animals fed a SRD ($p< 0,05$ vs. C), and a greater NAFLD activity score ($p<0.05$ vs. C). These parameters were not affected by hemin treatment. In summary, hemin treatment of IR-rats is associated with an improvement in insulin sensitivity in the liver, while no changes in systemic insulin resistance and histological parameters associated with tisular damage by SRD.

0908 - ABSENCE OF TRPCS LEADS TO INCREASED BODY WEIGHT

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TRPC genes encode non-selective Ca^{2+}-permeable cation channels implicated in the mechanism of store operated Ca^{2+} entry. TRPC channels have been increasingly linked to a diverse number of pathologies. The mutation, down or up-regulation of any of these channels may lead to diseases that include cardiopathies, neuronal disorders and immune deficiencies among others. This highlights the potential of TRPC channels to become novel therapeutic targets and also provides evidence of their physiological function. The absence of specific inhibitors limits the study of these channels and led to the development of knockout (KO) mice. KO of each and every one of the 7 TRPC genes, separately, has yielded mice with distinctive phenotypes, some favourable, others detrimental to the health of the mouse. It is important to note that TRPCs 1-7 have overlapping functions that could mask the effect of removing just one of these channels. HeptaKO mice are alive and breed descendants. Nonetheless, they present increased body weight compared to WT mice. Our laboratory has obtained RNA-seq data from eight tissues (liver, heart, spleen, testis, lung, kidney, midbrain and forebrain) of the heptaKO mice and their WT counterparts. This analysis may allow us to determine a possible link between TRPCs and signalling pathways that could explain the observed phenotype(s). To this end we performed a pathway enrichment analysis from which we determined that one of the most prevalent modified pathways is circadian rhythm. Interestingly, the latter has been increasingly related to metabolic syndrome that is characterised by localised fat around the waist, elevated blood pressure, high triglycerides, elevated blood sugar, and low HDL cholesterol. These results could thus present the TRPCs as potential therapeutic targets for this syndrome

0937 - PORPHYRINOGENIC AGENTS AFFECT MITOCHONDRIAL FUNCTION IN A MOUSE MODEL OF ACUTE INTERMITTENT PORPHYRIA

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Mitochondria plays a vital role in energy metabolism because oxidative phosphorylation and ATP synthesis occurred inside them. Heme is a prosthetic group of several hemeproteins such as Complex II, III, IV and mitochondrial nitric oxide synthase (mtNOS). Previously we demonstrated that porphyrinogenic agents affected several brain metabolisms including respiratory chain and Krebs cycle in CF1 mice and a murine genetic model of acute intermittent porphyria (AIP). The aim was to further investigate the effects of porphyrinogenic agents on mitochondrial metabolism in AIP mice. Complex IV, V and mtNOS expression and mitochondrial integrity were evaluated. Experiments were done in brain mitochondria of AIP male mice receiving Isoflurane (2 ml/kg), Sevoflurane (1.5

ml/kg), ethanol (30 %), allylisopropylacetamide (AIA, 350 mg/kg), Veronal (167 mg/kg) or starved (24 hours). Complex IV expression was only diminished by Veronal (50 %, p<0.05). Complex V expression was induced by Isoflurane (30 %, p<0.05) and inhibited by Veronal, ethanol and starvation (60-90 %, p<0.05). mtNOS expression and/or activity were induced (70-100 %, p<0.05) by anaesthetics and starvation. When Heme aa3 and cytochromes b, c, aa3 were quantified, no differences were observed due to anesthetic treatment; a significant reduction (70 %; p<0.05) was recorded by AIA or starvation, while Veronal and ethanol caused more than 100 % (p<0.05) induction. Mitochondrial integrity was assessed determining cytochrome c expresion and by MTT assay. A decrease in cytochrome c levels was caused by Sevoflurane (30 %; p<0.05), AIA (55 %; p<0.01) or ethanol (67 %; p<0.01). MTT trial showed significant alterations after Isoflurane, Veronal and AIA administration or under fasted conditions. In conclusion, xenobiotics caused a broad alteration in brain affecting simultaneously heme metabolism, respiratory chain, TCA cycle and nitric oxide metabolism, either by a direct action on enzymes or due to the relation among them.

0962 - ASSOCIATION BETWEEN THE DIETARY PATTERN AND INSULIN RESISTANCE IN A POPULATION OF PATIENTS FROM SAN LUIS CAPITAL, ARGENTINA

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IMIBIO-CCT-SL-CONICET-FQBYF-UNSL

The nutritional pattern has an important impact on public health quality becauseits relationship with the prevalence of obesity-associated chronic metabolicabnormalities. Herein we sought, for the first time, to determine whether, in alocal population of patients, there is a relationship between the nutritionalpattern and the potential risk of insulin resistance (IR). The sample included 29 female and 38 male patients, aged between 18 and 80 years-old, from theneighborhoods located at the downtown area of the Capital city of San Luis, Argentina. The nutritional pattern and IR were determined by analyzing avalidated food frequency questionnaire and anthropometric measurements, physical-activity questionnaire and biochemical data; respectively. Anthropometric measures included body mass index (BMI), waist circumference, waist/hip ratio, physical activity level questionnaire. Thebiochemical indicators of an increased IR risk included high fasting blood-glucose concentration, TG/HDL-cholesterol ratio and C-reactive proteinconcentration. Variables that were associated with a significantly high IR riskincluded high BMI, waist perimeters, triglycerides/HDL-cholesterol ratio and sedentary lifestyle. An unhealthy diet and poor physical activity were closelyassociated to IR when considering the percentages of the main food groups andthe parameters of IR. The data obtained allowed us to suggest that, in ourpopulation sample, the nutritional pattern is moderately healthy given the lowconsumption of healthy foods against the high consumption of high-energy foods. It is necessary to implement health policies aimed at strengthening theimportance of healthy diets and lifestyles to reduce the incidence of IR.

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0964 - DIETARY CONSUMPTION OF ZINC AND MODULATORS OF ITS ABSORPTION: A FIRST STUDY IN SAN LUIS CITY, ARGENTINA

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IMIBIO-CCT-SL-CONICET-FQBYF-UNSL

Dietary zinc (Zn) is an essential trace element that determines the incidence of obesity and its metabolic abnormalities. However, its bioavailability is affected by other nutritional factors involved in its absorption (facilitators and inhibitors). Herein we aimed at determining the dietary consumption of Zn and modulators of its absorption (facilitators/inhibitors) in a population of adults, of both genders between 18 and 59 years-old, residing in the Downtown District of the Capital City of San Luis in 2018. A validated food frequency questionnaire was used to evaluate dietary Zn consumption, as well as inhibitors (calcium, iron and fiber) and facilitators (proteins) of its absorption. The variables studied were gender, age group (young adult and mature adult), consumption of dietary zinc, calcium, iron and proteins. The dietary Zn consumption was insufficient in 35.67 % of the sample population. Regarding the consumption of inhibitors of Zn absorption, it was observed that dietary calcium intake was high in both young and mature adults. The most consumed sources of calcium were milk (49.43 %) and soft cheeses (22.40 %). It was observed that the intake dietary iron was optimal only in 6.25 % of mature women, and within its source foods, meat was the one with the highest intake (37.45 %). Fiber presented an insufficient consumption for young women, 72.73 %. The most consumed foods with high content of dietary Zn were potatoes (14.95 %) and tomatoes (14.50 %). However, mature adult women had an insufficient intake of dietary proteins, observing that among the most consumed protein source foods were red meats (46.61 %). The population studied had a sufficient consumption of dietary Zn, but a high consumption of dietary inhibitors of Zn absorption. This pattern may impact the prevalence of obesity and its associated metabolic abnormalities, consequently nutritional advice is needed in our population. Supported by PROICO 100218, PICT 3369, PIP 916 and PUE-IMIBIO-SL grants.

Reproducción / Reproduction III

Chairs: Carolina Mondillo | Mariel Núñez

0026 - PATERNAL EPIGENETIC PROGRAMMING: COCAINE TRIGGERS HISTONES POST-TRANSLATIONAL MODIFICATIONS IN MOUSE GERM CELLS

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Recent evidence indicates that paternal cocaine (coc) intake affects development and behavior of the offspring via epigenetic modifications. However, the mechanism by which coc alters germ cells epigenome has been poorly investigated. We previously showed that coc administration increases tyrosine hydroxilase expression and downregulates dopamine receptors DRD1 and DRD2 (doi:10.1371/journal.pone.0142713). Moreover, coc increased global 5-mC levels in DNA from germ cells and sperm, increased acetylated histone 4 (H4ac) and decreased class I deacetylases HDAC1/2 protein levels in germ cells (doi:10.1016/j.rbmo.2018.05.014). Here, we analyzed specific post-translational modifications (PTM) of histones in isolated germ cells of adult male mice treated with coc (10 mg/kg) or vehicle (veh), in an intermittent binge protocol (3 i.p. injections, 1 h apart, one day on/off for 13 days). To evaluate the involvement of DRD1 in the deleterious action of coc, DRD1 antagonist SCH23390 (SCH, 0.05 mg/kg) was injected 15 min before each coc or veh injection. Immunohistochemistry showed that H3K9ac, H3K27ac, H3K4me3 and H3K9me3 were localized in spermatogonia and early meiotic stages of spermatogenesis in veh and coc treated-mice. Coc increased H3K9ac and H3K9me3 and decreased H3K4me3 and

H3K27ac protein levels in germ cells ($p<0.05$). Pre-treatment with SCH had no effect on increased H3K9ac and H3K9me3 and decreased H3K27ac levels induced by coc, but prevented the decrease on H3K4me3 protein levels induced by coc ($p<0.05$). Coc also decreased HDAC1/2 proteins and pre-treatment with SCH reverted this effect ($p<0.05$). These results provide evidence that coc alters specific PTM of histones related to the activation of transcription of nearby genes and testicular DRD1 is able to mediate some of the epigenetic changes in male germ cells. Thus, the control of paternal lifestyle prior to conception could represent an upcoming hot topic in the management of male fertility and reproduction.

0611 - OVARIAN INTRAFOLLICULAR LIPID ENVIRONMENT OF PATIENTS INCLUDED IN ASSISTED REPRODUCTION PROGRAMS: IN SEARCH OF REFERENCE VALUES

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The functional role of ovarian follicular lipids and intracellular sites for neutral lipid storage (lipid droplets) is relevant for lipid homeostasis of cumulus-oocyte complexes but still an understudied topic. Until now, it is not clear if there is an association between plasma dyslipidemia and ovarian intrafollicular lipids or how this lipid environment affects oocyte maturation. In order to establish reference values or a reference metabolite profile that better define oocyte normal environment, we characterized follicular fluids (FF) and granulosa cells (GC) of control patients with measured plasmatic lipids (women), including male and tubal factor infertility, donors and women with desire to gestate genetically related children. We analyzed: (i) neutral lipid composition, hyaluronic acid and products of lipid peroxidation from FF and (ii) hydrophobicity of intracellular lipids of GC. Total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides (TG) content in plasma samples of 34 patients (33.5± 6.3 years) were compared to those in FF from the largest follicle and from a pool of smaller follicles. Lipid levels were lower in both FF than in plasma. Positive correlations were found between plasmatic and follicular HDL-cholesterol and between both types of FF for all lipids analyzed. Mean values of TG for both types of FF were statistically different between women with or without dyslipidemia. Hyaluronic acid (ELISA) and 4-hydroxinonenal (Western blot) were measured in FF from those patients without dyslipidemia (n= 21). A single target protein modified by 4-hydroxinonenal was found in all patients. Hydrophobicity of intracellular lipids was evaluated by fluorescence spectroscopy using the lipophilic dye Nile Red. Fluorescence spectra from Nile Red stained GC revealed a coincident emission maximum (578 nm). Human intrafollicular ovarian environment exhibits a distinctive lipid profile compared to plasma, with basal lipoperoxidation levels and yellow-orange Nile Red emission of GC indicative of hydrophobic lipid droplets.

0674 - CHARACTERIZATION OF MITOCHONDRIAL ACTIVITY DURING MURINE SPERM CAPACITATION

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Mammalian sperm need undergo a capacitation process in the female reproductive tract to acquire the ability to fertilize an egg. Overall, capacitation involves a series of structural and functional changes in sperm including membrane modifications, modulation of enzyme activities and protein phosphorylation. All these changes require an adequate supply of energy and, therefore, imply a fine energy administration. However, the energy sources and the molecular mechanisms of this regulation are still poorly understood. Based on this, the aim of this study was to determine the relevance of mitochondrial activity on mouse sperm capacitation. For this purpose, changes in mitochondrial membrane potential (MMP) were measured in live sperm during capacitation by flow cytometry using the probe TMRE. Results showed that only live sperm exhibited high MMP and, moreover, the percentage of sperm with high MMP increased during capacitation. When sperm were treated with increasing concentrations of the mitochondrial uncoupler CCCP during capacitation, there was a decrease in the percentage of cells with high MMP with no effects on sperm viability. Interestingly, we also observed a decrease in the average fluorescence intensity of sperm with high MMP compared to the control group. Next, we evaluated the need of capacitation for MMP increase by inhibiting the activity of PKA, a signaling molecule critical for this process. We observed that the percentage of sperm with high MMP did not increase in the presence of the PKA inhibitor H89. We next evaluated motility and MMP by fluorescence microscopy and observed that whereas motile sperm exhibited high MMP, cells with low MMP were immotile. In addition, treatment with CCCP produced a decrease in motility. In summary, we show, for the first time, that MMP increases during mouse sperm capacitation and that mitochondrial activity could be associated with the maintenance of sperm motility during this process.

0792 - C-X-C MOTIF CHEMOKINE RECEPTOR 4 (CXCR4) AND ITS LIGAND STROMAL CELL-DERIVED FACTOR 1 (SDF1) PROTEIN EXPRESSION IN THE OVARY DURING THE NATURAL ESTROUS CYCLE OF THE DOMESTIC CAT (FELIS CATUS)

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Expression of immune function genes (e.g. chemokine receptors) within follicle cells has been reported in ovaries from different species, suggesting that chemokine action in the ovary may extend beyond its role as a chemoattractant. In neonatal mouse ovaries, interaction between the chemokine receptor CXCR4 and its ligand SDF1 has been shown to prevent primordial-to-primary follicle transition. Our laboratory has previously shown that CXCR4 and SDF1 are expressed in the cumulus cells and oocyte of cumulus-oocyte complexes (COCs) retrieved from feline antral follicles, and that SDF1 significantly increases the expression of key ovulatory genes HAS2 and TNFAIP6 within the feline COC in vitro, through its main receptor CXCR4. Thus, the aim of this study was to analyze the protein expression and localization of the chemokine receptor CXCR4 and its ligand SDF1 in the ovary during the natural estrous cycle of the domestic cat (Felis catus). Ovaries from adult cats (n= 12) in different stages of the estrous cycle -estrus, diestrus and anestrus- were collected, fixed and processed for immunohistochemistry. Immunolocalization of CXCR4 was distinguished in the nucleus and/or cytoplasm of various cell types (oocyte, cumulus, granulosa, theca and luteal cells) within follicles -ranging from primordial to pre-ovalutory-and the corpora lutea. CXCR4 positive staining was non-homogenous and varied depending on the developmental stage of the follicles and the phase of the estrous cycle. We observed that CXCR4 was strongly noticeable in the interstitial cells from the stroma in all stages of the cycle. SDF1 showed a similar expression pattern to that of its receptor. In summary, the receptor CXCR4 and its ligand SDF1 are differentially expressed in various cell types within the feline ovary during the natural estrous cycle, supporting possible roles for them

in the ovary that surpass their chemoattractant function. Further analysis is needed to elucidate these potential novel roles.

0891 - HYPOTHYROIDISM IN AN ASSISTED REPRODUCTIVE TECHNOLOGIES (ART) POPULATION: IS OVARIAN FUNCTION AFFECTED?

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IBYME-CONICET

Thyroid dysfunctions are frequent in reproductive age women and can affect fertility. They are associated with spontaneous miscarriages, premature birth, anovulation and abnormal menstrual cycles. Moreover, TSH levels and thyroid autoimmunity are increased in infertile patients compared to healthy ones. However, the impact of hypothyroidism on ovarian function in adulthood is scarcely known. Our aim was to investigate the reproductive function of thyroid hormones by studying assisted reproductive technologies (ART) patients with a clinical history of hypothyroidism that are currently taking levothyroxine (LT4), a thyroid hormone replacement drug. We assessed serum hormone concentrations in 60 women (aged 27-39 years) undergoing controlled ovarian stimulation at the Reproductive Medicine Center Pregna. Written, informed consent was given by all patients. Patients were classified into: control (euthyroid) and those under treatment with LT4, indicative of thyroid deficiency (hypothyroid). hCG was injected when follicles reached 17 mm in diameter and oocytes were retrieved under vaginal ultrasound guidance 34 h later. Serum concentrations of estradiol (E2), progesterone, prolactin, TSH, T4 and T3 were measured and the number of oocytes retrieved was recorded. Grouped patients were matched for age. The number of retrieved oocytes was lower in patients undergoing LT4 treatment ($p<0.05$, Student's t-test), regardless of their current thyroid hormone levels being in a normal range. We did not find differences in serum TSH levels between the groups either. In addition, E2 levels were also decreased in the patients taking levothyroxine regularly ($p<0.01$, Student's t-test). Also, we found a positive correlation between E2 levels and retrieved oocyte number among the treated hypothyroid patients ($p<0.01$, Pearson). Nevertheless, there was no correlation between progesterone, prolactin or TSH levels and retrieved oocytes. The significant decrease in retrieved oocytes in the LT4-treated women during controlled ovarian hyperstimulation indicates a possible role of hypothyroidism in reproductive function, even after thyroid hormone levels are normalized. The decrease in E2 levels is also a sign of poor ovarian response. These alterations might be due to a lingering effect of past thyroid hormone deficiency on ovarian cell function, since thyroid receptors can be found in ovarian cells such as oocytes, granulosa and stromal cells.

0921 - GPR55 RECEPTOR ACTIVATION BY ENDOCANNABINOIDS REGULATES SIGNALING PATHWAYS ASSOCIATED TO SPERM MOTILITY

Raquel Maria LOTTERO (1) | Carlos Agustín Isidro ALONSO(1) | Camila ARROYO SALVO(1) | Eugenia BOGETTI(1) | Nicolás CHIARANTE(1) | Jessica PLAZA(2) | Marcelo MIRAGAYA(2) | Silvina PEREZ MARTINEZ(1)

CEFYBO, UBA-CONICET (1); INITRA, UBA (2)

The endocannabinoid system has been characterized in most mammalian spermatozoa and plays a crucial role in sperm function. Anandamide (AEA), the major endocannabinoid, is involved in sperm capacitation by activation of CB1 and TRPV1, but not CB2 receptors. An association between sperm motility and fertilizing competence has been shown, since motility is required for sperm to traverse the female genital tract. Recently, we characterized in bovine spermatozoa a novel cannabinoid receptor, G protein-coupled receptor 55 (GPR55), which is modulated by AEA. We found that GPR55 is localized to the equatorial segment and flagellum of capacitated sperm. Also, the activation of GPR55 regulates progressive motility in sperm incubated in capacitating conditions. Since GPR55 is coupled to different G proteins that activate a widespread of signaling pathways, in this work we studied possible molecular pathways triggered by the activation of GPR55 receptor in sperm incubated with different receptor agonists. Results of computer assisted sperm analysis supported that the increase in progressive motility by Meta-AEA (1.4 nM; non-hydrolysable analogue of AEA) was reverted by 10 µM CID16020046, a GPR55 antagonist ($p<0.005$). In addition, the incubation with Met-AEA induced an increase of phosphorylated PKA substrates that was also decreased by the presence of GPR55 antagonist ($p<0.05$). On the other hand, the activation of GPR55 also involves PLC/PKC pathway. Thus, we determined the induction of phosphorylated PKC substrates in sperm incubated with 1nM AEA, which levels were diminished in the presence of CID. This result was confirmed by using AM251 (0.2 µM), a GPR55 agonist (Gaq/11-coupled). In addition, the increase of phosphorylated PKC substrates by AEA was reverted by the presence of AM630 (CB2 antagonist; 0.1nM) but not by CB1 or TRPV1 antagonists. These results suggest that the activation of GPR55 involves PKA and PKC pathways and support its role in sperm motility in bovines.

Farmacología / Pharmacology I

Chairs: Ezequiel Nusque | María Inés Ragone

0182 - IS PHARMACOGENOMIC TESTING A GAME CHANGER IN PSYCHIATRY? AN OBSERVATIONAL STUDY

Ventura Alejandro SIMONOVICH(1) | Tomas ABUDARHAM(2) | **Nadia SAVOY** (1) | Daniel MATUSEVICH(2) | Paula SCIBONA(1) | Andrea Romina CAJAL(3) | Jose FACCIOLI(2)

SECCION FARMACOLOGIA CLINICA. SERVICIO DE CLINICA MEDICA. HOSPITAL ITALIANO DE BUENOS AIRES (1); SERVICIO DE PSIQUIATRIA. HOSPITAL ITALIANO DE BUENOS AIRES (2); INSTITUTO MEDICINA TRASLACIONAL E INGENIERIA BIOMEDICA (IMTIB) CONICET HIBA IUHI (3)

In 2017, the World Health Organization warned that "the prevalence of mental disorders continues to increase, causing considerable effects on people's health and serious consequences at the socioeconomic level and in the field of human rights in all countries". Psychiatry plays a fundamental role in the field of mental health, and the psychopharmacological treatment of these pathologies can be carried out through monotherapy strategies, combination of psychotropic drugs or their potentiation. Pharmacogenetic testing (PT) may help the individualization of therapeutic process, aiming towards higher efficacy and reducing toxicity. This constitutes a case report study, in which we reviewed the electronic medical records of 20 (18 to 85 y.o) patients who underwent PT in the context of therapeutic failure or toxicity. The allelic variants of CYP2D6, and CYP2C19 as well as the serotonin transporter SCL6A4 were analyzed. The aim of this work was to be able to evaluate if the results modified the behavior taken by the professionals who had requested them, or if they had been taken into consideration in the established treatment. The results showed that, from the medical records that were followed up, only in 6 cases were the test results recorded by the treating professionals, and involved a change in the treatment behavior. We identify obstacles in the implementation of pharmacological therapeutic plans according to the results of pharmacogenomic studies, the lack of communication being the main cause. This initial experience depicts the hurdles of requesting and implementing PT guidance for therapeutic approach in the context of prevalent mental illnesses in Argentina. Availability of the tests is not enough for successful implementation; we should rather aim towards day-

to-day collaborative work between Psychiatrists and Clinical Pharmacologists.

0343 - TOWARDS A BETTER METHOD OF CANNABINOID EXTRACTION AND PRESERVATION FOR CANNABIS-BASED PRODUCTS

Estefania CHAMORRO AGUIRRE(1) | **Ana Clara PASCUAL** (1) | Pablo Gustavo MILANO(1) | Marisol Claudia BOCETTI(2) | Susana Juana PASQUARE(1)

INIBIBB-CONICET, DEPTO. BIOLOGÍA, BIOQUÍMICA Y FARMACIA-UNS (1); SECRETARÍA DE SALUD DE LA MUNICIPALIDAD DE BAHÍA BLANCA (2)

Although cannabis is widely consumed for medicinal purposes, little is known about its content. In Bahía Blanca city, Argentina, we observed that 3.4% of 59 analysed cannabis oils failed to contain delta-9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN), and that 89.5 % had more THC than CBD. Several reports highlight the importance of quantifying cannabinoids in medicinal products in order to use an appropriate concentration. Here, we aimed to study the extraction, quantification and storage of the different cannabinoid products. To this end, cannabis female flowers were grinded and heated (115°C, 40 min) to decarboxylate acidic cannabinoids. Extractions were performed by adding 5 % (w/v) ethanol, vortexed, sonicated, and agitated by a shaking platform. The mixture was centrifuged and the supernatant was evaporated under stream of N_2. Resin, ethanol and oil preparations were stored at different temperatures and/or containers for up to 60 days. THC, CBD and CBN content was quantified by HPLC. Ethanol extracts from plants harvested in winter showed a 123 % increase in THC+CBN content with respect to a clone harvested in summer ($p<0.05$). The extraction method allowed obtaining 72, 24 and 4% of THC in the first, second and third extraction, respectively. Storage evaluation showed no changes in THC or CBD content when cannabis oils were kept at 25, 4 and -20°C in plastic tubes and ethanol extracts in glass at -20°C for up to 60 days. However, at 4°C, THC content decreased (13 %) at day 30 ($p<0.05$). On the other hand, ethanol extracts stored in plastic showed a decrease of THC at day 30 either at 4°C (17 %) or at -20°C (14 %) ($p<0.05$). In resins stored either in plastic or in glass, THC also decreased (14 and 10%, respectively) at day 30 ($p<0.05$). Our results show that although the extraction method employed is suitable to obtain different cannabinoid preparations, it is important to choose the appropriate storage method.

0354 - ASSESSMENT OF RITUXIMAB ADVERSE EVENTS IN PEDIATRIC DISEASES

Berta Lorelei CORNALÓ (1) | Natalia RIVA(2) | Maria Valentina SALVADOR(1) | Andrea SAVRANSKY(1) | Silvia TENEMBAUM(1) | María Marta KATSICAS(1) | Marta MONTEVERDE(1) | Paulo CÁCERES GUIDO(1) | Oscar IMVENTARZA(1) | Raquel STACIUK(1) | Agustín GONZÁLEZ CORREAS(1) | Pedro ZUBIZARRETA(1) | Eduardo LAGOMARSINO(1) | Paula SCHAIQUEVICH(2)

HOSPITAL DE PEDIATRIA JUAN P. GARRAHAN (1); HOSPITAL DE PEDIATRIA JUAN P GARRAHAN - CONICET (2)

Biological products are part of the targeted therapy used in several pediatric pathologies. Specifically, rituximab is a chimeric monoclonal antibody that binds to the CD20 antigen on the surface of mature B-lymphocytes, inducing cell depletion. Due to the increased use of this drug in pediatric patients and the limited information about its safety, the aim of this study was to evaluate Adverse Drug Rreactions (ADRs) to rituximab in patients with chronic and complex diseases at Hospital de Pediatria JP Garrahan. We carried out active pharmacovigilance since March 2019 and we prospectively evaluated ADRs of rituximab in pediatric patients with transplant, oncological, hematological, immunological and neurological pathologies, as part of an interdisciplinary project. The causality of ADRs was established using the Naranjo Algorithm. Moreover, ADRs were classified according to severity as mild, moderate, serious or lethal, and according Common Terminology Criteria for Adverse Events (CTCAE) v5.0. We analyzed 70 infusions given to 31 patients of (median, range) 12.3 years (1.9-18.7), with oncological diseases (n=5), immunological pathologies (n=11), solid organ transplantation (n=9) and neurologic diseases (n=6). Seventeen ADRs to rituximab were observed including fever (n=3), hypersensitivity reactions (n=8), hypotension (n=1), hypertension (n=2), hypogammaglobulinemia (n=2), and tachypnea (n=1). 94% of the ADRs were defined as probable or definite and 77% were moderate or severe. Two patients had severe ADR during the infusion that required its interruption. Grade 3 and 4 cytopenias [neutropenias (n=5) and thrombocytopenias (n=3)] were observed in patients with leukemia and lymphoma (n=5) during simultaneous treatment with chemotherapy. Rituximab administration may be associated with ADRs but are all clinically manageable. Thus, it is important to implement a program of pharmacovigilance to generate evidence and solid scientific data to support clinical decisions.

0357 - OPTIMIZATION OF THE EXTRACTION OF PHYTOMETABOLITES WITH ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES FROM LEAVES OF CASEARIA SYLVESTRIS

Matias Damian DUQUE DE ARCE | Ana María TORRES | Juan Jose RUIZ DIAZ | Gabriela Ana Leticia RICCIARDI | Gonzalo Adrian OJEDA

LABORATORIO DE PRODUCTOS NATURALES IQUIBA-UNNE

Casearia sylvestris Sw. is a widely distributed tree in South America. In Brazil´s popular medicine, the use of this plant is correlated with its pharmacological properties including anti-inflammatory, anti-ophidian and anti-ulcer activities. However, the development of a separation technique of bioactive compounds in the herbal extracts is particularly difficult, due to the high complexity of the matrix. The objective of this work was to determine the optimal conditions for the extraction of bioactive compounds with the software Design-Expert® 11, using a mixture design of three solvents (water/ethanol/acetone). Leaves of C. sylvestris were collected in Paso de la Patria (Corrientes, Argentina). The samples were dried, powdered and extracted by maceration under agitation for 2 days in the corresponding solvent. The extracts were filtered and concentrated under reduced pressure. Total phenolic, flavonoids and tannins contents were measured using Folin-Ciocalteau, aluminum trichloride and vanillin methods respectively. Antioxidant activity (AOA) was determined by DPPH and FRAP assays. In vitro anti-inflammatory activity (AIA) was investigated by the hypotonic hemolysis inhibition method. Ten different mixtures were evaluated by response surface methodology, in their ability to extract the bioactive compounds. To maximize biological activities a graphic and numerical optimization was performed, using desirability function (D). It was found that the maximum AOA was obtained by optimizing both, phenolic content and DPPH/FRAP assays (D= 1), in extracts with acetone:water 1:1. Evaluation of hemolysis inhibition indicated that the highest AIA, was achieved with flavonoids (D= 0.87) and tannins (D= 0.96), obtained with ethanol. In conclusion, from the analysis of these results, we can predict that in order to optimize the extraction of active metabolites with AOA and AIA, the extracts should be obtained with acetone:water 1:1 mixture and ethanol respectively.

0361 - INFLUENCE OF THE VEGETATIVE STATE AND PART OF THE PLANT ON THE ANTIOXIDANT ACTIVITY OF PLANTAGO TOMENTOSA (LLANTÉN) FROM NORTHEAST OF ARGENTINA

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OJEDA(1) | Eduardo Santiago DELLACASSA(2) | Ana María TORRES(1)

LABORATORIO DE PRODUCTOS NATURALES IQUIBA-UNNE (1); FACULTAD DE QUÍMICA- UNIVERSIDAD DE LA REPUBLICA (2)

Plantago tomentosa Lam. subesp. napiformis Rahn (Rahn) is an herb frequently found in Corrientes (Northeast of Argentina), but there is scarce information related to its chemical and biological activities. The abundance of this species and its traditional use as medicinal plant (digestive, antiulcer and healing), justifies to study more in deep its phytochemistry. In order to evaluate the phytochemistry and antioxidant properties, plant material was collected at two vegetative stages: spring (Sp, flowering stage) being samples divided in roots (R), leaves (L) and flowers (F). The second colleect was performed on summer (Su) where flowers were absent. Extracts of different polarity [aqueous (A), ethanolic (E) and hexanic (H)] were obtained by maceration. Total flavonoids (AlCl3), phenols (Folin-Ciocalteau) and tannins (vainillin-HCl) were evaluated. The antioxidant capacity was studied by free radical scavenging using DPPH (2,2-diphenyl-1-picrylhydrazyl) and by the oxide-reduction ability using potassium ferricyanide (FRAP). The antioxidant activity found was like that of gallic acid for all the ethanolic extracts from Sp samples, while only L ethanolic extract from Su showed similar activity. By FRAP, ethanolic extract of L from Sp and Su presented the highest oxide-reduction capacity (17.7 and 16.9 mg/g extract, respectively), while the other extracts (different organs and polarity) did not show significant antioxidant activity. Quantitation of flavonoids showed that L and F ethanolic extracts presented the main levels (488.2 and 238.2 mg/g, respectively) in Sp, while for Su the value was 167.6 mg/g in L. Phenols levels increased in the ethanolic extracts: 193 mg/g in L and 193.3 mg/g in F at Sp compared to 162,7 mg/g extract in L for Su. Tannins predominated in Su ethanolic extracts of L and R (212,2 and 292,2 mg/g). Results indicated P. tomentosa possess promissory antioxidant activity depending on the growth state and part of plant considered.

0429 - IMPACT OF PHARMACOGENETIC AND THERAPEUTIC DRUG MONITORING IN THE SAFETY AND EFFICACY OF FIRST LINE ANTIRETROVIRAL THERAPY IN PATIENTS WITH HIV INFECTION.

Paula Belen AMARILLA CASSORATTI (1) | Lucas VICHIARINO(1) | Paula SCIBONA(1) | Cintia Valeria CRUZ(1) | Lorena ABUSAMRA(2) | Marisa SANCHEZ(3) | Cecilia FRECHA(4) | Leandro FATUZZO(4) | Ezequiel CORDOVA(5) | Mariana DE PAZ SIERRA(3) | Francisco Javier OLIVER MARTOS(4) | Veronica CISNEROS(6) | Diego SALUSSO(6) | Gustavo LOPARDO(6) | Maria Isabel GIMENEZ(4) | Ventura Alejandro SIMONOVICH(1) | Waldo BELLOSO(1)

SECCION FARMACOLOGIA CLINICA. SERVICIO DE CLINICA MEDICA. HOSPITAL ITALIANO DE BUENOS AIRES (1); DIVISION INFECTOLOGIA. HOSPITAL FERNANDEZ (2); SECCION INFECTOLOGIA. SERVICIO DE CLINICA MEDICA. HOSPITAL ITALIANO DE BUENOS AIRES (3); LABORATORIO CENTRAL. HOSPITAL ITALIANO DE BUENOS AIRES (4); DIVISIÓN INFECTOLOGÍA. HOSPITAL ARGERICH (5); FUNCEI (6)

Although the life expectancy of HIV patients and access to treatment today resembles that of the general population, more than 20 % of these patients discontinue treatment at standard doses, mainly due to adverse effects. The incorporation of therapeutic individualization in daily practice could help to identify the most appropriate dose for each patient of Atazanavir and Efavirenz, thus preventing some known toxicities and avoiding their eventual early discontinuation. The aim of the present study was to evaluate the factibility and usefulness of individualization treatment guided by pharmacogenetic (PG) and therapeutic drug monitoring (TDM) in the selection of the most adequate initial dose of efavirenz and atazanavir in patients with HIV treatment naive in Argentina. We conducted a prospective, multicentric and randomized study. Patients were randomized in a control group ('SC': Standard of Care) and pharmacological adequacy group ('PA': TDM+FG). PA patients were given an initial dose according to the pharmacogenomic index (the index was made according to the population prevalence of polymorphisms and the clinical relevance, patients were divided into 'Low Impact' (standard initial dose) and 'High Impact' (reduced initial dose) and the TDM was evaluated on days 14, 28 and 168. All patients were monitored for viral load, CD4 and adverse effects. Ninety five patients who were enrolled, 47 were randomized to PA and 48 to the SC. From de PA group, 31 were indicated Efavirenz and 16 Atazanavir, 37 an initial standard dose (Low Impact) was indicated and 10 the initial dose was reduced (High Impact). In the group SC, 31 patients were indicated EFV and 17 patients were indicated ATZ. The overall dropout rate was 18.8 % for the PA group and 29.4 % for the SC group. In the PA group, therapeutic monitoring of the plasma levels was carried out. The monitoring in the low impact group, 14 (59.9 %) patients were in range, 8 (29.6 %) weren´t and 5 (18.5 %) had not data. Of 4 patients in the high impact group, 3 had plasma levels of the drug in range. In the evaluation of the viral load carried out in the 24th week of treatment in the low impact group, 18 (66.6 %) patients had undetectable viral load or less than 50 copies. In the high impact group, all patients had undetectable viral load or less than 50 copies. Eight (16.6 %) patients of the PA group had adverse effects between weeks 2 and 23. The viral load at 24th week of treatment was undetectable for 4 (13.6%) patients and 13 (38.2%) had less than 50 copies. In the group SC, 10 patients had adverse effects. All patients in both groups had CD4 values greater than 200 cells/µL. Conclusions. In summary, the pharmacogenetics adaptation of initial and ongoing doses of first-line antiretroviral drugs seems feasible and useful for the individualized therapeutic approach of patients with HIV infection who begin treatment.

0435 - ANTIBIOTIC DOSAGE ADJUSTMENT IN PATIENTS WITH ACUTE RENAL FAILURE AND RENAL REPLACE THERAPY

Florencia Ines AIELLO (1) | Paula SCIBONA(1) | Cintia Valeria CRUZ(1) | Lucas Agustin VICHIARINO(1) | Cecilia LOSADA(1) | Rosario LUXARDO(2) | Maria Isabel GIMENEZ(3) | Guillermo ROSA DIEZ(2) | Waldo BELLOSO(1) | Ventura Alejandro SIMONOVICH(1)

SECCION FARMACOLOGIA CLINICA. SERVICIO DE CLINICA MEDICA. HOSPITAL ITALIANO DE BUENOS AIRES (1); SERVICIO DE NEFROLOGIA. HOSPITAL ITALIANO DE BUENOS AIRES (2); LABORATORIO CENTRAL. HOSPITAL ITALIANO DE BUENOS AIRES (3)

Acute renal failure (ARF) under renal replace therapy (RRT) is a high prevalent entity among critically ill patients, and is often associated with sepsis. Current dosage recommendations come from extrapolations of data in chronic kidney disease or from small case series. Around 25-60 % of patients treated with RRT present sub therapeutic levels of antibiotics. In order to analyze the pharmacokinetics of vancomycin and meropenem in critically ill patients with ARF and RRT, 8 critically ill patients with ARF (stage 3 KDIGO) and extended hemodialysis were included. Six patients were treated with vancomycin 1 g/ 12 h, two of them received also meropenem 0.5g/ 24 h and one of them meropenem 1 g/ 12 h over one hour. Two patients received meropenem 1 g/ 12 h over 3 h. Meropenem and vancomycin plasma concentrations were determined by liquid chromatography and immunoassay, respectively, for 12 h after antibiotic administration. For vancomycin, only one in six patients presented trough levels within range (15-20 mg/L), five had trough levels between 20-30 mg/L and one had trough level >30 mg/L. For meropenem, the T>MIC for susceptible bacteria (4 mg/l) was <40 % in two of three patients who received meropenem 1 g/ 12h over 3 h. The one who received 1 g/ 12 h but in one hour infusion time had a T>MIC >40 % for susceptible and intermediately susceptible bacteria (8 mg/l). The MIC for susceptible and intermediately susceptible bacteria was covered >40 % in all patients who received meropenem 0,5 g/ 24 h. Critically ill patients with ARF and extended RRT present potentially

toxic vancomycin levels with doses of 1 g/ 12 h. A meropenem dosage of 1 g/ 12 h over 3 hours provides subtherapeutic levels. Adequate levels were achieved with meropenem 1 g/ 12 h but over one hour or with doses of 0,5 g/ 24 h. Therapeutic drug monitoring may contribute to optimise individual dosing.

0498 - GLOBAL HEART FAILURE HOSPITALIZATION RATE REDUCTION DUE TO SODIUM-GLUCOSE COTRANSPORTER 2 INHIBITORS TREATMENT IN DIABETIC PATIENTS: A SYSTEMATIC REVIEW AND META ANALYSIS.

Ezequiel ZAIDEL | Francisco BAGNATO | Valentina RODRIGUEZ ROHWAIN | Rodrigo VISCIGLIA | Samantha VILA | **Héctor Alejandro SERRA**

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Sodium-glucose cotransporter 2 inhibitors (SGLT2i) were the last treatment introduced in Type 2 Diabetes Mellitus. Certain evidence indicates that they may be beneficial in heart failure Our aim was to performs a systematic review and meta analysis to evaluate efficacy SGLT2i in reducing HF hospitalizations (HFH) both in randomized clinical trials (RCT) and real-world observational studies (RWD). PubMed was searched for SGLT2i studies in any language until November 1, 2018. Cohorts with follow up <24 weeks were excluded. Authors independently reviewed abstracts and extracted data for eligible full texts. A random-effects model was used to estimate the odds between SGLT2i and their controls in terms of efficacy and safety. Mantel-Haenszel chi-square test was used to prove differences. From 244 records, 9 studies met the eligible criteria and were included. In 4 RCT (34,642 patients), SGLT2i significantly reduced HFH risk compared with placebo (4.3 vs. 6.3 %; OR= 0.65; 95 % CI= 0.55-0.76; p<0.001; I2= 48%). In 5 RWD (1,731,061 patients from 12 countries), the pooled SGLT2i therapy reduced HFH risk almost in half compared with other glucose lowering agents (OR= 0.55; 95 % CI= 0.39-0.69; p<0.001; I2= 98 %). Among the studies that provided safety information, the risk of ketoacidosis and genital infections were increased (OR= 2.16 and 5.74, respectively) in SGLT2i group, but the risk of other serious adverse events (hypoglycemia, urinary tract infections, amputations or fractures) was not significant. In this meta analysis on almost 1.8 million diabetic people, SGLT2i were confirmed as strong HFH risk reduction drugs. Although different pathophysiological mechanisms are currently being investigated to explain this association, a greater utilization of SGLT2i could be beneficial to reduce the epidemic of HFH in diabetic patients.

0675 - ANTIOXIDANT ACTION OF CANNABIS SATIVA RESIN AND CBD AND SYNERGISM INDUCED BY LARREA DIVARICATA AND TILIA X VIRIDIS EXTRACTS

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Cannabis sativa L. (Cannabaceae) is a medicinal plant used as anticonvulsant in refractory epilepsy in kids. Convulsions induce oxidative stress (OS) in cerebral tissues which affects the normal function of the central nervous system altering cognitive functioning. The synergistic effects of plants could not only improve therapeutic effects but also mitigate adverse effects. Larrea divaricata Cav. (Zygophylliaceae) is an autochthonous plant with a well documented antioxidant activity and Tilia x viridis ssp moltkei (Tiliaceae) is a plant used for its anxiolytic effects. The purpose of this work was to evaluate the antioxidant activity of an ethanolic extract (EEC) of C. sativa, studying the participation of the cannabinoid CBD in its action and to evaluate for the first time the possible synergistic combination of EEC with aqueous extracts of L. divaricata (AEL) and Tilia x viridis (AET). The antioxidant activity was determined by the capacity of scavenging the radical 1,1-diphenyl-2-picryhydrazyl (DPPH) by a spectrophotometric assay. CBD was identified and quantified by HPLC. Quantification of CBD: 0.273 mg CBD/ mg EEC. All the extracts presented antioxidant activity. Results expressed as inhibitory medium concentration IC50 (µg/ml) represent Mean ± SEM of three experiments made by duplicate (*p<0.05; **p<0.01; ***p<0.001 respect to extracts alone in accordance to Student´s T test). CI: Combination Index. IC50 extracts alone: EEC: 15.63 ± 1.22; AEL: 24.18 ± 0.54; AET: 25.18 ± 0.71; CBD: 12.49 ± 0.79. IC50 combinations: EEC + AEL: 2.91 ± 0.09**; EEC + AET: 3.48 ± 0.56*; CBD + AEL: 4.19 ± 0.11**; CBD + AET: 3.04 ± 0.19***. CI Larrea/Tilia: 0.87; CI Cannabis/Larrea: 0.43; CI Cannabis/Tilia: 0.28; CBD/Larrea: 0.40; CBD/Tilia: 0.30. Conclusions: CBD is involved in the antioxidant activity of EEC. AEL and AET could synergize the effect of EEC (CI <1), allowing in a future their possible association to increase therapeutic effects and mitigate adverse effects.

0899 - ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES OF AN ETHANOLIC EXTRACT OF NECTANDRA ANGUSTIFOLIA "YELLOW LAUREL"

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Nectandra angustifolia (N.a.) is a native plant whose leaf and bark extracts have been used in ethnomedicine for the treatment of rheumatism, arthritis, chronic pain and as an antivenom in snakebites. Therefore, the objective of this work was to characterize its possible biological activities. Leaves and stems of N.a. were air-dried and an extract (NaE) was further obtained by maceration with ethanolic. Total flavonoid content and antioxidant activity were determined on NaE using the aluminum trichloride technique and DPPH respectively. The chromatographic profile of NaE was subsequently determined by HPLC identifying peaks compatible with flavonoids. The non-cytotoxic concentration of NaE (50 µg/mL) was determined for in vitro biological assays using Raw 264.7 (murine macrophages) cell cultures through cell counting with Trypan-blue and XTT Kit® assay. Subsequently, the cell cycle of RAW 264.7 cells exposed to NaE (10, 50 and 100 µg/ml) was analyzed for 24 h, revealing a dose-depending decrease in S-phase cells, as well as, an enhancement of the sub-Go population compared to control cells. These data are compatible with a NaE induction of cell apoptosis. Additionally, the anti-inflammatory capacity of NaE was evaluated by RT-qPCR. Briefly, proinflammatory cytokine gene expression (IL-1 and IL-6) were determined in cells preincubated with NaE and, subsequently stimulated with LPS compared to those treated with LPS alone. Experimental data show that NaE decreases the release of proinflammatory cytokines at different exposure times (4 and 24 h). These preliminary results show that NaE contains natural components with antioxidant and anti-inflammatory properties that would be an attractive option for the future design of phytotherapeutics with different applications in biomedicine.

0903 - PASSIFLORA CAERULEA ADMINISTRATION REVERSES DEPRESSIVE-LIKE BEHAVIOR INDUCED BY BILATERAL OLFACTORY BULBECTOMY IN MICE

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UNIVERSIDAD NACIONAL DE CÓRDOBA (1); INSTITUTO DE INVESTIGACIONES DE CIENCIAS DE LA SALUD - CONICET (2); INSTITUTO MULTIDISCIPLINARIO DE BIOLOGÍA VEGETAL (IMBIV)-CONICET-FCAULTAD DE CIENCIAS QUÍMICAS-UNC (3); DEPARTAMENTO DE QUÍMICA ORGÁNICA, FCQ-UNC, INFIQ-CONICET (4)

The genus Passiflora is widely used in medicinal folklore for the treatment of different diseases. However, not all the species have been studied at the same way and between them there are different effects. Passiflora caerulea (P. caerulea) is in our country and it is consumed as a tea by the population mainly to treat anxiety. Previous studies showed the effect from isolated chrysin (a flavonoid which is in P. caerulea) as an anxiolytic and antidepressant. The possible effects of P. caerulea on depressive-like symptoms and its role as an alternative therapeutic tool for the treatment of depression have not been clarified yet. The aim of this study was to examine the antidepressant-like action of P. caerulea using the open-field test (OFT), and to investigate the possibility that P. caerulea could reverse some of the depressive-like behavior induced by bilateral olfactory bulbectomy in male mice. Swiss albino mice (N: NIH) with bilateral olfactory bulbectomy (BOB-animal depression model) were used in comparison to mice without extraction of olfactory bulbs (Sham, controls), n= 6-3 per group/treatment. The locomotors activity in OFT was evaluated with registers of central crossings behavior, peripheral crossings behavior and the rearing numbers before BOB surgery (basal conditions or preoperative period), 14 days after the surgery (postoperative period) and after 5 days of oral administration (gavage) of passionflower (10 mg/kg) in the open-field test (post-treatment period). The data were analysed by two-way ANOVA followed by an LSD test. The results show that BOB induces an increment in the locomotor activity comparing with SHAM animals and that sub-chronic treatment with P. caerulea in bulbectomized animals (BO) decreased locomotors activity in relation to BO - saline animals ($p<0.05$) as similar as Sham-saline values. No significant differences were observed in Sham animals treated with P. coerulea with respect to Sham - saline. In conclusion, this study provides new evidence about the effects of P. caerulea in a depression animal model, where P. caerulea managed to reverse the hyperactivity characteristic of BO animals.

0973 - OMEGA-3 FATTY ACIDS IN THE TREATMENT OF PERIPHERAL NEUROPATHIC PAIN: NEW PHARMACOLOGICAL STRATEGIES

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INSTITUTO NACIONAL DE BIOTECNOLOGÍA, CENIIT, UNIVERSIDAD DE LA RIOJA

Chronic pain has a marked negative impact on the quality of life. In recent years, it has been increasing interest in the development of new safer more effective treatments of neuropathic pain, which is presented with sensory abnormalities such as allodynia and hyperalgesia. Recent studies have demonstrated the analgesic effect of omega-3 fatty acids (O3). The aim of this study was to evaluate thermal hyperalgesia, mechanical allodynia and nerve regeneration after treatment with O3 in an animal model of peripheral neuropathic pain of chronic sciatic nerve constriction (CCI). Thermal hyperalgesia, mechanical allodynia and nerve regeneration were evaluated in male Wistar rats, which were administered with: oral O3 (0.32 or 0.72 g/kg) 24 h after ICC and for 21 days (chronic treatment), oral saline (control group with ICC), local O3 (10 µl, O3 30 %) on the sciatic nerve at the end of tissue constriction (acute treatment) and oral O3 (0.32 or 0.72 g/kg) 24 h after surgery but without ICC for 21 days. The tests of thermal hyperalgesia (hot plate test), mechanical allodynia (Von Frey test) and the functional motor recovery test (walking track analysis) were performed on days 3, 7, 14 and 21 post-surgery. In all groups, a neuropathological examination was performed on day 21 post-surgery with or without CCI. Oral O3 treatment blocked of thermal hyperalgesia, decreased the mechanical allodynia (50 %) and allowed for the motor function recovery (100 %). In addition, neuropathological examination revealed that the nerves conserved the axonal density and architecture while the CCI control group showed loss of continuity of perineurium, neuroma formation and abundant inflammatory infiltrate. In contrast, local O3 administration failed to modify the parameters tested. Oral O3 administration relieves thermal hyperalgesia effectively and improves the recovery process in rats with CCI, suggesting that they could constitute a potential treatment for the relief of neuropathic pain in humans, with minimal risk of adverse effects.

Biología celular y molecular de procesos fisiológicos y patológicos / Biology III

Chairs: Fernando Correa | Manuel Wolfson

0247 - LIPID METABOLISM REGULATION BY THE PROINFLAMMATORY CYTOKINE TNF

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The crosstalk between the cancer cells and those from the tumor microenvironment are important in cancer biology. In breast cancer, there is a deregulated secretion of cytokines and chemokines from the adipose tissue (AT) that can contribute to tumor onset and progression. In a previous work, we have seen that the human AT surrounding breast cancer has a greater TNF expression compared to AT adjacent to normal mammary. Since TNF can induce adipocyte lipolysis and, hence, support the anabolic tumor metabolism, we measured triglyceride secretion in murine mammary gland AT conditioned media (C57BL-6j) after 48 h with or without TNF 10 ng/ml. We found that TNF increased the triglyceride secretion (5.8 g/l vs. 0.93 g/l basal; $p<0.01$). In addition, we observed by gas chromatography that the pattern of secreted fatty acids was altered by TNF, being oleic and linoleic acid the most predominant. Otherwise, glucocorticoids are also involved in metabolic adaptation and their action on the AT is complex since the lipid release or storage depends on the physiological or pathological state of the tissue. We did not see any difference in the triglyceride secretion after 48 h with or without dexamethasone 500 nM (1.12 g/l vs. 0.93 g/l; $p>0.01$). We also performed a total extraction of lipids from the tissue of all the above conditions. We observed a decreased in the total content of lipids only under TNF stimulus (TNF: 0.77 respect to basal, $p<0.01$). Then, to studied the interaction of AT with the tumor in the metabolism of the tumoral cells, we analyzed the expression of genes involved in lipogenesis of human breast cancer cells lines MCF7 and MDA-MB-231 injected in mice (public database GSE66744). The expression of fatty acid synthase decreased in vivo in both cell lines respect to basal (MDA-MB-231: 0.43; MCF7: 0.64 respect to basal; $p<0.05$). These results support to further study the interaction of AT cells and breast cancer cells and the role of TNF in their lipid metabolism.

0257 - ANTI-AGING EFFECT OF YERBA MATE IN RETINAL PIGMENTED EPITHELIUM

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The increase of persons older than 65 years is a global trend. Ageing of the population goes hand in hand with a greater prevalence age-related macular degeneration (AMD), a progressive retinal disease leading to irreversible loss of vision. Oxidative stress in the retinal pigmented epithelium (RPE), a cell layer essential for photoreceptor survival, is a major AMD cause. RPE oxidative damage leads to premature cellular senescence and reduction of oxidative stress could have a preventive or therapeutic role in AMD. Polyphenols, powerful natural antioxidants, could protect against degenerative diseases. Yerba mate (YM) is an important source of polyphenols, since it has large amounts of caffeic (CAF) and chlorogenic (CHL) acids. The aim of this work is to verify the protective effects of YM, CAF and CHL on RPE cells damaged by oxidative stress in vitro. RPE cells (ARPE-19 cell line) were incubated for 2 hours with YM (125 µg/ml or 250 µg/ml), CAF (70 µM), CHL (100 µM) or appropriate controls. Premature cell senescence, induced by exposure to H_2O_2 (150 µM) for 90 minutes, was detected by ß-galactosidase (ß-GAL) activity. Cells were collected at different time points following damage. Reactive oxygen species (ROS) levels and the phosphorylated histone H2AX (p-H2AX) were evaluated using fluorescence microscopy. CREB phosphorylation and SIRT1 expression were analyzed by Western blot and qPCR, respectively. In cultures exposed to oxidative stress, YM promoted RPE cell survival and decreased ß-GAL+ cells by 50 % ($p<0.05$). CAF or CHL also increased survival and decreased senescence. These effects were associated with increased CREB-phosphorylation and SIRT1 mRNA expression ($p<0.05$). YM and its main polyphenols, CAF and CHL, protected ARPE-19 cells from oxidative stress. These compounds could prevent AMD, and possibly other age-related conditions, through the activation of pro-survival and anti-oxidant cell signaling pathways.

0278 - ROLE OF ACYL-COA SYNTHETASE 4 IN MITOCHONDRIAL BIOENERGETICS IN BREAST CANCER CELLS

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Acyl-CoA synthetase 4 (ACSL4) catalyzes acyl-CoA synthesis from long chain fatty acid, being arachidonic acid its preferred substrate. In breast cancer, ACSL4 promotes tumor aggressiveness by increasing migration, proliferation and invasion. In cancer cells, there is evidence of dysregulation of mitochondrial function, mitochondrial mass and subcellular spatial organization. The aim of this work is to study whether mitochondrial metabolism is regulated by ACSL4 in breast cancer cells. We evaluated levels of specific proteins in mitochondrial fraction of the MCF-7 stable breast cancer cell line overexpressing ACSL4 (MCF-7 tet-off/ACSL4) and control cells (MCF-7 tet-off/empty vector). We observed a significant increase in Complex III ($p<0.01$) and VDAC1 ($p<0.001$) levels in cells that overexpress ACSL4 compared to control. Mitochondrial bioenergetic function was studied using the Seahorse XF Cell Mito Stress Test. Our results did not show significant difference in oxygen consumption rate between cell lines. Furthermore, respiratory parameters showed no significant differences in basal (coupled mitochondria) and non-mitochondrial respiration, ATP synthesis and coupling efficiency percentage. However, significant increase was observed in maximal respiration, proton leak and respiratory reserve capacity ($p<0.05$) in MCF-7 tet-off/ACSL4 cells. The maximal respiration could be related to the induction of complex III, promoted by the expression of ACSL4. The increase in respiratory reserve capacity could indicate that expression of ACSL4 promotes a greater capacity to respond to energy demand. Also, mitochondrial DNA (mtDNA) and mitochondrial mass were measured by real time PCR and flow cytometry respectively. No significant differences were found in mtDNA but significant decrease of mitochondrial mass ($p<0.05$) was observed in breast cancer cells that overexpress ACSL4. This work expands knowledge of the function of ACSL4 in breast cancer cells and mitochondrial metabolism.

0332 - SNAKE VENOM PHOSPHOLIPASE A2 (SVPLA2) FROM CROTALUS DURISSUS TERRIFICUS INHIBITS TUMORAL CELL ADHESION

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Snake venoms are natural sources of bioactive substances with therapeutic potential. In particular, different types of phospholipases have been shown to possess antitumor and antiangiogenic properties. The venom of the South American Rattlesnake, Crotalus durissus terrificus (C.d.t.), is a complex mixture of proteins. Crotoxin, the main toxin of this venom, contains a basic phospholipase A2 (PLA2) and a non-toxic acidic protein, crotapotin. Thus, the aim of this study was to evaluate the potential effect of a PLA2 isolated from C.d.t. (Cdt-PLA2) on tumoral cell adhesion. Cdt-PLA2 was purified by two chromatographic steps, a gel filtration and a reversed phase HPLC C-18 chromatographs. The purity of the enzyme was verified by SDS-PAGE. Firstly, cytotoxicity of Cdt-PLA2 (5 - 250 µg/mL) was assessed in cultured murine tumoral epithelial cell line, LM3, grown in DMEM 5 % FBS at 37°C, 5 % CO_2. Non-cytotoxic concentrations were selected for adhesion inhibition assay. Briefly, LM3 cells (3×10^4/well) were preincubated for 30 min at 37°C with Cdt-PLA2 (5, 10, 20, 25 and 50 µg/mL) or culture medium (control) and then added to 96-well plates. After 1.5 h, non-adherent cells were removed by careful washing and aspiration with PBS. Adherent cells were fixed and stained with crystal violet. The percentage of cell adhesion was determined by comparison of the absorbance readings (620 nm) with the mean absorbance of control cells (not exposed to the PLA2), considered as 100% adhesion. The obtained results showed a concentration-dependent adhesion inhibition effect of Cdt-PLA2 using non-cytotoxic concentrations. A 50 % of cell inhibition adhesion was observed with the maximum dose assayed. These findings are the beginning of the study of the potential use of this enzyme as an antitumoral.

0386 - ANALYSIS OF THE REGULATORY MECHANISMS OF MITOFUSIN 2 GENE EXPRESSION IN H295R HUMAN ADRENAL CELLS

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Mitofusin 2 (Mfn2) is one of the most relevant mitochondrial proteins controlling mitochondrial fusion. Mfn2 accurate expression is involved in mitophagy, apoptosis, mitochondrial metabolism in mammalian cells and it is down regulated in obesity and in type 2 diabetic patients and mutated in Charcot-Marie-Tooth type 2A neuropathy. We have previously shown that angiotensin II (Ang II) promotes Mfn2 mRNA and protein expression and that Mfn2 is necessary for steroid synthesis in H295R human adrenal cells. It has been described that Mfn2 gene expression regulation involves several transcription factors, such as Sp1 and estrogen-related receptor-alpha (ERRalpha). We have observed that ERRalpha up-regulates Mfn2 expression in H295R cells. Then the aim of this study was to analyze the mechanisms involved in the regulation of basal and hormone-stimulated Mfn2 gene expression, and a possible role of ERRalpha in adrenal human cells. For this

purpose, two reporter constructs were generated: a full length Mfn2 promoter including exon 1 (large) and a Mfn2 promoter with a deletion of the 5' end from position –2700 to –700 pb (short), both constructions containing ERRalpha binding sites. We observed that both promoters display activity in H295R human adrenal cells as detected by luciferase assay, but the short promoter presents three times more activity than the large one (***p<0.001). Then, Mfn2 short promoter was used to evaluate Ang II transcriptional effect and we observed that Mfn2 promoter activity increases under Ang II stimulation (Ang II vs. control, **p<0.01). By means of a shRNA against ERRalpha, we showed that this factor is involved in basal Mfn2 transcription (mock vs. shRNA***p<0.001). These results suggest that Mfn2 basal expression depends on the presence of ERRalpha and that Ang II is involved in Mfn2 transcriptional regulation. In H295R human adrenal cells Mfn2 promoter contains cis-elements that negatively regulate basal transcription.

0417 - ROLE OF TUMOR SPECIFIC PROTEIN MAGEB2 IN PROTEIN TRANSLATION

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Protein synthesis has a major role in cell proliferation. Many oncogenes affect the translation machinery making aberrant translation a common characteristic of tumor cells. We recently published that expression of the tumor-specific protein MageB2, induces cell proliferation in human cell lines as well as in an experimental mouse model. Our ongoing research showed that endogenous expression of MageB2 enhanced pre-rRNA transcription and ribosome biogenesis. Besides, we also observed a lower rate in protein synthesis in CRISPR/CAS9 MageB2 KO HCT116 cells with respect to WT. Our objective here is to investigate an additional role of MageB2 in protein synthesis regulation, alternative to rRNA transcription regulation, but associated to a functional interaction with the translation machinery and associated pathways. Our first observation obtained from Immunoprecipitation followed by Mass Spectrometry approach was that MageB2 interacts with different ribosomal proteins (RP). We then investigated whether the interaction of MageB2 to RP was linked to its nucleolar localization. Ultracentrifugation in sucrose cushion allowed us to detect MageB2 in cytoplasmic ribosome fraction, together with other RP (S6, S13, L23 and RPLP2). These results suggest that MageB2 could play a role in the nucleolus (rRNA transcription regulation) but also form part of the nascent ribosome that will be involved in protein synthesis as a mature cytoplasmic complex. The eukaryotic Initiation Factor 4E (eIF4E) is a key regulator of protein synthesis, that is activated by phosphorylation and associated to mature ribosomes. We observed a 30 % reduction in phospho-eIF4E levels in MageB2 KO cells when compared to WT. This is not due to transcriptional regulation, as quantification by RT-qPCR did not show differences in eIF4E mRNA levels between HCT116 MageB2 KO and WT cell lines. Based on results presented here we propose that MageB2 could form part of tumor cell ribosomes and promote protein synthesis.

0418 - MKP-3 SPLICE VARIANTS DIFFERENTIALLY REGULATE FOXO1 TRANSCRIPTION FACTOR.

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MAP kinase phosphatase-3 (MKP-3) is dual-specificity phosphatase up-regulated by proliferative stimuli and specific for the MAP kinases ERK1/2. However, it has been described that MKP-3 also dephosphorylates the transcription factor forkhead box protein 1 (FOXO1). The human MKP-3 gene generates the full length transcript, variant L, and an alternative splice product, or variant S. Our work focuses on the analysis of potential differences between isoforms and their effects on cellular functions. Given that FOXO1 dephosphorylation is required for its translocation to the nucleus, where it promotes the transcription of specific genes, we proposed that S and L variants of MKP-3 could differentially regulate its subcellular localization. Here, HEK293 cell lines stably expressing S or L variants (HEK-S and HEK-L respectively) and control HEK293 cells (HEK-C), were transfected for mVenus-tagged FOXO1 expression in order to analyze FOXO1 subcellular localization by fluorescence microscopy. The results showed that in serum-starved cells, mean FOXO1 fluorescence intensity ratio between nuclear and cytoplasmic regions in HEK-L cells is significantly higher than in HEK-S and HEK-C cells (L= 6.55 ± 0.8, S= 0.53 ± 0.5, C= 0.47 ± 0.5, p<0.001). These results suggest that only the over-expression of L variant promotes FOXO1 translocation. Next, förster radius energy transfer (FRET) strategy was used to evaluate the interaction of each MKP-3 variant with FOXO1. These studies revealed a significant interaction between both mCerulean-tagged MKP-3 variants and mVenus-tagged FOXO1 against not fused mVenus and mCerulean fluorophores (p<0.001), though the level of interaction was equivalent among variants (p>0.05). Collectively, our results show that both MKP-3 variants are able to interact with FOXO1 but only L variant is able to regulate the localization of FOXO1.

0464 - HEART ON A CHIP: CARDIOTOXICITY STUDIES IN MICROFLUIDIC DEVICES USING CARDIOMYOCYTES DIFFERENTIATED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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UNIVERSIDAD TECNOLÓGICA NACIONAL (UTN) FACULTAD REGIONAL HAEDO (1); FLENI-CONICET (2)

Microfluidic devices may have several advantages over traditional cell cultures, including replication of in vitro microenvironment and less needs of reagents. The objective of this work was to evaluate cardiotoxicity in our cardiac model in vitro using a microfluidic device with a dilution module. To test our experimental setting, cardiomyocytes (CMs: day 21) obtained by differentiation of human induced pluripotent stem cells (hiPSCs: day 0) were seeded in a culture chamber of microfluidic device to obtain an appropriate biophysical tissue architecture. The CMs were incubated with different concentrations of doxorubicin (DOX) created by the dilution module of microfluidic device starting from an initial concentration of 0.5 µM for 24 h with continuous flow (0.1 µl/min) to simulate the blood flow. We evaluated the cardiotoxicity by fluorescence microscopy after using live/dead assay. In the same way, we tested different concentrations of FGF-2 formed from 10 ng/ml as a protection mechanism against DOX following a similar evaluation methodology. Our results showed that CMs obtained from differentiation protocol beat spontaneously at physiological rhythms (70-80 bpm). These CMs expressed cardiac marker cardiotroponin (cTnT) detected by immunostaining and real-time qPCR and quantified by flow cytometry obtaining 80-90% of positive cells. By exposing the CMs to six different DOX gradient concentrations generated by the dilution module, we saw a direct relation between increase DOX concentration and CMs cell death. Then, effect of pretreatment with different FGF-2 concentrations before DOX incubation showed that there is a significant decrease in the number of death CMs suggesting an increment in cell survival. In conclusion, our "lab on a chip" technology with hiPSC-CMs is a promising tool that could significantly improve the ability

to test drug efficacy and toxicity in vitro, due to the use of human cells, easy use, reducing cost and duration with respect to conventional methods.

0503 - REGULATION OF MITOCHONDRIAL DYNAMICS PROTEINS IN MA-10 LEYDIG CELLS: THE ROLE OF MITOFUSIN 2 ON ACYL-COA SYNTHETASE EXPRESSION, A KEY ENZYME IN STEROIDOGENESIS

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Mitochondrial fusion is driven by GTPases like Mitofusins 1 and 2 (Mfn1/2) and Optic Atrophy 1 (OPA1) whereas mitochondrial fission mainly involves Dynamin-related protein 1 (Drp1). Mitochondrial dynamics (fusion/fission events) participate in many physiological processes and in different human pathologies. Steroidogenesis depends on several mitochondrial-associated proteins as PKA, StAR, ERK/MEK, SHP2 (tyrosine phosphatase) and Acyl-CoA synthetase 4 (Acsl4). We have previously demonstrated that Mfn2 participates in steroids production and in StAR expression and mitochondrial localization, in MA-10 murine Leydig cells. However, the regulation of mitochondrial dynamics proteins under hormonal stimulation has been little explored. The aim of this study was to evaluate the hormone-regulation of Mfn2, OPA1 and Drp1 and a possible role of Mfn2 in Acsl4 expression and mitochondrial localization, in MA-10 Leydig cells. We observed that Mfn2 levels significantly increase after 8Br-cAMP (cAMP analogue) stimulation, in a time-dependent manner up to 24h (control vs. 4h, ***p<0.001). We detected by immunoblot two main OPA1 isoforms that are regulated by 8Br-cAMP. Mitochondrial Drp1 is significantly phosphorylated by human chorionic gonadotropin (hCG) and 8Br-cAMP stimulation in serine 637 (control vs. 1h, ***p<0.001), a PKA-phosphorylation site associated with a decrease in Drp1 fission activity. By decreasing Mfn2 expression with a siRNA, we observed that the presence of Mfn2 is necessary for the expression and mitochondrial localization of Acsl4, detected by real time PCR and immunoblot (mock vs. siRNA, ***p<0.001); whereas mitochondrial localization of PKA did not depend on Mfn2. Phospho-Drp1 levels were not significantly affected by Mfn2 down-regulation, in agreement with the presence of mitochondrial PKA. Our results indicate that several mitochondrial dynamics proteins are hormone-regulated and Mfn2 plays a role in the expression and mitochondrial localization of Acsl4, in MA-10 cells.

0514 - T7 (Y639F) RNA POLYMERASE EXTRACTION AND PURIFICATION TO PRODUCE HIGH YIELD 2'FLUORO-MODIFIED RNA APTAMERS THROUGH CELL-SELEX PROCEDURES

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Nucleic acids are susceptible to ubiquitous serum nucleases. Chemical modifications to the 2' position of ribose can be used to stabilize oligonucleotides, specifically RNA aptamers. Aptamers are single stranded oligonucleotides which bind specifically to a variety of targets. The 2'-fluoro modified RNA aptamers can be generated in high yield using the Y639F variant of T7 RNA polymerase. Thus, the main goal of this work is to extract and purify the T7 (Y639F) RNA polymerase, so it could be used in a near future for the production of 2'F-modified RNA aptamers through cell-SELEX. The BL21(DE3) E. coli bacteria was used for transformation, together with the kanamycin-resistant 6xHis-Tagged T7 Y639F polymerase vector. First, chemically competent E. coli cells were produced using MgCl2 and CaCl2. Next, these competent bacteria were incubated with 100ng of the vector. At the end, the cells were streaked in LB-kanamycin resistant plate. The Y639F T7 RNAP bacteria was then inoculated with Lysozyme (10 mg/mL), PMSF (20 mg/mL), Leupeptin (5 mg/mL), 8 % Na-Deoxycholic acid and protease inhibitor cocktail. Cells were lysed and transfer to Nickel NTA magnetic agarose beads. The beads were washed to eliminate non-specifically bound protein, and then, 6xHis-Tagged T7 RNAP protein were eluted from the magnetic beads. The purified protein was used for in vitro-transcription of dsDNA template to generate an RNA aptamer. Our results demonstrated that our purified T7 polymerase could be successfully used for in vitro RNA aptamer transcription. Then, we confirmed that the dilution 1:1, and even, 2:1 of T7 RNAP are the optimal concentration for in vitro transcriptions with phenol-chloroform extraction. To conclude, it is often a challenge to get T7 polymerases that incorporate nucleotides containing modifications. Higher yields of 2'-fluoro-pyrimidine transcripts can be produced using the Y639F mutant T7 RNA polymerase purified by our group in the concentrations mentioned above.

0526 - STUDY OF THE EXPRESSION OF UDP-GLC:GLYCOPROTEIN GLUCOSYLTRANSFERASE (UGGT) ISOFORMS IN AN ISCHEMIA-REPERFUSION MODEL IN RAT HEART

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The first reported isoform of UGGT plays a central role in the endoplasmic reticulum glycoprotein-folding quality control. We have previously reported the existence of a second isoform in the mouse (UGGT2) that showed in vitro biological activity. The aim of this study was to analyze their expression in rat heart and their regulation under an ischemia (I)/reperfusion (R) protocol. Male Wistar rats (2 mo. old) were divided into 4 groups: (I), (R), (Ct) and (C). Rats were anesthetized and heparinized. After loss of reflexes and muscle relaxation, the thorax was opened to quickly excise the heart. The organ was perfused (6 $ml.min^{-1}.g^{-1}$) by the Langerdorff technique with Krebs solution, continuously bubbled with 95 % O_2 and 5 % CO_2, at 37°C and electrically stimulated (5 V, 5 ms) at 3 Hz while the left intraventricular pressure (LVP) and calorimetric response were monitored. After 60 min of equilibration, perfusion was stopped during 30 min (I group) and re-perfused for 45 min (R group). Ct hearts were perfused during 75 min, while hearts before "I" stimulus were considered the C group. Expression of UGGTs was investigated in heart sections by immunohistochemistry (IHC) and in left ventricles by dot- and Western- blot (D/WB), n= 3/group. Protein expression was determined by densitometry of the blot bands by Image J 1.42q software. Our results showed that both isoforms are expressed in (C) hearts. IHC studies showed that UGGT1 was upregulated while UGGT2 diminished in I and R groups. D/WB studies confirmed that LV from I group expressed the UGGT1´s highest expression followed by (R), I vs. Ct: p<0.001; R vs. Ct; p<0.05, while UGGT2 was mainly detected in Ct group. This study demonstrated UGGTs expression in rat heart and their differential regulation under I/R stimuli. The upregulation of UGGT1 in an I/R model is consistent with its role during an unfolded proteins reponse (UPR). The downregulation of UGGT2 after I/R stimuli suggest that both isoforms might play different roles in cardiomiocytes.

0528 - INDUCED DECREASE OF E-CADHERIN TRIGGERS A PARTIAL EPITHELIAL-

MESENCHYMAL TRANSITION IN HUMAN EMBRYONIC STEM CELLS

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FLENI-CONICET

Epithelial-to-mesenchymal transition (EMT) is a fundamental cellular process implicated in cell differentiation, embryonic development, tissue repair and cancer metastasis. During this event, epithelial cells lose cell junction, apico-basal polarity and acquire migratory properties and other traits of mesenchymal phenotype. A plethora of stimuli can trigger this event and most of them converge in loss of E-cadherin, a key process in the initiation of EMT. E-cadherin is a homophilic adhesion molecule involved in cell-cell contact. Its expression is decreased during EMT and also loss of function of this protein promotes this transition. Based on this background, our hypothesis is that an induced decrease of proteins involved in intercellular junctions could activate EMT process. In the present work, our aim was to analyze the effect of induced down regulation of E-cadherin in EMT. For this, we generated a stable human embryonic stem cell line (hESC) containing doxycycline-inducible CRISPR-dCas9-KRAB system to repress transcription of E-cadherin. Our results indicated that incubation with doxycycline at different times produced gradual decrease of E-cadherin mRNA and a reduction in immunofluorescence intensity suggesting a lower presence of this protein. Additionally, we observed increased collective cell migration in these conditions. Downregulation of E-cadherin also produced morphological changes since a proportion of cells progressed to spindle shape cell morphology. However, cells maintained the characteristic colony disposition of hESC. Finally, we analyzed gene expression profile of pluripotency and EMT. Although these cells maintained their pluripotency state, SNAI1 and SNAI2, key genes involved in the initiation of EMT, were significantly increased. In accordance with our results, we suggest that downregulation of E-cadherin could induce a partial EMT but not the complete process.

Endocrinología/ Endocrinology II

Chairs: Sol Kruse | Nora Saraco

0162 - IN VIVO AND IN VITRO DEXAMETHASONE TREATMENT ENHANCES WHITE ADIPOCYTE PRECURSOR CELLS COMPETENCY.

María Guillermina ZUBIRÍA (1) | Alejandra Paula GIORDANO(1) | Eduardo SPINEDI(2) | Andrés GIOVAMBATTISTA(1)

IMBICE (1); CENEXA (2)

Dexamethasone (DXM) is a synthetic glucocorticoid widely recognized as an adipogenic inducer in both, in vivo and in vitro models. However, there is scarce information about the DXM role in adipocyte precursor cells (APC) before differentiation induction. Our aim was to evaluate the DXM effect on APC number and competency, using in vivo and in vitro approaches. Therefore, we injected two groups of adult male rats for 2 or 7 days with either DXM (30 µg/kg of weight ip, DXM-d2 and DXM-7d, respectively) or vehicle (CTR-2d and CTR-7d). Retroperitoneal adipose tissue (RPAT) was dissected and stromal vascular fraction (SVF) cells were isolated for Fluorescence-Activated Cell Sorting (FACS) analysis (APC profile: CD34+/CD45-/CD31-) and expression of competency markers (PPARg2 and Zfp423). Two days after DXM treatment PPARg2 and Zfp423 expression levels were increased ($P<0.05$), whereas on day 7 showed normal levels. FACS analysis showed that DXM did not affect APC percentage after 2 or 7 days of treatment. Additionally, we evaluated in vitro if DXM effects on APC were mediated by mineralocorticoid (MR) or glucocorticoid (GR) receptor or both. Thus, confluent SVF cells were incubated (48 h) in the absence or presence of DXM (0.25 µM; CTR and DXM, respectively) in combination with GR antagonist (1 µM RU486, DXM-R) or MR antagonist (10 µM spironolactone, DXM-S) or both (DXM-R-S). FACS analysis and expression profile were performed. DXM increased PPARg2 expression ($p<0.05$), effect fully prevented by the simultaneous blockade of both receptors. Zfp423 expression also increased in DXM cells ($p<0.05$), an effect abolished regardless of the blocking condition. APC number was not modified by any treatment. Overall, our results suggest that DXM primes APC for differentiation by enhancing cell competency, both in vitro and in vivo, mainly through the increase in Zfp423 and PPARg2 expressions. Also, we have demonstrated that in vitro DXM was able to act through GR and MR.

Supported by PICT2015-2352 grant.

0209 - ALTERATIONS IN THE CONCENTRATIONS OF NITRIC OXIDE, CORTISOL, ANGIOTENSIN AND ALDOSTERONE IN DOGS WITH CUSHING'S DISEASE AND ITS EFFECT ON ARTERIAL HYPERTENSION

Patricia Noemí VIDAL (1) | Diego Daniel MICELI(2) | Víctor Alejandro CASTILLO(3)

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Cushing's disease (CD) is a very common endocrinopathy in dogs, characterized by a state of chronic hypercortisolism that causes metabolic and systemic alterations. One of them with high prevalence is high blood pressure, which together have a great impact on the body, such as the development of kidney disease, which is a cause of death. For this, we studied the concentrations of cortisol, aldosterone, angiotensin II (ang II), nitric oxide (NO) and blood pressure in dogs with CD, then we compared with healthy dogs. We studied 20 dogs with CD properly diagnosed with no other pathologies at the FCV-UBA School Hospital and a group of 12 control dogs from caniles FCV-UBA. We measured to all of them blood pressure: systolic (SP) and diastolic (DP) and blood samples were taken, fasting, for cortisol, NO, aldosterone and ang II. Most of the variables studied had non-parametric distribution. Comparisons between the medians of CD and control groups were made by Mann-Whitney test. Spearman test was used for the correlation. It was considered significant $p<0.05$. Eighty % of the CD dogs had elevated DP and 60 % elevated the SP. Cortisol was significantly elevated in EC dogs ($p<0.0001$). NO was significantly decreased in dogs with CD ($p<0.0001$). Cortisol and NO correlated inversely ($r= -0.67$; $p<0.0001$). SP and DP correlated with cortisol positively ($r= 0.46$, $p=0.008$and $r= 0.63$, $p<0.0001$; resppectively) and negatively with NO ($r= -0.87$, $p<0.0001$ and $r= -0.81$, $p<0.0001$, respectively). Aldosterone did not show significant differences between controls and CD dogs ($p=0.8$), but ang II was significantly elevated in CD dogs ($p<0.0001$). Alterations in cortisol levels and NO have a direct impact on blood pressure in CD. Aldosterone would not be involved among the mechanisms that develop hypertension in dogs with CD, but angiotensin would be involved indeed. These results have great relevance to determine the antihypertensive drug to be used to achieve its control and avoid its impact, for example at the renal level.

0229 - PHENOLIC ACIDS PREVENT DIABETIC COMPLICATIONS ABOLISHING THE ACTIVATION OF ALDOSA REDUCTASA BY TUBULINA

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INBIAS/CONICET. DEPARTAMENTO DE BIOLOGÍA MOLECULAR-UNIVERSIDAD NACIONAL DE RIO CUARTO

Previously in our laboratory we describe that that the overexpressed and purified human aldose reductase (AR) interacts directly with tubulin, mainly whit the 3-nitro-tyrosinated isotype. When the Tubulin/AR complex is incorporated into a growing microtubule, the AR activity is increased more than 6 times. More recently we show that tyrosine (Tyr), 3-nitrotyrosine (Ntyr) and different compounds derived from phenolic acids (CAFs) like tyrosine prevent the formation of Tub/AR complex and enzyme activation in vitro. In cells in culture the CFAs prevent: i) the formation of the tubulin/AR complex, ii) the induction of microtubule formation by high glucose, and iii) activation of AR and inhibition of Na^+, K^+-ATPase (NKA) activities by tubulin. This show that tubulin regulates AR activity by a direct interaction, which can be prevented in vivo and in vitro by CAFs. In this work we investigate in a model of diabetic rats the effect of CAFs (Tyr, NTyr and vanilic acid) on the development of four secondary complications of diabetes mellitus in which it is recognized the participation of the activation of AR: i) diabetic cataracts, ii) alterations in erythrocyte deformability, iii) development of arterial hypertension and iv) development of renal insufficiency. The results obtained show that the CAFs prevent the development of cataracts and increase the survival of diabetic rats. This is correlates with better renal functioning, the maintenance of a normal erythrocyte deformability and the prevention of the development of arterial hypertension. This suggest that regulation of enzymatic activity AR by tubulin in vivo by CAFs were able to attenuate the development of secondary pathologies of DM due to their preventive action of Tub/AR interaction and the consequent NKA activation.

0241 - SPEXIN, FRIEND OR FOE? STUDY OF THE ROLE OF SPEXIN DURING THERMOGENESIS OF WHITE ADIPOSE TISSUE.

Sabrina Eliana GAMBARO | María Guillermina ZUBIRÍA | **Alejandra Paula GIORDANO** | Ezequiel Alejandro HARNICHAR | Andrea Estefanía PORTALES | Andrés GIOVAMBATTISTA

IMBICE

Spexin (SPX) is a novel adipokine related to appetite and weight control, glucose homeostasis, and lipidic metabolism. Plasma levels are reduced in obese and type II diabetes patients. Recent reports showed that SPX inhibits white adipogenesis and stimulate lipolysis. Our aim was to study SPX effects during the thermogenic process of white adipose tissue (AT). This process has been widely proposed as a possible therapy for obesity. C57BL/6J male mice were treated or not with SPX for ten days (ip. 29 µg/kg/day; CTR and SPX). At day 3 animals were divided and a group was kept at room temperature (RT) and the other at 4°C (CTR-C and SPX-C). Body weight and caloric intake were recorded every day. At the end of the experiment, plasma was collected for metabolic parameters measurement and Epididymal AT (EAT) and Inguinal AT (IAT) were dissected for quantification of thermogenic markers (UCP1, PGC1a and COX8b) by qPCR. Total caloric intake was increased upon cold stimulation ($p<0.001$), but no SPX effect was observed. Also, plasmatic glucose and triglycerides were no changed by SPX treatment, however both were decreased by cold ($p<0.001$). At RT, SPX produced a decrease in the UCP1 and COX8b expression in both AT depots ($p<0.01$). As expected, after cold stimulation mRNA expression of UCP1, PGC1a and COX8b in EAT and IAT depots were increased ($p<0.001$), and SPX treatment prevented these enhancement ($p<0.001$). Moreover, we performed in vitro cultures from the stromal vascular fraction of AT depots from CTR animals and differentiated to beige adipocytes in presence of SPX (0.5, 1, 10 ng/ml) during all the differentiation process. UCP1 expression decreased with all SPX concentrations ($p<0.05$). In conclusion, SPX reduced the browning process of IAT and EAT, being more markedly upon cold exposure, and inhibited UCP1 expression in beige differentiated adipocytes. Overall, our results support the inhibitory role of SPX in browning of AT.

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0273 - GABAB RECEPTOR DELETION IN KISS1 NEURONS/CELLS AFFECTS THE HYPOTHALAMIC EXPRESSION OF KISS1 IN MALE MICE

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IBYME-CONICET (1); UNIVERSITY OF BASEL (2)

It is known that Kiss1 cells/neurons co-express GABAB receptors (GABABRs), and our group has previously demonstrated that global GABAB1KO mice show a dramatic increase in Kiss1 expression in male and female extrahypothalamic areas (amygdala, BNST, lateral septum), but its expression does not differ in AVPV-PeN/ARC. To establish the impact of GABABRs on kisspeptin physiology we developed a strain of mice with specific deletion of GABABRs in Kiss1 cells/neurons and characterized them from reproductive perspectives. Kiss-Cre mice (Jackson's Lab) were crossed with GABAB-floxed mice (donated by Dr. Bettler) to obtain Kiss1-GABAB1KO (KO) mice. Male Kiss1 expression was evaluated by qPCR in amygdala, BNST, AVPV-PeN and ARC micropunches; serum hormones were determined by RIA. Conversely to GABAB1KO mice, no significant changes were observed in extrahypothalamic areas [amygdala (fold change): KO= 0.95 ± 0.08 (n= 10) vs. WT=0.98 ± 0.08 (n= 9), p ns; BNST (fold change): KO= 3.36 ± 1.48 (n= 9) vs. WT= 1.05 ± 0.15 (n= 6), p= 0.07]. Interestingly, KO males showed increased Kiss1 expression in the ARC [fold change: KO= 1.32 ± 0.16 (n= 8) vs. WT= 0.9 ± 0.1 (n= 8), $p<0.05$] and decreased expression in the AVPV-PeN [fold change: KO= 0.46 ± 0.1 (n= 7) vs. WT= 1.19 ± 0.2 (n= 7), $p<0.01$]. The hypothalamic alterations in Kiss1 expression did not affect basal LH levels [serum LH (ng/ml): KO= 0.77 ± 0.20 (n= 15) vs. WT=0.95 ± 0.29 (n= 13), p ns], and were not a consequence of a difference in testosterone-induced negative feedback [serum testosterone (ng/ml): KO= 0.73 ± 0.08 (n= 13) vs. WT= 0.62 ± 0.09 (n= 9), p ns]. Altogether, results show the direct influence of GABABRs on Kiss1 expression levels in critical nuclei involved in the control of reproduction in mice. Further evaluation of male Kiss1-GABAB1KO reproductive efficiency will increase our understanding of GABAB involvement in the control of reproduction and Kiss1 physiology. Funding: CONICET, ANPCYT, ISN-CAEN, UBA, Fundación René Barón, Fundación Williams.

0401 - PRE-PUBERTAL ANDROGEN DEPLETION IMPROVES THE WHITE ADIPOSE TISSUE BROWNING

Alejandro Ezequiel HARNICHAR | Maria Guillermina ZUBIRIA | Alejandra GIORDANO | Eduardo SPINEDI | Andres GIOVAMBATTISTA

IMBICE

It is known that cold exposure induces the generation of beige adipocytes in white adipose tissue (AT) depots. We previously described that the addition of androgens (A) during in vitro differentiation of beige adipocytes inhibits its thermogenic capacity. Now, we aim to study if in vivo absence of A affects the in vitro beige adipocyte generation and browning potential of white AT after cold stimulation. For these purposes, we used the following groups of S-D male rats: controls (CTR), bilaterally orchidectomized at 27 days of age (ODX) and pair fed controls (CTR-PF, equal caloric intake as ODX group). In the first assay, adipocyte precursor cells (APCs) were isolated from inguinal AT (IAT) and retroperitoneal AT (RPAT) from different groups and were cultured with a pro-beige cocktail. At day 8, beige adipocyte markers (UCP-1, PGC1a, and PRDM16) were measured by RT-qPCR. We found an increase in UCP-1 ($p<0.001$), PGC1a ($p<0.01$) and PRDM16 ($p<0.01$) in differentiated cells obtained from IAT and an increase in UCP-1 ($p<0.05$) and PRDM16 ($p<0.01$) in RPAT cells from the ODX group. In another experiment, 60 day-old animals from the different groups were kept for 7 days at either room temperature (RT) or at 4°C (CTR, CTR-PF, ODX and CTR-C, CTR-PF-C, ODX-C, respectively). IAT and RPAT were isolated for subsequent measurement of

thermogenic genes (UCP-1, PGC1a and ARß3) by RT-qPCR. We found that IAT and RPAT UCP-1 expression was elevated in ODX rats at RT and in cold-stimulated conditions ($p<0.05$), this was accompanied by an increase in PGC1a only in IAT from cold-stimulated ODX rat ($p<0.05$). Although no change was found in ARß3. We conclude that depletion of A in ODX rats primes APCs for further adipocyte thermogenic activity in vitro. In addition, castration improves the AT browning in cold-stimulated condition, mainly through an increase in thermogenic markers in both AT depots. Thus, our study supports the inhibitory role of A in AT thermogenesis.

Supprted by PICT 2017-2314 grant.

0462 - LACK OF ENDOGENOUS ESTRADIOL DID NOT WORSEN GENERAL METABOLIC STATE NOR ADIPOSE TISSUE FUNCTION, EVEN THOUGH ADVERSE DIET CONSUMPTION.

Ignacio MIGUEL (1) | Ana ALZAMENDI(1) | Florencia MARTIN(1) | Amanda REY(1) | Eduardo SPINEDI(2) | Andrés GIOVAMBATTISTA(1)

IMBICE (1); CENEXA (2)

We previously studied the role of estrogen deficiency in 60-day-old female rats ovariectomized (OVX) on prepubertal age (27-day-old). It was concluded that OVX animals have improved some metabolic-endocrine functions. Our aim was to determine consequences of fructose rich diet (FRD) intake on female rats OVX at adult age. Therefore, OVX rats were fed FRD (fructose 10 % w/v in tap water) between 60-81 days of age. Thus groups studied were: animals that consumed water and chow ad libitum: control basal (CTR B), ovariectomized basal (OVX B), and considering higher food intake by OVX animals, OVX pair-fed rats (OVX PF B) were incorporated; finally, rats in similar conditions but fed FRD (CTR FRD, OVX FRD, OVX PF FRD) were studied. Trunk blood was collected to measure plasma metabolites and retroperitoneal adipose tissue (RPAT) pads were dissected out, weighed and processed for histological and gene expression analyses. FRD treatment did not increased body weight in any group, despite energy intake. RPAT mass decreased in OVX PF B vs CTR B animals ($p<0.05$, as previously published). In accordance with RPAT mass values, OVX PF adipocyte size was smaller (OVX PF B, $p<0.01$ vs. CTR B and OVX PF FRD, $p<0.05$ vs. CTR FRD). FRD treatment only produced plasmatic triglycerides increase in CTR FRD ($p<0.05$ vs. CTR B), and lowest values were found in OVX PF animals (OVX PF B, $p<0.05$ vs. CTR B and OVX PF FRD, $p<0.01$ vs. CTR FRD). None difference in plasma glucose levels was noticed. RPAT resistin gene values decreased in OVX groups fed with FRD (OVX PF FRD, $p<0.05$ and OVX FRD, $p<0.01$, vs. CTR FRD). While those of leptin increased in CTR FRD animals (vs. OVX FRD, $p<0.05$) no changes were seen in OVX PF FRD rats. Our study indicates that prepubertal OVX do not metabolically worsen individual condition, even when fed with an adverse diet.Supported PICT 2017-2314 and -2334 grants.

0506 - MOLECULAR DIAGNOSIS OF RARE THYROID PATHOLOGIES.

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Resistance to thyroid hormone (RTH) is a rare syndrome, with decreased sensitivity to thyroid hormone which leads to elevated serum TH concentrations, but inappropriately normal or elevated thyroid stimulating hormone concentrations. This disease is mostly caused by mutations of thyroid hormone receptor beta (THRB) gene. The incidence is estimated to be 1 in 40,000-50,000. Thyroxine-binding globulin (TBG) is the main transporter of thyroid hormones and is encoded by the TBG gene. Several mutations have been reported in TBG gene causing partial TBG deficiency (TBG-PD) whose prevalence is 1:4000. Molecular diagnosis has been carried out in 34 and 16 unrelated argentinian families with clinical evidences of RTH and TBG-DP respectively. Genomic DNA was isolated from blood cells and the exons 7-10 of the THRB gene and exons 0-5 of TBG gene were amplified by PCR and sequenced by Sanger technique. The novel missense mutations identified were analyzed by in silico studies to elucidate a correlation between structural disturbances and putative functional commitment. 26 mutations in THRB have been identified; 11 novel mutations: p.K306T, p.N331D, p.A335P, p.L341P, p.L346F, p.D351E, c.1276_1277insTGA (p.V425_T426insM), p.I431M, p.A433CfsX28, p.P447T and p.P453L and 15 previously reported mutations: p.I250T, p.A268G, p.A317T, p.R320H, p.G332R, p.R338W, p.G345R, p.H435P, p.R438H, p.K443N, p.P452L, p.P453T, p.F459C, p.F459L, p.E460K. The more frequent mutations are p.P453T, p.R338W, p.A268G and p.R320H identified in 5, 3, 2 and 2 families respectively. 10 mutations in TBG gene have been identified; 9 novel mutations: g.IVS1+2delT, g.IVS1+6T>C, p.A64D, p.N154Y, p.A188T, p.L237R, p.Y241X, p.Q256X, p.A333S and a known mutation: p.T38TfsX13. g.IVS1+2delT, g.IVS1+6T>C and p.A188T are present in 5, 2 and 2 families respectively. This work contributes to elucidate the molecular basis of RTH and TBG-PD and the improvement of the diagnosis avoiding unnecessary therapy and side effects.

0718 - DIET COMPOSITION AND SOMATIC GROWTH IN PEJERREY (ODONTESTHES BONARIENSIS)

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Pejerrey is a South American fish with a high potential for aquaculture. Due to its flesh quality, it has been considered a good candidate for aquaculture production since the beginning of the 20th century. However, this is still on hold as a consequence of both economical and biological factors. Among the latest we can find a low growth rate in culture and the lack of a specific and economical formulated diet. Therefore, the aim of this study was to determine the dietary protein:lipid ratio to ensure the best growth rate in early developmental stages. Pejerrey fry were fed for 60 days with four experimental diets containing low (400 g Kg^{-1}) or high (500 g Kg^{-1}) protein (LP or HP, respectively) and low (120 g Kg^{-1}) or high (200 g Kg^{-1}) lipid (LL or HL, respectively), in the following combinations: LP-LL; LP-HL; HP-LL and HP-HL. Growth parameters were determined every 15 days and at the end of the trial total mRNA of head and trunk of pejerrey fry (post-larva) were obtained to determine the expression levels of some genes involved in lipid metabolism, food intake and growth regulation. Fry fed with diets LP-LL and HP-LL showed the highest growth rate and growth hormone (gh) mRNA expression levels. Gene expression of Δ6-desaturase was high in head of fry fed with diet LP-HL. Gene expression of nucb2/nesfatin-1 and gh in head, and of nucb2/nesfatin-1 and the ghr-I and ghr-II in body followed the same patterns. In conclusion, diets with LL ensure pejerrey fry growth compared to those that contain HL. In these LL groups, mRNA expression of genes from the GH-IGF axis were associated with the observed somatic growth promotion. The expression of nucb2/nesfatin-1 seems to indicate some effect of this peptide not related to food intake regulation, e.g. a regulatory role on GH expression, that would warrant future research. Our results contribute to stablish the pejerrey's nutritional requirement for the development of its sustainable aquaculture production.

0741 - EFFECT OF NOX4 AND SELENIUM IN THYROID AUTOREGULATION

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Na/I symporter (NIS) mediates iodide (I) uptake in the thyroid gland, the first and rate-limiting step in the biosynthesis of thyroid hormones. Thyrotrophin (TSH) and insulin/IGF-I control the expression and function of NIS. While TSH has stimulatory effects, insulin/IGF-I exerts its inhibitory effects through the PI3K/akt pathway. Iodine excess inhibits NIS expression through PI3K/akt pathway activation which involves ROS production. Others factors likes TGFB1 and the iodolipid, 2-iodohexadecanal (2-IHDA), have inhibitory effects on NIS expression while Selenium (Se) upregulates it. We reported that NADPH oxidase NOX4 knockdown prevents the increase of intracellular ROS and reverse the inhibitory effect on NIS induced by iodide excess. The aim of this work was to analyze the role of NOX4, Iodohexadecanal (2-IHDA) and Selenium on PI3K/akt pathway activation and NIS expression in FRTL-5 cells. siRNA targeted knockdown of NOX4 reversed the phosphorylation of akt induced by iodine ($p<0.05$). 2-IHDA induced akt phosphorylation (1.8 fold, $p<0.05$) and a PI3K inhibitor (LY294002) reversed the inhibitory effect on NIS mRNA and gene transcription. 2-IHDA also induced TGFB1 (3 fold, $p<0.05$), however, treatment with TGFB inhibitor (SB431542), did not reverse the inhibitory effect of 2-IHDA or iodine on NIS expression. Se reverted akt phosphorylation induced by iodide and reversed the inhibitory effect on NIS expression. Besides it increased GPX-1 (1.5 fold) and NOX4 (3.2 fold) and decreased TGFB1 ($p<0.05$) expression. Our results suggest that NOX4 and Se could play a role in thyroid autoregulation mechanism mediated by PI3K/akt pathway.

0749 - DIFFERENTIALLY EXPRESSED MIRNAS IN ZONA RETICULARIS (ZR) CELLS OF THE HUMAN ADRENAL CORTEX

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The human adrenal cortex, involved in adaptive responses to stress, fluid homeostasis, and secondary sexual characteristics, arises from a tightly regulated development of a zone and cell type-specific secretory pattern. However, the molecular mechanisms governing adrenal zonation, particularly postnatal ZR development, which produce adrenal androgens in a life-time-specific manner, remain poorly understood. The hallmark of ZR is the low expression of type 2 3β-hydroxysteroid dehydrogenase (HSD3B2). However, the mechanisms underlying HSD3B2 downregulation in the ZR remain unknown. MiRNAs are seen as regulators of cell phenotypes. The objective of the study was to compare miRNA expression profiles in human adrenal ZR and zona fasciculata (ZF). ZF and ZR were microdissected from 5 human adrenals tissues by laser capture microdissection. Total RNA was extracted from 10 ZF/ZR pairs and next-generation sequencing (NGS) was used to perform the microRNA expression profiling. Two hundred eighty one mature microRNAs were identified in human adrenal cortex. Among them 7 microRNAs were significantly different by 2-fold or greater in the ZF and ZR. The expression of miR-375-3p, miR-483-3p and miR-7-5p was higher in ZR compared with its paired ZF. Multiple available boinformatic algorithms (TargetScan, miRanda, DianaLab, MicroCosm and PicTar) were employed to search for the target genes. Among predicted target genes, key HSD3B2 regulators genes (GATA-6, GATA-4 SF1, NR4A2, and IGF-1), were revealed. Adrenal zone-specific micro-RNA profiling revealed novel regulatory modules for androgen production at the posttranscriptional level that will open further research on the regulation of adrenal androgen production in health and disease.

0783 - LONG-TERM ADMINISTRATION OF IMT504 GREATLY IMPROVES THE DIABETIC CONDITION IN FEMALE NOD MICE. IMPACT ON THE IMMUNE SYSTEM

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Due to the importance of type I Diabetes, it has become crucial to find a treatment that would not only normalize blood glucose, but also restore insulin secretion by stopping the autoimmune destruction of beta cells. Our approach for the reversion of the diabetic condition consists of the use of an immunomodulatory oligonucleotide, IMT504 (IMT). Previously, we have shown that IMT treatment promotes a marked recovery of toxic diabetes in rats, immunodependent diabetes in mice and in a short-term treatment in diabetic NOD mice. Here we evaluate a continuous and chronic IMT treatment in NOD diabetic mice, more similar to what could eventually be used in humans. We have previously shown that this treatment improves the metabolic condition and we aim here to study its immunological effects. Diabetic female NOD mice were implanted with constant drug release pumps, loaded with either IMT (total dose released per day: 20 mg/kg BW) or saline. After 28 days of treatment, mice were sacrificed, and their spleens and pancreases harvested for gene expression analysis by RT-qPCR and leukocyte infiltration determination (haematoxylin and eosin staining) respectively. Ongoing results show that IMT induces a decrease in leukocyte infiltration in Langerhans islets ($p<0.05$). Regarding gene expression in splenocytes, we observed in the treated group, an up-regulation of Indoleamine 2,3-deoxygenase 1 ($p<0.0001$) and Galectin-3 genes ($p<0.05$), down regulation of Interleukin-4 ($p<0.001$) and Interferon-γ ($p<0.05$), but no differences in Transforming growth factor-β expression compare to controls. We conclude that a continuous and prolonged IMT treatment alters the immune response of diabetic mice, by changes in the expression of genes coding for pro and anti-inflammatory proteins involved in the immune pathways of the disease, and thereby results in a decrease in the immunological infiltration of pancreatic islets. Funding: CONICET, ANPCYT, UBA, Johnson&Johnson Arg, Fund R Barón, Fund Williams

Neurociencias / Neurosciences IV

Chairs: Carla Caruso | Andrea de Laurentis

0327 - EARLY IGF-1 GENE THERAPY ASSOCIATED WITH NANOTECHNOLOGY REVERSES COGNITIVE DEFICITS AND OXIDATIVE STRESS INDUCED BY TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) is a global public health concern. It is the leading cause of death in the population under forty years old and after that, it remains as one of the main. TBI-related injuries

are divided in 2 subcategories: primary injury, which occurs at the moment of trauma and secondary injury, which occurs immediately after trauma and produces effects that may continue for a long period of time. The latter is attributable to further cellular damage from the effects of primary injuries and related to inflammatory mechanisms and oxidative stress (OS) and may in themselves have severe consequences. Secondary injuries may develop over a period of hours or days following the initial traumatic assault and this delayed nature suggests that there is a window for therapeutic intervention to prevent progressive tissue damage and improve functional recovery after injury. Among survivors, neurological impairment may vary from subtle symptoms to severe long term sequelae, either physical as long as cognitive. To date, there is no treatment to target mechanisms of secondary injury and the therapeutic arsenal is limited to decrease intracranial pressure. Non-steroidal anti-inflammatory drugs and steroids are not effective in the treatment of TBI because of their limited access to central nervous system. A therapeutic alternative of increasing interest in the treatment of brain injuries, is the use of neurotropic factors such as Insulin-like growth factor 1 (IGF-1), since they are neuromodulators associated with neuroprotection and anti-inflammatory effects. The aim of the present investigation is to evaluate the effectiveness of IGF-1 in the treatment of TBI in both reversing OS as well as improving cognitive deficits.

0465 - GROWTH HORMONE SECRETAGOGUE RECEPTOR SIGNALING IN DOPAMINE NEURONS MEDIATES HIGH FAT DIET INTAKE IN A BINGE-LIKE EATING PROTOCOL

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IMBICE

Ghrelin is a peptidic hormone that regulates a variety of biological processes, including energy homeostasis. Ghrelin induces food intake when administered to rodents and humans and this orexigenic effect is exerted through binding of the hormone to the growth hormone secretagogue receptor (GHSR), a G protein-coupled receptor mainly expressed in the brain. Ghrelin regulates both the homeostatic and the hedonic components of food intake, acting on hypothalamic and mesolimbic neuronal circuits, respectively. GHSR is present in different neuronal populations and, particularly, GHSR-expressing dopamine neurons of the mesolimbic circuit are involved in ghrelin's regulation of food reward. In this study, we investigated the role of GHSR-expressing dopamine neurons in the regulation of the different ghrelin's biological effects. We utilized a genetically modified mouse model in which Cre recombinase is expressed exclusively in dopamine neurons (DAT-Cre mice). We crossed DAT-Cre mice to a mouse model in which GHSR expression is blocked by a LoxP-flanked transcription blocking cassette (GHSR-deficient mice) in order to generate mice expressing GHSR selectively in dopamine neurons (GHSR-deficient/DAT-Cre mice). We first tested if GHSR-deficient/DAT-Cre mice were useful to study the effect of GHSR expression exclusively in dopamine neurons. Then, we studied the effect of peripheral and central ghrelin administrations to GHSR-deficient/DAT-Cre mice. Finally, we used a binge-like eating and a conditioned place preference protocol in order to determine the role of GHSR-expressing dopamine neurons in the regulation of ingestive behaviors. Our results indicate that ghrelin receptor signaling in dopamine neurons mediates complex feeding behaviors while the selective expression of GHSR in dopamine neurons is not sufficient to restore ghrelin-induced food intake and locomotor activity.

0525 - DESIGN AND FACE VALIDATION OF A MURINE MODEL OF SOCIAL VULNERABILITY

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Social vulnerability refers to conditions of social, economic and cultural disadvantages experienced by some population groups as a result of a prevailing social order. These conditions increase the risk of setting up disorders such as anxiety, depression, aggressiveness and lack of inhibitory control, among others. This work aims to design and validate a murine model of social vulnerability (VS) to, later on, study molecular mechanisms that mediate the development of behavioral disorders in dams and offspring produced by a set of adverse environmental factors. The model tries to simulate unfavorable conditions to which mothers and children of the most disadvantaged social sectors are frequently exposed: -limited protection of the mother during pregnancy and nursing: cages are frequently intervened during pregnancy and dams are physically restrained during the nursing period -inadequate habitability conditions: cages contain little bedding and without nesting material -early mother-child separation for long periods: daily separation between PD6 and PD16, as well as early weaning: -harassment-violence: pups are exposed to a dominant male mouse between PD20-PD30. Dams and offspring were evaluated in different behavioral tests. Dams exposed to VS offered less care to their pups (quantity and quality of activities such as nursing, grooming and liking between PD1-PD5), displayed depression-like traits (forced swimming test) and held more risk behaviors (elevated plus maze test) than dams in the control group. Regarding offspring, VS treated mice presented dominant behavior over mice of control group (dominance tube test), deficiency in spatial recognition memory (novel object place recognition test) and aggressiveness signs (intruder–resident test). All indicated differences were statistically significant. Results obtained from dams and offspring sustain the face validity for the proposed model of social vulnerability, which allows us to approach its target validity.

0533 - FASTING INDUCES REMODELING OF THE OREXIGENIC PROJECTIONS FROM THE ARCUATE NUCLEUS TO THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS IN A GROWTH HORMONE SECRETAGOGUE RECEPTOR-DEPENDENT MANNER

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Some hypothalamic circuits are known to undergo morphological and functional remodeling in order to ensure the control of the body homeostasis. Ghrelin is a stomach-derived hormone that acts on the growth hormone secretagogue receptor (GHSR), and is known to play a key regulatory role on the energy balance. Here, we hypothesized that the up-regulation of the GHSR system during fasting at the orexigenic Agouti-related peptide (AgRP)/neuropeptide Y (NPY)-producing neurons of the arcuate nucleus (ARC) would promote a morphological remodeling of the ARC projections to the hypothalamic paraventricular nucleus (PVH) in adult mice, and that such structural changes mediate the fasting-induced activation of the PVH neurons. We showed through immunostaining analysis that the total amount of the orexigenic neuropeptides AgRP and NPY (mean intensity), the density of fibers containing these neuropeptides (area) and the amount of AgRP and NPY per fiber (integrated density) were increased in the PVH of fasted mice. Similarly, analysis of fluorescent signal in the PVH of NPY-GFP mice also showed that ARCnpy-->PVH projections increase under fasting. In addition, tracing studies confirmed that ARC-->PVH projections increase under fasting. Importantly, fasting-induced activation of PVH neurons was impaired in ARC-ablated

mice in which the density and strength of ARCagrp/npy-->PVH projections is not increased under fasting. Additionally, we show that fasting-induced remodeling of these projections from the ARC to the PVH and the fasting-induced activation of the PVH neurons is impaired in mice with pharmacological or genetic blockage of the GHSR signaling suggesting that ghrelin signaling controls these adaptations. To our knowledge, these are the first evidence that the connectivity between hypothalamic circuits controlling food intake can be remodeled in the adult brain, depending on the energy balance conditions, and that GHSR activity is a key regulator of this phenomenon.

0545 - ACUTE FE-DEXTRAN TREATMENT AND REDOX BALANCE IN RAT WHOLE BRAIN AND CORTEX

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An acute Fe-dextran treatment produced oxidative stress in rat brain that lead to the translocation of Nrf2 to the cell nucleus, producing the activation of genes involved in the glutathione metabolism in the cellular environment. Previous reports have shown that the acute Fe overload produced by a single injection of Fe-dextran resulted in a significant decrease in total thiol and glutathione content in rat cortex area after 6 and 8 h post injection (p.i.). In the whole brain, enzymatic activities of glutathione-S-transferase (GST) and glutathione peroxidase (GPx), and total thiol content were increased as compared to control tissues at 6 or 8 h p.i., respectively. The aim of this study was to determine the effect of acute Fe overload on glutathione-dependent enzymatic metabolism in cortex rat brain. A single dose of 500 mg Fe-dextran/kg body weight was administrated intraperitoneally to male Sprague Dawley rats. Total brain samples or cortex area were obtained from control and treated animals after 6 or 8 h p.i.. Glutathione reductase (GR) was determined spectrophotometrically. Reduced glutathione (GSH), oxidized glutathione (GSSG) and malondialdehyde (MDA) content were determined by reverse phase HPLC. MDA content showed a significant increased ($p<0.05$) at 8 h p.i. in whole brain. A significant decrease in cortical GSH ($p<0.05$), and a significant increase in cortical GSSG ($p<0.05$) was observed at 8 h p.i. A slight but non-significant reduction in the activity of the enzyme GR was seen at 6 and 8 h p.i. in brain cortex. Taking as a whole, these results suggested that the increase in the GSSG/GSH ratio could be associated to the increase in the activities of GST and GPx without any change in GR activity in brain cortex. Moreover, it seems that the alteration in the redox status caused by the Fe treatment in the cortex could contribute to the lipid peroxidation changes detected in the whole brain.

0546 - EXPRESSION OF CCL2 BY REACTIVE ASTROCYTES AFTER COMPLETE SPINAL CORD INJURY

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As it is known, spinal cord injury (SCI) involves different degrees of disruption in the sensitive, autonomic and motor systems. Therefore, the goal in this field is finding some therapeutic approach that could promote the recovery of the previously lost functions. Keeping this in mind, the main research carried out by our group, is focused on the design of a treatment that promotes different kind of locomotor recovery (using a transection SCI rat model). One of these treatments involves the intra-spinal administration of Netrin-1, an embryological chemoattractant protein proved to participate in axonal navigation. Netrin-1 treated animals showed a significant improvement in the recovery of locomotion, as well as their autonomic functions. In line with this, the present project focuses on the study on how the inflammation at the lesion site evolves during a complete SCI. For this matter, each specimen was studied sequentially day to day after the injury, observing the evolution of the inflammatory process. CCL2, a chemokine secreted by astrocytes that promotes the activation of macrophages after the SCI, is one of the main markers for inflammation in this pathology. Histologically, we detected and quantified CCL2's localization and expression in reactive astrocytes. We also measured the number of macrophages at the lesion site. These results were obtained by performing an immunofluorescence microscopy each day after the SCI, starting at day 1 until day 20. Our results show a scar healing at the lesion site; a sharp increase in the number of macrophages, with a maximum reached at the third day after the SCI and a following decrease of them. CCL2 expression varies among days after SCI. CCL2 increases as days go by, upstream of the lesion site. However, its expression starts to decrease at the epicenter of the lesion coinciding with macrophage's migration. Finally, our results show an increase of the inflammation at the lesion site after SCI. This process significantly decreases in rats treated with Netrin-1, whose number of macrophages, expression of CCL2 and scar area are significantly lower.

0549 - AUTOPHAGY PROTECTS BV-2 MICROGLIAL CELLS FROM MANGANESE-INDUCED CELL DEATH

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CONICET - UNIVERSIDAD DE BUENOS AIRES, INSTITUTO DE QUÍMICA BIOLÓGICA DE LA FACULTAD DE CIENCIAS EXA

Manganese (Mn) is a trace metal required for human health. As a micronutrient, Mn is needed only in small quantities and can become toxic at higher concentrations. Chronic exposure to Mn, in occupational or environmental settings, causes a parkinsonian-like syndrome known as Manganism. More than a century after its discovery, Mn neurotoxicity is still considered a public health concern. Like neurons, glial cells are susceptible to Mn-induced injury. We have previously demonstrated that Mn triggers microglial cell death by regulated necrosis, involving both parthanatos and lysosomal disruption. Autophagy is a catabolic pathway in which cellular components are degraded by lysosomes in response to stress conditions. Nevertheless, autophagy activation may have both beneficial and detrimental effects, depending on the context. Evidence indicates that Mn activates autophagy in microglial cells. However, the role of autophagy on microglial cell death remains unknown. To address this question, we exposed BV-2 cells to Mn and analyzed the time-course of autophagy activation and the effect of its modulation on cellular fate. We detected a time-dependent increase in LC3-II protein levels (WB and ICC; $p<0.001$). The expression of SQTM1/p62, an autophagic substrate, followed the same kinetics as LC3-II ($p<0.001$), suggesting an impairment of autophagy. Nevertheless, both LC3-II and p62 levels significantly increased after bafilomycin A1 treatment ($p<0.001$), indicating an active autophagic flux. Induction of autophagy with both rapamycin (200 nM) and melatonin (10 µM) partially prevented Mn toxicity at 24 h (MTT; $p<0.01$ and $p<0.001$, respectively). Surprisingly, autophagy inhibition (wortmannin, 50 nM) had no effect on cell survival, at any time-point tested. Our results suggest that autophagy could play a protective role in Mn-induced cell death. Thus, evidence obtained represent a valuable contribution to the design of novel therapeutic tools for the treatment of Manganism.

0550 - NEUROPROTECTIVE EFFECTS OF 17ß-ESTRADIOL ADMINISTRATION ON DOPAMINERGIC SYNTHESIS IN AN ANIMAL MODEL OF PARKINSON DISEASE.

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CONICET

We investigate estrogen neuroprotective actions in adult male rat brain tissue after neurotoxic injury. Using inmunohistochemical techniques (IHQ), we label and localize neurons that express tyrosine hydroxylase (TH) on substantia nigra (SN) and their projections to corpus striatum (CPu). TH is the first enzyme in dopamine (DA) biosynthesis and catalyses the conversion of L-tyrosine to L-DOPA. We use it to quantify neuron loss in SN and analyze striatal dopamine depletions. On postnatal day 60 (D= 0) rats were injected with 6-hydroxydopamine (6-OHDA) or vehicle (V) in the left CPu. From D 7-17 (10 days), they received a chronic treatment with 17ß- Estradiol (E= 0.1 µg/kg/day s.c.) or corn oil (O). Groups were conformed as HP (6-OHDA lesion, O treatment n= 18); HP+E (6-OHDA lesión, E treatment n= 18); E (V lesion, E treatment n= 15); C (V lesion, O treatment n= 15). On day 60 all animals were euthanized for TH IHQ. CICUAL approval 86/2016. In SN, the number of TH+ cells (NN) on both hemispheres was statistically lower in HP animals in comparison to the other groups (C= 26 ± 1.4, E= 29.8 ± 3.7, HP= 12.5 ± 1.1, HPE= 28.7 ± 2.3; p<0.0001). To calculate the size of the lesion (SL) we used NN mean values on both hemispheres and compared the mean difference to control group. There was a significant increase in HP group compared to C (mean diff= 13.60, p<0.001). This represents an expansion of the 18.2 % in the size of lesion. In the case of E (mean diff= -3.7) and HP+E (mean diff= -2.6) there was no statistical difference between means in comparison to C, but the SL was diminished in 18.4 % for E and 19.7 % for HPE group. We made a plugin to automatically count and label TH+ neurons in SN, using this approach we calculated regression analysis of NN vs. stained area (mm^2). Results indicate a linear and positive relation for all groups; goodness of fit (r^2) for C was 0.54 and perfect (r^2= 1) for E, HP and HP+E. Linear regression analysis shows that, NN is a good predictor for the stained area proportion. What is more, treatment with E improved CPu dopaminergic projections and neuron arborization. Staining was more intense and better distributed in HPE group compared to HP. While 6-OHDA administration diminishes NN and increases lesioned size in HP; evidence shows that E administration attenuates loss and almost reverts tissue detrimental effects in HPE group. In conclusion, E has neuroprotective effects on the nigrostriatal dopaminergic pathway which may stimulate dopamine synthesis.

0654 - DISTINCTIVE SYNAPTIC REMODELING PROPERTIES OF HIPPOCAMPAL NEURONS IN THE VALPROIC ACID RAT MODEL OF AUTISM

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Autism spectrum disorders (ASD) are characterized by impairments in social interaction and repetitive-stereotyped behaviors. These core symptoms imply alterations in brain areas of the limbic system including the hippocampus. Previously, we reported hippocampal alterations using the well-validated ASD animal model by prenatal exposure to valproic acid (VPA, 450 mg/kg ip). The hippocampus of juvenile VPA rats displayed decreased synaptic marker synaptophysin (SYN) along with an increased expression of the neural cell adhesion molecule (NCAM) and a decrease in its polysialylated form (PSA-NCAM). Also, neurons from VPA animals showed a smaller dendritic tree and fewer glutamatergic synapsis which also depicted the NCAM/PSA-NCAM imbalance in vitro. The aim of this study was to evaluate the remodeling properties of primary hippocampal neurons either from VPA or control male pups. After neuronal treatment (DIV13 or 7), cytoskeletal and synaptic markers were evaluated (DIV14) by immunocytochemistry. While in neurons from control animal glutamate (5 µM-3min, DIV13) induced an NMDA-dependent dendritic retraction and synaptophysin (SYN) puncta number reduction, neurons from VPA animals were only capable of dendritic retraction without any change in synapse number. When evaluating the response to fluoxetine (0.1 µM, DIV13), neurons from VPA animals were unable of remodeling their dendritic tree but SYN puncta number decreased. These changes were accompanied with increased PSA-NCAM expression only in VPA neurons. Similar effects were generated by a functional PSA mimetic peptide (DIV13) in the VPA group but not in the control one. A sialic acid precursor (DIV7) normalized synapse number and dendritic tree in neurons from VPA animals without affecting the control group. To sum up, our results indicate distinctive remodeling features of neurons from VPA animals and suggest that NCAM/PSA-NCAM balance modulation may play a key role in restoring synaptic and dendritic profile.

0679 - RECRUITING SPHINGOLIPIDS TO PROMOTE MIGRATION OF RETINAL PIGMENT EPITHELIUM CELLS

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Retinal proliferative diseases, frequent causes of vision loss, involve excessive migration and proliferation of Müller glial cells (MGC) and retinal pigmented epithelium (RPE) cells. Bioactive sphingolipids, as sphingosine-1- phosphate (S1P) and ceramide-1-phosphate (C1P), are established mediators of inflammation and fibrosis and we have shown that S1P stimulates MGC migration (Simon et al; 2015). We now analyzed if they promote RPE migration. We supplemented ARPE19 cells, a human RPE cell line, with 5 µM S1P or 10 µM C1P. We pretreated them with SphkI2 or NVP, inhibitors of sphingosine kinase-1 (SphK1) and ceramide kinase (CerK), respectively to analyze if endogenous S1P or C1P stimulate cell migration, and with W146 and BML241, S1P1 and S1P3 antagonists, respectively to investigate if S1P activated these receptors. Migration was analyzed by the scratch wound assay. Exogenous S1P or C1P significantly promoted RPE cell migration, but their combined addition had no additive effect. Inhibiting S1P synthesis significantly reduced cell migration, and exogenous S1P and C1P partially restored it. In contrast, NVP treatment had no effect on RPE migration. Pretreatment with W146 reduced RPE migration both in control and S1P-supplemented cultures, while BML241 only reduced it in S1P-treated cultures. Our results suggest that endogenous synthesis of S1P activates S1P1 to induce RPE cell migration whereas exogenous S1P stimulates migration by activating both S1P1 and S1P3. Notably, exogenous C1P enhances RPE cell migration but its endogenous synthesis does not. Noteworthy, when added together, S1P and C1P had the same effect on migration as when added separately, implying they may share common signaling pathways. Hence, sphingolipids appear as central regulators of cell migration, and targeting their metabolism might provide tools for treating proliferative retinopathies.

0695 - MECHANISMS OF NEURONAL DEGENERATION INDUCED BY THE CYANOTOXIN ß-N-METHYLAMINO-L-ALANINE (BMAA)

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The non-proteic aminoacid BMAA is a cyanotoxin released by many cyanobacteria occurring in most dams and water resources around the world. Its chronic intake has been linked with neurodegenerative diseases, like amyotrophic lateral sclerosis (ALS), Parkinson and Alzheimer diseases. We showed that BMAA affects retinal neurons and Muller glial cell viability in vitro. Here we investigated the signaling pathway involved in BMAA deleterious effects on cultured photoreceptors and amacrine neurons, its effects on axonal architecture and whether it affects other non-retinal neuronal types. Pure rat retinal neuronal cultures and the neuronal-like, PC12 cell line, cultured in chemically defined media to promote differentiation, were treated with 400 nM and 1 µM BMAA, respectively, for three days. Neuronal cultures were pretreated with MK-801, an N-methyl-D-aspartate (NMDA) receptor antagonist. Cell death was evaluated by DAPI staining and axonal outgrowth by immunocytochemical methods. Pretreatment with MK-801 significantly prevented the increase in the percentage of pyknotic or fragmented nuclei induced by BMAA in amacrine neurons. BMAA enhanced axonal outgrowth in neurons. We then analyzed BMAA effects on PC12 cells at two different stages of differentiation, evaluated morphologically and by anti-ß III tubulin expression. PC12 cell differentiation increased with time in culture, reaching 52 and 77 % at 7 and 12 days, respectively. BMAA increased similarly the percentage of cell death at both differentiation stages, from 33 in controls to 58 % in BMAA-treated cultures. Noteworthy, BMAA also promoted axonal outgrowth in PC12 cells, while simultaneously reducing ß III tubulin levels. These results suggest that BMAA provokes degeneration and axonal changes in different neuronal types, activating NMDA receptors to promote amacrine cell death, and identify BMAA as a potential inducer of neurodegenerative damages, with its consequent deleterious effects on human health.

0812 - REGULATION AND ROLE OF ACYL-COA SYNTHETASE 4 IN NEUROSTEROIDOGENIC CELLS

Melina Andrea DATTILO (1) | Lucía Manuela HERRERA(1) | Yanina BENZO(1) | Jesica Giselle PRADA(1) | Juan Manuel COHEN SABBAN(1) | Corina Ileana GARCÍA(2) | Cristina PAZ(1) | Paula Mariana MALOBERTI(1)

INBIOMED- INSTITUTO DE INVESTIGACIONES BIOMÉDICAS. UBA-CONICET (1); FUNDACIÓN INSTITUTO LELOIR - IIBBA (2)

The brain is a neurosteroidogenic organ in which neurons, astrocytes and microglia express enzymes related to neurosteroidogenesis. Acyl-CoA synthetase 4 (ACSL4) enzyme expression in the brain has been described. Although the role of ACSL4 in neurons and in the development of the nervous system has been studied, nothing had been described about its role in neurosteroidogenesis and its regulation in glia cells. Here we show that in primary astrocyte cultures incubated with 1mM 8Br-cAMP for 12 h, ACSL4 mRNA levels are increased compared to untreated cells ($p<0.001$). This treatment also significantly stimulated P4 production ($p<0.001$) measured by radioimmunoassay. P4 synthesis was significantly inhibited when astrocytes were previously treated with Triacsin C, an ACSL4 inhibitor ($p<0.05$). In turn, cAMP increased StAR mRNA levels ($p<0.001$) and Triacsin C inhibited this increment ($p<0.05$). Also, we showed that ACSL4 is involved in migration, proliferation and process elongation of astrocytes. Triacsin C significantly inhibited cell proliferation ($p<0.01$) and cell migration ($p<0.001$) measured by wound healing assay. Astrocytes process elongation induced by AMPc was inhibited by Triacsin C ($p<0.001$). Results were confirmed in a rat glioma model by stable silencing of ACSL4 in C6 cells with a specific siRNA (C6-ACSL4 siRNA). P4 production ($p<0.001$) and StAR mRNA levels were decreased in C6-ACSL4 siRNA ($p<0.001$) compared to control cells. Moreover, cell migration, cell proliferation and colony formation ($p<0.001$) were also affected by ACSL4 knockdown. In conclusion, as in classical steroidogenic systems, we demonstrate that ACSL4 is involved in the regulation of StAR expression and in the production of steroid hormones. Moreover, we show that ACSL4 could also participate in the migration, proliferation and process elongation of neurosteroidogenic cells.

0825 - PURINERGIC REGULATION OF THE SYNAPTIC ACTIVITY BY NON-VESICULAR ENDOGENOUS ATP AT THE MOUSE NEUROMUSCULAR JUNCTION

Maximiliano HURTADO PASO | Tamara Vanesa FRONTERA | Juan GUARRACINO | Luciano GALIZIA | Adriana LOSAVIO

INSTITUTO DE INVESTIGACIONES MEDICAS A LANARI (UBA - CONICET)

At mammalian neuromuscular junction, ATP and its metabolite adenosine modulate ACh release via presynaptic inhibitory P2Y13, A1 and A3 receptors and facilitatory A2A receptors. It is accepted that the major source of purines in the synaptic cleft is vesicular ATP released together with ACh. However, it was recently proposed that ATP can be secreted from skeletal muscle fibers through pannexins. Considering that high K^+ concentration increases the activity of pannexins, the aim of this work was to evaluate whether the ATP released from muscle fibers contribute to the purinergic modulation upon neurotransmitter secretion when membranes are depolarized by increasing K^+ concentrations. So, in phrenic-diaphragm preparations (CF1 mice) incubated in solutions containing 10-30 mM K^+, we studied the miniature end-plate potential (MEPP) frequency when pannexins were blocked by probenecid (100 µM). At 10 and 20 mM K^+, probenecid increased MEPP frequency (K+ 10: control 2.1 ± 0.1/s, probenecid 3.4 ± 0.6/s, n= 6, $p< 0.05$; K^+ 20: control 162.0 ± 11.25/s, probenecid 224.2 ± 16.3/s, n= 4, $p< 0.001$), whereas at 30 mM K^+, probenecid decreased MEPP frequency (Control 286.2 ± 17.3/s, probenecid 233.8 ± 10.8/s, n= 5, $p<0.05$). These results suggest that, at mammalian neuromuscular junction, non-vesicular endogenous ATP coming from muscle fibers through pannexins contribute to the modulation of ACh release. The increase in MEPP frequency observed at 10-20 mM K^+ when pannexins were blocked could indicate the lack of ATP/adenosine action on inhibitory receptors. On the other hand, as A2A facilitatory receptors are only activated when high adenosine concentration is present at the synaptic cleft (30 mM K^+), the reduction of MEPP frequency recorded at this K^+ concentration in the presence of probenecid, would suggest that these receptors are being less activated.

Infectología y Parasitología / Infectology and Parasitology III

Chairs: María Fernanda Franke | María Elena Nader | Carolina Poncini | Alan Talevi

0045 - ANTIPROLIFERATIVE EFFECT OF TRICLABENDAZOLE AND CLOFAZIMINE ON TOXOPLASMA GONDII GROWTH, A REPURPOSING APPROACH.

Agustina GANUZA(1) | Lucas ALBERCA(2) | Roque DIETRICH(2) | Luciana GAVERNET(2) | Alan TALEVI(1) | **Maria CORVI** (1)

INSTITUTO TECNOLÓGICO DE CHASCOMÚS (INTECH) (1); FACULTAD DE CIENCIAS EXACTAS, UNIVERSIDAD NACIONAL DE LA PLATA (2)

Toxoplasmosis is an infection caused by the parasite Toxoplasma gondii. Although healthy individuals present few symptoms, the disease could have a high impact in immunocompromised individuals and in congenital infection, leading to serious health problems. Although the combination of pyrimethamine with a sulfonamide is still very effective for treatment of toxoplasmosis, the use of these two drugs in immunocompromised individuals for

long periods of time frequently leads to adverse reactions. As such, there is a need for alternative therapeutic options. Recently, by application of in silico drug repurposing it was reported that cisapride (gastroprokinetic agent), cinnarizine (antihistamine used to treat travel sickness), clofazimine (antimycobacterial compound), triclabendazole (antihelminthic drug) and paroxetine (antidepressant) inhibit putrescine uptake in Trypanosoma cruzi. Given that T. gondii is auxotroph for polyamines, here we evaluated these compounds on T. gondii growth in vitro. All the tested compounds presented anti-toxoplasmic effect. The calculated IC50 for paroxetine, cinnarizine and cisapride were 2.42, 3.12 and 4.72 µM, respectively. However, triclabendazole and clofazimine presented a higher selectivity towards T. gondii inhibition growth: selectivity index of 15.67 and 10.3, respectively (IC50 0.61 µM for triclabendazole and 0.3 µM for clofazimine) without showing a cytotoxic effect on host-cells. Our results suggest that target and drug repurposing are valid approaches for the study of putative antiparasitic compounds, especially for neglected diseases.

0055 - IDENTIFICATION OF POLYAMINE TRANSPORT INHIBITORS: REPURPOSING ANTIPSYCHOTIC DRUGS FOR CHAGAS DISEASE

Chantal REIGADA | Edward VALERA VERA | Melisa MARTINEZ SAYE | Mariana MIRANDA | Claudio PEREIRA

INSTITUTO DE INVESTIGACIONES MEDICAS A LANARI (UBA - CONICET)

In Trypanosoma cruzi, the etiological agent of Chagas disease, the uptake of polyamines constitutes a promising target to design specific inhibitors with tripanocidal effects, since it is essential for parasite survival. In a previous study, Ant4, a 9-anthracenylmethyl-putrescine conjugate, designed for cancer treatment, inhibited the polyamine transport in T. cruzi parasites and also presented a strong trypanocidal effect on trypomastigotes, the bloodstream stage of T. cruzi. Considering the effects of Ant4 in the parasite, and that is not approved for use in humans, in this work we proposed to identify, using in silico and in vitro strategies, trypanocidal drugs approved for the treatment of other diseases that have similar structure and activity to Ant4. Initially, we performed a similarity ligand-based virtual screening in the SWEETLEAD database containing world's approved drugs and natural products, using Ant4 as reference molecule. Applying this strategy, four antipsychotic tricyclic drugs were identified to be used in experimental assays in T. cruzi parasites. Three of them; promazine, chlorpromazine and clomipramine, showed to be effective inhibitors of polyamine uptake in epimastigotes and trypomastigotes. The drugs also revealed a high trypanocidal activity against amastigotes (IC50 values of 3.8, 1.9 and 2.9 µM, respectively) and trypomastigotes (IC50 values of 3.4, 2.7 and 1.3 µM, respectively) while in epimastigotes the IC50 were significantly higher (34.7, 41.4 and 39.7 µM, respectively). Taking advantage of the intrinsic fluorescence signal of Ant4 and chlorpromazine, we demonstrated that both compounds are incorporated into the parasite, suggesting the existence of additional intracellular targets. In conclusion, these polyamine transport inhibitors are promising trypanocidal drugs, in addition they are approved for use in humans, which could reduce significantly the requirements for their possible applications in the treatment of Chagas disease.

0135 - BROADENING THE SPECTRUM OF IVERMECTIN: EVIDENCES OF ITS EFFECT ON EPIMASTIGOTES OF T. CRUZI

María Daniela RUIZ | Agustina CLAUSI | Luciana LAROCCA | Verónica DE PINO | Carolina CARRILLO | **Laura FRACCAROLI**

ICT MILSTEIN - CONICET

Chagas disease is an endemic parasitosis originally from Latin America, caused by the protozoan Trypanosoma cruzi (T. cruzi). The current therapies are limited in efficacy and show multiple side effects. Thus, there is a need to identify new effective and specific trypanocidal strategies. Ivermectin (IVN) is a broad-spectrum antiparasitic drug of human and veterinary use. It is used for both ecto- and endo-parasite treatments and presents low toxicity in humans. These factors, along with its relative low cost, make IVN an interesting drug candidate for Chagas disease treatment. In previous studies, IVN has shown an effect against T. brucei and Leishmania in animal infection models. Beginning our evaluation of IVN as a potential trypanocidal drug, the aim of this work was to analyze the effects of IVN on T. cruzi epimastigotes and other trypanosomatids proliferation and viability. To approach this aim, we performed growth curves of epimastigotes of the Y-GFP strain in the presence of IVN (0 - 200 µM). The cultures were evaluated both by cell counting in Neubauer chamber and optical density at 630 nm for 8 days. IVN dose dependently reduced the proliferation of the parasites. The relative density and the viability (assessed by MTT) significantly decreased while duplication time increased at day 4 of culture. The IC50 calculated at day 4 of culture was 12.53 µM (10.83 - 14.49 µM). In related trypanosomatids, preliminary results showed that IVN affected the proliferation of Phytomonas Jma, with an estimated IC50 of 5.5 µM, while it did not affect to Crithidias fasciculata. The results presented herein showed that IVN affects the proliferation and viability of T. cruzi epimastigotes suggesting that Ivermectin could be a potentially viable drug to study in the Chagas disease context.

0141 - EFFECTS OF MELIA AZEDARACH EXTRACT ON T. CRUZI EPIMASTIGOTES PROLIFERATION

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ICT MILSTEIN - CONICET

Chagas disease is an endemic parasitosis caused by the protozoan Trypanosoma cruzi (T. cruzi). The current therapies are limited in efficacy and show multiple side effects. Thus, there is a need to identify new effective and specific trypanocidal strategies. Melia azedarach (MA), native of Asia but widely distributed in several countries, known as "Paraíso", has been described to have therapeutic properties such as antifungal and antihelmintic. The aim of this work was to evaluate the effect of extracts obtained from ripe fruits from MA in the proliferation of T. cruzi epimastigotes. To approach this aim we performed MA extracts using water, ethanol and DMSO as solvents. We tested the extracts in cultures of epimastigotes from the Y-GFP strain in concentrations between 0 and 6 mg/ml. We observed that only DMSO extracts dose dependently decreased the proliferation of the parasites. The IC50 calculated at day 4 of culture was 0.94 mg/ml (0.81-1.09 mg/ml). To evaluate the stability, we stored the extract at 4 and -20 ºC during 15 days. Then, we calculated the IC50 of both in Y-GFP epimastigotes observing that the storage at -20 ºC maintained the activity while the extract at 4 ºC decreased its activity by half. We tested the citotoxicity of the DMSO extract in Vero cell line with MTT assay, calculating a selectivity index of 1.2. While it is not optimal, it is proximal to those obtained for Nifurtimox or Benznidazole. We performed an HPLC separation of the extract recollecting different fractions. Preliminary results showed that the individual fractions did not decrease epimastigotes proliferation, while the pool of those fractions restored the effect. The results present herein propose that the extracts obtained from ripe fruits of MA have bioactive compounds that affect the proliferation and viability of T. cruzi epimastigotes suggesting that the citotoxic activity may be the result of the interaction of different compounds present in the extract.

0216 - MACHINE LEARNING APPLICATION TO ASSESS TARGET DRUGGABILITY. TRYPANOTHIONE SYNTHETASE AS A STUDY CASE.

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Trypanothione synthetase (TryS) has been reported as a promising drug target in trypanosomatids [1]. Metabolic control analysis has confirmed its key role in the redox metabolism of the parasites [2; 3]. Whereas some years back potential drug targets were binarily classified into druggable/non-druggable, recent work suggests that proteins could display different degrees of druggability [4; 5]. Though TryS is generally considered druggable in the sense that a (relatively small) pool of compounds capable of inhibiting the enzyme have been reported, high-throughput screens and our own experience on the target suggest that it might be indeed druggable, but "difficult to drug". Here, we have resorted to a wide range of binding pocket prediction methods to explain the low levels of success in high-throughput and in silico screen for TryS inhibitors. Tools that evaluate druggability scores, such as PockDrug [6], DoGSiteScore [7] or CavityPlus [8] as well as tools to identify ligand-binding regions or to estimate binding pocket size (e.g. FTMaps [9]) were jointly applied. Ten proteins, including TryS and nine highly druggable ones (e.g. COX-2, cruzipain, falcipain-2 and others), were studied and compared. The analysis shows that TryS, independently of the method used, is predicted as a low druggable target in comparison to the other nine, validated, highly druggable ones, which tend to display comparatively large binding pockets and better druggability scores. Therefore, we propose that, in spite of its crucial biological role in trypanosomatids, TryS might be, in fact, a challenging target to develop new therapeutic options. References: [1] Comini, MA. et al. Free Rad Biol Med 36: 1289-1302 (2004). [2] Saavedra, E. et al. Curr. Med. Chem. 25: 1-25 (2018). [3] Gonzalez-Chavez, Z. et al. Redox Biology 26: 11231 (2019). [4] Barril, X. et al. Comput Mol Sci 3: 327-338 (2013). [5] Kana, O. et al. J Comput. Alded Mol Design 33: 509-519 (2019). [6] Hussein, HA. et al. Nucleic Ac. Research 43: 436-442 (2015). [7] Volkamer, A. et al. J. Chem. Inf. Model. 50: 2041-2052 (2012). [8] Xu,Y. et al. Nucleic Acids Research. 46: 374-379 (2018) [9] Kazokov, D. et al. Nature Protocols. 10: 733-755 (2015)

0305 - EXPLORING THE LINK BETWEEN ADENINE DNA METHYLATION AND 3D GENOME ORGANIZATION IN THE PARASITE TRICHOMONAS VAGINALIS

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Trichomonas vaginalis is a common sexually transmitted parasite that colonizes the human urogenital tract causing infections that range from asymptomatic to highly inflammatory. Chronic infections have been associated with high risk pregnancies, increased risk of acquiring HIV and higher susceptibility to developing cervical or prostate cancer. Despite their importance in other organisms, the epigenetic mechanisms involved in gene regulation in the parasite remain poorly understood. Recent works have highlighted the importance of histone modifications in the regulation of transcription and parasite pathogenesis. However, the nature of DNA methylation in the parasite remained unexplored. Using a combination of immunological techniques and UHPLC, we analyzed the abundance of DNA methylation in strains with differential pathogenicity demonstrating that N6-methyladenine (6mA), and not 5-methylcytosine (5mC), is the main DNA methylation mark in T. vaginalis. We performed an adapted methylated immunoprecipitation assay followed by high-throughput sequencing (MeDIP-seq) on a patient-derived strain to obtain genome-wide distribution of 6mA mark. Our results revealed that this mark is enriched at intergenic regions, with a preference for certain superfamilies of DNA transposable elements. We show that 6mA in T. vaginalis is associated with silencing when present on genes. Interestingly, bioinformatics analysis revealed the presence of transcriptionally active or repressive intervals flanked by 6mA-enriched regions and results from chromatin conformation capture (3C) experiments suggest these 6mA flanked regions are in close spatial proximity. These associations were disrupted when parasites were treated with the demethylation activator ascorbic acid. This finding revealed a new role for 6mA in modulating 3D chromatin structure and gene expression in this deep-branching eukaryote.

0371 - IN SILICO-GUIDED DRUG REPURPOSING: IDENTIFICATION OF NON-COMPETITIVE INHIBITORS OF TRYPANOSOMA CRUZI AND PLASMODIUM FALCIPARUM CYSTEINE PROTEASES

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Trypanosoma cruzi and Plasmodium falciparum are the etiologic agents of Chagas disease and Malaria, respectively. Cysteine proteases play key roles in the pathogenesis and survival of these parasites, such as cell/tissue penetration, hydrolysis of host or parasite proteins, autophagy, and evasion or modulation of the host immune response, being considered attractive chemotherapeutic targets. Cruzipain (Cz) and Falcipain-2 (FP-2) are two essential cysteine proteases of such organisms. Previously, we have found that methacycline (a member of tetracycline family) is a non-competitive inhibitor of FP-2 (Alberca et al. 2019). In this study our objective has been the characterization of six tetracycline analogues (tetracycline, minocycline, doxycycline, oxytetracycline, chlortetracycline and methacycline) as inhibitors of these cysteine proteases by in silico and in vitro determinations. First, we used bioinformatic tools to predict possible allosteric binding pockets; subsequently, we studied their possible interactions with these proteases by molecular docking simulations. The structures of the enzymes were obtained from the Protein data bank. Finally, we proceed to inhibition studies on the purified enzymes, which confirmed that these family of antibiotics inhibit cysteine proteases in a reversible, non-competitive manner, with Ki values in the mid-micromolar order. Our results provide further evidence on the utility of computational tools as a rational basis for systematic drug repurposing.

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0453 - EVALUATION OF A POTENTIAL ALTERNATIVE TO THE TREATMENT OF HUMAN NEUROCYSTICERCOSIS: ALBENDAZOLE-LOADED LIPID NANOCAPSULES ENHANCE THE BIOAVAILABILITY OF ALBENDAZOLE IN THE BRAIN OF HEALTHY MICE

Julia FABBRI (1) | Juan Pablo ESPINOSA(2) | Patricia Eugenia PENSEL(1) | Clara María ALBANI(1) | Sandra Karina MEDICI(2) | Gabriela ULLIO GAMBOA(3) | Jean Pierre BENOIT(4) | María Celina ELISSONDO(1)

LABORATORIO DE ZOONOSIS PARASITARIAS, IIPROSAM, FCEYN, UNMDP - CONICET (1); FARES TAIE INSTITUTO DE ANÁLISIS. (2); UNITEFA-CONICET. FCQ, UNC. (3); INSERM U1066, MINT–MICRO ET NANOMÉDECINES BIOMIMÉTIQUES, IBS-CHU ANGERS (4)

Neurocysticercosis (NCC) is a zoonotic disease caused by encystment of Taenia solium larvae in the central nervous system. Pharmacological treatment is performed with albendazole (ABZ). However, the slow rate of dissolution of ABZ produces a poor and erratic absorption of the drug at the gastrointestinal level. This produces wide variability in plasma and cerebrospinal fluid concentrations of the drug among patients, with variations in treatment efficacy. Lipid nanocapsules (LNCs) are drug carrier nanosystems designed to solubilize lipophilic drugs. The aim of the

present work was to characterize and compare the brain pharmacokinetic behavior of a suspension of ABZ (ABZ-SUSP) or ABZ-LNCs in healthy mice. Animal procedures and management protocols were approved by the Institutional Animal Care and Use Committee (RD 148/15) of the FCEyN, UNMdP. CF-1 mice were separated into two groups (n= 44): ABZ-SUSP and ABZ-LNCs. In both cases, a single dose of 5 mg/kg of ABZ was administered orally. The brains were recovered at different post-treatment times until 16 h. The samples were analyzed by high performance liquid chromatography to quantify ABZ and its metabolites. Enhanced ABZ sulfoxide concentration profile was obtained in brains from ABZ-LNCs treated animals. Higher metabolite brain exposure was obtained after administration of ABZ-LNCs formulation to mice (area under the concentration versus time curve (AUC)= 0.82 ± 0.17 mg.h/kg and peak concentration (Cmax)= 0.14 ± 0.03 mg/kg) compared to the ABZ-SUSP (AUC= 0.29 ± 0.05 mg.h/kg and Cmax= 0.072 ± 0.01 mg/kg) ($p<0.05$). The improvement of the brain availability of ABZ observed after the administration of ABZ-LNCs to healthy mice worth to be evaluated on an animal model of NCC. This new nanocarrier as drug delivery system for ABZ could be a suitable alternative for treating NCC in humans.

0472 - ADVANCES IN THE STUDY OF ANTHRACYCLINES EFFECT ON TRYPANOSOMA CRUZI

María Daniela RUIZ(1) | **Laura FRACCAROLI** (1) | Darío BALCAZAR(1) | Cristina VANRELL(2) | Luciana LAROCCA(1) | Patricia ROMANO(2) | Carolina CARRILLO(1)

ICT MILSTEIN - CONICET (1); IHEM-UNCUYO (2)

Trypanosoma cruzi (T. cruzi) is the etiological agent of Chagas disease. As current therapies are limited in efficacy, there is a need to identify new specific trypanocidal compounds. Our previous work showed that anthracyclines (antitumor agents) decreased survival and proliferation of T. cruzi epimastigotes and interfered with its putrescine uptake. The aim of this work was to deepen the study about anthracyclines effect on polyamine metabolism in epimastigotes and to analyze their effect on different T. cruzi life cycle stages. Daunorubicin (Dnr) and Doxorubicin (Dxr) were the anthracyclines selected to evaluate their effect on T. cruzi (strain Y-GFP). We performed growth curves, and measured intracellular content of polyamines, by HPLC analysis, on epimastigotes cultured in putrescine depleted medium for 1 to 15 days. Under these conditions, intracellular putrescine diminished and T. cruzi epimastigotes became significatively more sensitive to Dnr and Dxr (IC50 of 0.1 µM for Dnr and 2 µM for Dxr), not depending on the nutritional stress length. On the other hand, although Dxr and Dnr interfere with polyamine uptake, sub-IC50 doses of these did not change the intracellular concentration of putrescine during 15 days of culture. Anthracyclines effect was tested in in vitro metacyclogenesis, infectivity and amastigotes proliferation assays. Differentiation from epimastigotes to metacyclic trypomastigotes diminished by Dnr treatment (from 14.8 % in control condition to 8.9 % with Dnr). Dnr did not affect the number of infected H9C2 cells nor total number of these cells but reduced by half the number of amastigotes per cell. The findings presented herein showed that Dnr and Dxr affect T. cruzi epimastigotes survival and proliferation, metacyclogenesis and replicative capacity of amastigotes. This effect could be related to the decrease of polyamine uptake by anthracyclines and their intracellular toxic effects.

0478 - IVERMECTIN IMPROVES THE EFFECT OF ETIDRONATE AS ANTI-HELMINTHIC AGENT

María Laura GERSTISER (1) | Alejandra JUÁREZ VALDEZ(2) | Oscar JENSEN(3) | Emilio Aj ROLDÁN(2) | Alicia Graciela FUCHS(2)

CENTRO DE ZOONOSIS/ BECA "ABRAAM SONIS" (1); UNIVERSIDAD ABIERTA INTERAMERICANA (2); CENTRO DE ZOONOSIS (3)

MLG and AJV equally contributed. In the world, ~$2x10^{12}$ people are infected by helminths and 61% of the human`s diseases are zoonotic. Hydatidosis produced by Echinococcus granulosus (Eg) has a complex life cycle, the dog is the definitive host releases fertile proglottids (pg) in the environment and intermediate infected host's developed the hydatid cyst. Prevention is performed deworming the dog, but alive eggs are released with feces. Bisphosphonates (BP) have an antiproliferative effect on EGPE, a cell line from Eg protoscoleces G1 (Echeverria et al., 2010; Fuchs et al., 2014 and Ferrulli et al., 2019) and they decreased viability on Taenia hydatigena (Th) eggs treated ex vivo. In this study was searched the effect of etidronate (EHDP) in combination with ivermectin (I) on EGPE colonies and on Th eggs incubated ex vivo with feces. Microscopic examination of treated and untreated samples was performed in parallel. EGPE cell colonies were treated with 30 µM EHDP (GADOR, SA) and/or 20 µM I (SIGMA) and controls with excipient (E), during 5 days, 30 microscopic fields of each were analyzed. Gravida's pg, obtained after dog diagnostic deworming, were incubated with feces and treated with 0.6 mL KCl containing 1.5 % HEDP and/or 0.48 % I during 3 days, control was E, 450 microscopic fields of each were analyzed. Results were evaluated by ANOVA and "Chi-square". EGPE colonies treated with I or EHDP decreased ($p<0.05$) the number, size and cellular densities compared with E. I+EHDP; decreased ($p<0.05$) the number and size of colonies compared with drugs alone and 38.7 % of colonies were empty. On Th pg EHDP decreased the number of mature eggs and increased the percentage of anembryonic eggs, compared with E ($p<0.05$). EHD+I decreased of eggs in formation and mature, compared with those treated with HEDP alone ($p<0.05$). EHDP+I has a synergic effect decreasing cell proliferation or increasing death in vitro and ex vivo and it could be a promising way to decreased eggs fertility.

0479 - EFFECTS OF BENZNIDAZOLE MICROPARTICLES ON THE MURINE EXPERIMENTAL INFECTION BY TRYPANOSOMA CRUZI.

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Benznidazole (BNZ) is one of the drugs of choice for Chagas disease treatment, although it has several drawbacks including the high doses required and the adverse effects that it usually produces in patients. In search of new formulations, with greater safety and efficacy in infection control, recently in our laboratory, we have shown that BNZ nanocrystals were able to reduce cellular infection by T. cruzi in vitro (Scalise et al., 2016) and in mice infected with an isolated T. cruzi Nicaragua (TcN) in acute and chronic stages of the infection (Rial et al., 2017). In this work we evaluated the toxicity, the possible hemolytic effect and the efficacy of new polymeric microparticle formulations (RS, RL and RL/RS) in mice with acute TcN infection. The toxicity of the microparticles was evaluated in vitro in VERO cells by the MTT method and the possible hemolytic effect on human blood. C3H/HeN mice were infected with 1,000 TcN and treated in the acute phase, with 30 daily doses of 50 mg/kg/day of BNZ microparticles. The effectiveness of the treatments was evaluated by observing daily survival, measuring the parasitemia by qPCR, the serology by ELISA and the histopathology of the hearts by staining with hematoxylin and eosin. BNZ microparticles were not toxic to mammalian cells and did not cause erythrocyte lysis. All TcN infected treated mice showed no changes in their general condition or behavior and survived until euthanasia (92 dpi), while only 15 % of untreated infected mice survived. The parasitic load in peripheral blood was undetectable; IgG levels significantly decreased, and were negative

in 71 % RL, 86 % in RS and 67 % in RL/RS treated mice. The cardiac tissue of untreated animals had multiple and extensive inflammatory foci that were reversed with drug treatments. Therapy with BNZ microparticles could be useful to efficiently treat the murine T. cruzi infection, decreasing the amount of BNZ administered to provide a promising clinical treatment.

0486 - POLY(ADP-RIBOSE)GLYCOHYDROLASE ACTIVITY IS IMPORTANT FOR LYSOSOME FORMATION DURING T. CRUZI INVASION

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Chagas´ disease stands as one of the main public health problems in Latin America. Trypanosoma cruzi, protozoan parasite responsible for this disease, has a complex life cycle comprising several stages that allow it to multiply and disseminate. During the cell invasion step, the parasite modulates several metabolic pathways in the host cell; therefore, drugs that operate on these pathways could be used with therapeutic aims. Signaling though PI3k/Akt elicited in the host cell during T. cruzi infection. Activation of this pathway is important since it has anti-apoptotic effects that operate in favor of the infection and is also crucial for the regulation of the lysosome dependent and independent invasion mechanisms. Recently, it has been reported that Poly(ADP-ribose)Glycohydrolase (PARG) participates in the regulation of the PI3k/Akt route by downregulating Akt phosphorylation in cancer cells. In our infection model, PARG inhibition by DEA 1 µM or silencing by iRNA (shPARG) in Vero cells, the % of phosphorylated Akt is not altered when compared to wild type infected cells, but the upregulation of Akt levels on wild type (35 % at 15 min and 80 % at 6 h PI) cells could not be observed in cells where PARG activity is absent. Lysosome formation in response to parasitic infection is also altered when Vero cell PARG is inhibited or silenced: at 1 h PI, LAMP-1 (lysosome marker) signaling diminishes in DEA 1 µM-treated or shPARG cells in comparison to wild type cells. The reduction in lysosome density can also been observed in the absence of infection, indicating that lysosome formation regulation by PARG might be operating also in physiological conditions. Previous results obtained by our group showed that PARG inhibition led to a marked decrease in T. cruzi infection in vitro. These new findings could indicate that the downregulation of the lysosome invasion pathway could partially account for the reduction in T. cruzi infection when PARG activity is absent.

0490 - COMPUTATIONAL REPOSITIONING OF BIOACTIVE COMPOUNDS FROM LARGE CHEMOGENOMIC SCREENS: IDENTIFICATION OF CONSERVED DRUGGABLE MODULES BETWEEN YEASTS AND TRYPANOSOMES

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Detailed characterization of the cellular response to chemicals is fundamental to understand the mechanism of action of drugs. One strategy to do this is to analyze the growth capacity (fitness) of gene mutants exposed to different drugs. Recently, a number of genome-wide fitness profiling assays were performed on Saccharomyces cerevisiae. These chemical-genomics screens were based on whole-genome collections of heterozygous and homozygous deletions and quantified the growth fitness of each strain in the presence of different chemicals. Now, several such chemogenomic datasets are available, providing a rich source of pharmacogenomic associations between drugs and genes ("druggable modules"). In contrast, in trypanosomes pharmacogenomic associations are scarce, hence these yeast chemogenomic screens may serve as good starting points to guide repurposing opportunities. The aim of this project is the curation and standardization of yeast-based chemogenomic assays from published studies, and the development of an orthology mapping pipeline. Using this pipeline to find conserved druggable modules between yeasts and T. cruzi, we obtained 93,758 gene-drug interactions, with a set of 3,005 unique genes and 2,430 unique drugs. Further filters were applied to each set. For drugs, filters were applied to retain compounds that are drug-like, novel, commercially available, and with low potential promiscuity; with a final iteration to maximize chemical diversity within the set. For genes, we selected those that have T. brucei orthologs with significant fitness phenotypes when knocked down (through an orthology mapping between T. cruzi genes and T. brucei whole-genome RNAi essentiality assays described in Alsford et al., 2011). After standardization and filtering we obtained a library of 50 compounds, associated with 78 candidate protein targets in T. cruzi.

References: Alsford S (2011) Genome Research 21: 915-24. DOI:10.1101/gr.115089.110

0492 - TDR TARGETS: DRIVING DRUG DISCOVERY FOR HUMAN PATHOGENS THROUGH INTENSIVE CHEMOGENOMIC DATA INTEGRATION

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The volume of biological, chemical and functional data deposited in the public domain is growing rapidly, thanks to highly-automated sequencing and screening technologies. However, there is still a large data imbalance between well-funded model organisms and pathogens causing neglected diseases. We developed a chemogenomics resource, (TDR Targets, tdrtargets.org), that aims to organize and integrate heterogeneous large datasets with a focus on drug discovery for human pathogens. The database also hosts chemical and genomic data from other organisms to leverage data for comparative and inference-based queries. One of the major impacts of TDR Targets is to facilitate target and chemical prioritizations by allowing users to formulate complex queries across diverse data spaces. In this communication we will highlight new data and functionality updates in TDR Targets. In this release, the database has been updated to integrate data on >2 million bioactive compounds; 20 pathogen genomes; and 30 complete genomes from model organisms and other related pathogens. Furthermore, the data was also used to populate a recently developed network model (Berenstein et al., 2016) to produce i) a novel druggability metric for targets based on the connectivity in the network to bioactive compounds, ii) guide new prioritization strategies for both targets and compounds, and iii) visually aid in the navigation across target/compound spaces in the web interface. This network model connects protein (target) nodes to compounds, based on curated bioactivity annotations. It also connects proteins to other proteins based on shared annotations, and compounds to other compounds based on chemical similarity and substructure metrics. This chemogenomic network facilitates a number of inferences, such as inferring plausible targets for orphan drugs or candidate compounds for orphan targets.

References: Berenstein AJ et al (2016) PLOS Negl Trop Dis 10: e0004300. DOI: 10.1371/journal.pntd.0004300

0538 - ANTI-TRYPANOSOMA ACTIVITY OF HYBRIDS OF BILE ACIDS AND NATURAL ALKALOIDS

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Chagas disease, caused by Trypanosoma cruzi, is a major public health problem in Latin America. According to the World Health Organization, around 20 million people are infected and another 40 million are at risk of acquiring the disease. The current treatment for the Chagas disease continues to be one of the main reasons why this disease is not yet eradicated. First choice drugs, Benznidazole and Nifurtimox, have a bad performance, including no pediatric formulation and high toxicity, especially in the chronical stage of the disease. For these reasons, it is necessary to find alternative compounds to treat the disease. To this end, we have produced a series of hybrids of Cinchona alkaloids and bile acids, expecting to combine their anti-parasitic activity and the known properties as drug transporters, respectively. These chimeric compounds were synthetized by a Barton-Zard decarboxylation reaction and have shown promising activity against T. brucei, L. mexicana mexicana and P. falciparum. Moreover, we have recently demonstrated that these compounds have antiparasitic activity against different Trypanosoma cruzi DTUs. In this work, new hybrids, with modifications in the bile acid fraction, have been tested for cytotoxicity assayed on HeLa cells. Half maximal inhibitory concentration (IC50) against these cells were estimated ranging between [1.0 – 3.0 ug/ml]. Besides, we tested the compounds against two T. cruzi strains (DTUs II and VI) tripomastigotes forms and the IC50 were estimated between [0.15 – 0.6 ug/ml]. Compounds activity against amastigote forms was less effective than the observed against tripomastigote forms. Selectivity of the hybrids were calculated as IC50HeLa/IC50tripomastigote form and some compound showed values of 10 or higher. This study opens the door to new possibilities in the screening of alternative drugs used traditionally in the Chagas disease's treatment.

0564 - METABOLOMIC ANALYSIS OF TRYPANOSOMA CRUZI PARASITE BY ^{1}H-NMR SUBJECTED TO VARIATIONS IN HEME AVAILABILITY: EXPERIMENTAL SETUP

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Trypanosoma cruzi, the causative agent of Chagas disease, displays auxotrophy for heme cofactor being essential to acquire it from the hosts. According to our results, the parasite takes heme in the replicative stages, where TcHTE protein plays an essential role. It is well accepted that heme is imported to supply the cofactor for several hemeproteins, like mitochondrial cytochromes, however there is no evidence about which metabolic routes are modulated by heme. We designed and performed a non-targeted metabolomic assay as the key approach to answer that fundamental question. We described the experimental setup and preliminary results of the first metabolomics study by ^{1}H-NMR spectroscopy reported in T. cruzi. Specimen preparation is a crucial step for metabolomics since rapid changes in metabolite levels may occur in response to external perturbations. Several protocols evaluating metabolic quenching, cell disruption and number of parasites were assayed to establish the most accurate method for sample preparation. Briefly, T. cruzi DM28c parasites growing in LIT-10% FBS with 5 µM hemin were collected and resuspended in fresh medium containing 0uM, 5uM or 20uM hemin for 48h. Then, 50 millons cells were pelleted and washed twice with ice cold NaCl 0.9 % solution in order to quench cellular metabolism. Intracellular metabolites were extracted with chilled 50 % acetonitrile aqueous solution. Samples were then subjected to freezing-unfreezing cycles and sonication in a water bath. Acetonitrile was dried under N_2 flow and samples were lyophilized. Nine replicates of each condition were submitted for non-targeted metabolomics analysis by ^{1}H-NMR. A multivariate study was performed using principal component analysis revealing metabolic differences among samples subjected to diverse hemin supplementation in the culture medium. Supervised methods will allowed us to establish the identity of the metabolites whose content was affected by variation in heme concentration.

0573 - ANTI-TRYPANOSOMA CRUZI ACTIVITIES OF N,N´-SUBSTITUTED DIAMINES

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Chagas disease is transmitted by the Trypanosoma cruzi kinetoplastid parasite, which possess a complex life cycle alternating between an invertebrate and a vertebrate hosts. Looking to identify new chemical entities, N,N'-substituted diamines have been proposed as an interesting scaffold for antiparasite drug development. Previously, we have prepared a collection of derivatives with that scaffold. The incorporation of 4-benzyloxy group onto the benzyl group resulted in a noticeable enhancement of the growth inhibitory activity on CL Brener T. cruzi epimastigotes. The compounds showed negligible cytotoxicity towards Vero cells. In this work we compared the activity on Dm28c strain with the seven most active analogs previously reported. The best candidate was N1,N6-bis(4-(benzyloxy)benzyl)hexane-1,6-diamine (AR-Tc-3) with an IC50 of 0.99 µM. Looking to elucidate the action mechanism, metabolomic study was performed tracing secreted metabolites variations on parasites under 1 µM AR-Tc-3 treatment. Parasites ($0.5x10^6$ epimastigotes/mL) were incubated over 96 h in DMEM-high glucose supplemented with 5 µM hemin and 2 % fetal bovine serum (FBS) with or without the compound. The conditioned media were separated from epimastigotes by centrifugation and the supernatants were filtered through 0.22 µm acetate membranes. These samples were analysed by 1H NMR adding deutered DMSO as internal standard and deutered water to lock the sample. The metabolites were unequivocally identified by spiking the culture medium with the pure metabolites to properly determine its chemical shift in these experimental conditions. We observed a three-time fold decrease in the amount of acetate and alanine released to the medium by AR-Tc-3 treated epimastigotes. No changes in ethanol and lactate excretions were observed. Conclusion: N.N'-substituted diamines, are promising candidates to develop new anti-T. cruzi chemotherapies and AR-Tc3 affects the energy metabolism.

0582 - SYNTHESIS, CHARACTERIZATION AND IN VITRO EVALUATION AGAINST TRYPANOSOMA CRUZI EPIMASTIGOTES OF A NEW THYMOL DERIVATIVE

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Neglected Infectious Diseases (NIDs) are a group of infectious diseases, many of them parasitic, which mainly afflict the poorest people and with limited access to health services. NIDs have received limited attention despite their magnitude and impact on global public health. Chagas disease is a NID and is the major health problem in Latin America, affecting nearly 6 million people. The current treatment for this disease has numerous disadvantages:

low therapeutic index, high cost, prolonged therapeutic schemes, side effects (such as skin rashes, nausea, and gastrointestinal disorders), teratogenicity and drug resistance. This is why the search for new drugs that are more effective and better tolerated by patients is essential. The aim of the present study was to evaluate the synthesis, characterization and in vitro biological performance of a new chemical entity derived from the monoterpenic compound thymol against Trypanosoma cruzi. The derivative was obtained from the conjugation of the free hydroxyl group of thymol with the aliphatic alcohol n-pentanol. The synthesis of the derivative was carried out in two consecutive stages, the first involves the reaction of thymol with N,N-carbonyldiimidazol under nitrogen atmosphere in dichloromethane and the second, the reaction of the intermediary formed with the n-pentanol alcohol. Once the reaction is finished, successive extractions are made in distilled water. The reaction was monitored by thin layer chromatography (TLC) with a mobile phase Hexane: Ethyl Acetate, 6:4. The evaluation of cytotoxicity was carried out by means of the MTT colorimetric test on line U-937 (ATCC CRL-1593.2) with four serial dilutions of the derivative (50-12.5-3.125-0.78 µg/mL). The evaluation of the antitripanosomic activity in vitro was made by the colorimetric method with human macrophages U-937 infected with epimastigotes of T. cruzi strain of Tulahuen. The derivative was added in a series of concentrations (50-12.5-3.125-0.78 µg/mL). The synthesis was simple, economical and with good yields. Thymol and the new derivative were unequivocally identified by nuclear magnetic resonance spectroscopy (1H-RMN, 13C-RMN, COSY, HSQC and HMBC) and infrared and both showed moderate activity against T. cruzi. The new derivative showed lower cytotoxity than the starting compound. The structural modification allowed to improve its efficiency on the model used, presenting a higher selectivity index than the starting compound thymol.

0602 - PROTECTIVE EFFECT OF ENTEROCOCCUS FAECALIS CECT7121 AGAINST TRICHINELLA SPIRALIS INFECTION IN MICE.

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Trichinellosis is an important parasitic zoonosis produced by the ingestion of raw or undercooked meat infected by Trichinella spiralis larvae. At the present, the pharmacological treatment against Trichinellosis in human is unsuccessful. Therefore, the potential of viable Enterococcus faecalis CECT7121 culture, administered by oral route, in mice against T. spiralis infection was evaluated. Eighteen BALB/C mice were divided in two groups (n= 9) as follow: A- Experimental Group: E. faecalis CECT7121 strains were administered daily in a dose of 10^9 UFC/mL in 300 µL for five consecutive days. B-Negative control Group: animals received 300 µL of sterile sodium chloride. On day 5 of treatment, whole mice were infected with 450 T. spiralis larvae. Four mice, from both experimental and control group, were sacrificed on day 6 post infection for assessing the amount of T. spiralis adults from the small intestine. At day 28 post infection the remaining mice from each group were sacrificed and the tongue processed in order to estimate the muscle larval burden in that tissue, used as surrogate marker of the number of larvae per g (LPG) of tissue. The test used for groups statistical comparison was Mann- Whitney. A difference of $p<0.05$ was considered significant. The average number of recovered T. spiralis adult worms' from the intestinal content was 105 ± 46 for the treated group and 80 ±42 in control group, resulting in non- significant 24 % of reduction ($p>0.05$). However, the percentage of LPG reductions obtained in tongue tissue after 28 days post- infection increased 62 % ($p <0.01$) in mice treated with E. faecalis CECT7121 as preventive. The quantification of T. spiralis adults in the intestinal content alone was not indicative of presence or absence of protective response when compared with samples obtained from mice tongue tissue. The protective response of E. faecalis CECT7121 against T. spiralis infection will be a contribution to improve the conventional therapeutic against Trichinellosis.

0604 - HIT OPTIMIZATION OF A TRYPANOSOMA CRUZI BROMODOMAIN INHIBITOR IDENTIFIED USING COMBINATORIAL CHEMISTRY

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Recently, our group has used Dynamic Combinatorial Chemistry targeting the Trypanosoma cruzi Bromodomain factor 3 (BDF3), as a strategy for the identification of a parasite inhibitor, this hit is an acylhydrazone with a Kd of 1.7 µM and and IC50 for epimastigotes, amastigotes and trypomastigotes between 13 and 23 µM. TcBDF3 interacts with acetylated alpha-tubulin present in the cytoskeleton and flagella of T. cruzi and is essential for the viability. TcBDF3 is an interesting target for the development of new trypanocidal drugs that disrupt the bromodomain-acetylated ligand interaction during the parasite differentiation. There are six other proteins with bromodomain in T. cruzi, among which TcBDF2 has also been shown to be essential for the parasite. Today it is a challenge to find selective inhibitors that can distinguish between the different bromodomains, and are more effective and less toxic than the trypanocidal drugs currently use. We prepare a small library of acylhydrazones synthesized from an acylhydrazide nucleus and various aldehydes selected according to the hit previously described by our group for TcBDF3, with the goal of finding more potent and selective inhibitors against TcBDF2 and TcBDF3. The interaction of each hydrazone with TcBDF2 and TcBDF3 from T. cruzi was determined by microplate protein fluorescence quenching assays and Thermal Shift. The results obtained so far allow us to conclude that i) all synthesized hydrazones interact with the hydrophobic pocket of TcBDF3, ii) none of them interact with TcBDF2 up to the highest concentration tested (20 µM), iii) two of the synthesized hydrazones are attractive due to its affinity to TcBDF3 and iv) only one of these hydrazones inhibits the development of epimastigotes of T. cruzi (IC50 <10 µM).

0608 - EXPLORING CLINICAL SEROLOGICAL CORRELATIONS AT LARGE SCALE: CHANGES IN THE ANTIBODY REPERTOIRE OF PATIENTS WITH CHAGAS DISEASE CARDIOMYOPATHY

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During an infection, the immune system produces antibodies against pathogens. With time, the immune repertoires of infected individuals become specific to their clinical history and thus represent a rich source of diagnostic markers. How these serological markers correlate with pathology is an open question. Chagas Disease (CD) is caused by the protozoan parasite Trypanosoma cruzi and develops into Chagas Disease Cardiomyopathy (CCD) in ~30 % of cases. CCD can be mild (stage B1) or evolve to cause arrhythmias and bundle branch blocks (stage B2) progressing to dilation (stages C, D) which can result in further symptoms and complications leading to mortality. In this work we investigated serological correlations in 17 individuals at different stages of CCD. Using high-density arrays displaying 2.8 million peptides from the complete proteomes of two T. cruzi strains (CL-Brener + Sylvio X10) we examined antibody repertoires using pooled samples from three groups of CCD patients: stage B1, stage B2, and stages C+D. These pools, were used to map reactive

antigens in these patients and search for epitope markers that could correlate with disease progression. Next, using a subset of ~400,000 peptides, we assayed serum samples from a 42 year old patient that showed progression of CCD. At age 39 the heart ejection fraction (EF) was 52 % accompanied by electrocardiogram with sinus rhythm, right bundle block and left anterior hemiblock (Sample1). One year later EF worsened to 39 % accompanied by complex ventricular arrhythmia (Sample2), maintaining similar values until today. Analysis of these two serum samples showed ~320 T. cruzi antigens with significant serological changes between both stages (threshold= 50 % signal change). Similar fractions of antigens increased or decreased measured antibody levels. To our knowledge, this is the first and largest collection of Chagas Disease antigens and epitopes correlated with pathology, providing a rich source of serological biomarkers.

0664 - CHAGAS DISEASE: PHARMACEUTICAL NANOVEHICLES ENCAPSULATING BENZNIDAZOLE.

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Chagas disease is an endemic parasitosis in Latin America whose etiologic agent is Trypanosoma cruzi. Benznidazole, the first-line therapy, eliminates trypanosomes in the circulatory system but seemingly does not fully eliminate parasites in tissue reservoirs, presenting a low cure rate in the chronic stage of the disease. An interesting approach to be considered is antichagasic drug delivery in novel nanotechnological vehicles. In other pathologies, nano systems are known to modify drug pharmacokinetics, improve delivery to target cells, increase drug concentration in tissues and protect circulating drug from elimination mechanisms, enhancing the drug safety profile and promoting adherence to the treatment. Our project aimed to obtain and evaluate pharmaceutical nanovehicles encapsulating benznidazole, taking advantage of the previously mentioned advantages. Here, nanoparticles were synthesized by the homogenization/ultrasonication method using two distinct matrixes: one lipidic (fatty esters) and one polymeric (ethyl acrylate, methyl methacrylate). This were evaluated in terms of drug loading and other physicochemical characteristics, with interest on developing future hybrid particles. Both types of nanoparticles presented spherical shape and encapsulation efficiencies of approximately 60 %. Mean diameter size was measured by DLS and TEM. Polymeric and lipidic particles sizes were 156.5 and 153.8 nm, respectively. Polydispersity index was around 0.2 which indicates an adequate size distribution to perform biological assays. In vitro drug release assays showed controlled release profiles in different dissolution mediums (phosphate buffer, hydroalcoholic solution and lauryl sulfate solution), with and average release of around 80 % of the drug load at 24 hours. As the next step in our research, thermal and spectroscopical methods will be included, as well as cell permeability/toxicity assays, and evaluation of trypanocidal effects in cell models.

0774 - EFFECT OF PUTATIVE PARG INHIBITORS IDENTIFIED BY AN IN SILICO APPROACH ON TRYPANOSOMA CRUZI INFECTION

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Poly-ADP-ribose polymer signaling is common to various nuclear processes related to DNA metabolism. As a reversible modification, it is regulated by a delicate balance of synthesis and degradation, being poly(ADP-ribose)glycohydrolase (PARG) the major hydrolase. We have demonstrated in mammalian cells that PARG inhibition or silencing is essential for the successful infection by the T. cruzi, making them an interesting target for the search of new treatments for Chagas disease. T. cruzi PARG inhibition also causes a delay in cell cycle. We have compiled a database of molecules tested against human PARG from which, we have inferred 1,000 ligand-based classificatory models capable of recognizing hPARG inhibitors using a semicorrelation approach and a random subspace approximation. We have generated a ligand-based model ensemble capable of recognizing human PARG inhibitors, with excellent behavior in the in silico validation step. The ensemble was applied in VS campaign, finding 26 drugs that could potentially behave as PARG inhibitors. Six drugs were chosen based on their solubility and availability and were tested both on infected cells and T. cruzi epimastigote. We analyzed the infection in Vero cells using trypomastigotes expressing ß-gal at 96 h PI. At 50 µM, Bromhexine and Rosuvastatine caused a marked reduction on the infection (96 and 55 % compared to DMSO infection), while Sulfazalasine and Doxycycline led to a mild reduction in the infection (20 % for both drugs). At the indicated concentration, these drugs did not affect host cell growth significantly. When tested on epimastigotes, only Doxycycline demonstrated to affect viability at concentrations ranging from µM. These results indicate that while Bromhexine, Rosuvastatine and Sulfazalasine might modulate the infection by affecting the host cell PARG, Doxycycline could possibly be affecting both the parasite and the host cell enzyme, although effects on other molecular targets can not be disregarded.

0780 - SYNTHETIC TETRAHYDRO-ß-CARBOLINES DERIVATIVES IN THE MURINE MODEL OF CHAGAS DISEASE

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Tetrahydro-ß-carbolines (ßC) have shown a variety of pharmacological activities including anti-trypanosomatids effects. We studied in vitro anti-T. cruzi activity of 12 ßC derivatives, selecting 4 of them by their selectivity indexes. The aim of this work was to evaluate their in vivo effect on the murine model. BALB/c mice were infected with T. cruzi RA or K98. ßC 253, 268, 269 or 274 (1 mg/kg/day) were administrated by ip route when parasitemia became detectable 10 days post infection. Controls were treated with Benznidazole (Bz) or vehicle (C). Parasitemia, clinical condition and survival were evaluated for 30 days in RA-groups and for 60 days in K98-groups. At the end of the experimental period, K98-groups were submitted to histopathological analysis and the myopathy-linked enzyme marker creatine kinase (CK) was also evaluated. ßC 253 and 269 provoked a 58.5 and 45.6 % reduction of circulating parasites respectively at the peak of parasitemia. As well, 253 elicited an increase in survival ($p<0.05$ vs. C). Surprisingly, although 274 was not effective controlling parasitemia, a significant decrease in skeletal and heart muscle infiltration (vs. C) was observed with an improved survival. Mice treated with 253 and 268 showed significant lower tissue infiltration than C and Bz. All treated mice presented lower seric CK activity compared to C ($p<0.05$) in coincidence with histopathologic findings. The nature of the various substituent groups could influence the biological activity of the compounds. ßC 268, has a strong electron donor group, while 269 and 274, are substituted with strong donor and weak attractor electron groups. ßC 253, which has only a weak electron attractor group, was able to reduce parasitemia, increase survival and promote lower tissue injury. Our studies therefore provide a good starting point since ßC 253 could be considered a promising lead compound for the development of new therapies for Chagas disease.

0806 - PROFILING THE HDAC INHIBITORS ACTIVITY AGAINST THE MODEL OF CESTODE PARASITES MESOCESTOIDES CORTI

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Echinococcosis and cysticercosis are neglected diseases (1) caused by the cestode parasites Echinococcus ssp. and Taenia solium, respectively. These diseases affect socioeconomically disadvantaged population and represent a significant problem in human and animal health. There is a scarce availability of compounds approved for chemotherapy. Thus, it is imperative to identify new drugs. In this work, we investigated histone deacetylase (HDAC) enzymes as potential drug targets to develop new therapies against neglected diseases caused by cestodes. We previously reported the presence and expression of HDACs in several cestode parasites. Furthermore, we showed that Trichostatin A (TSA), a pan-HDAC inhibitor, produces a decrease in parasite viability, alterations on the tegument and morphology and an increment of the total amount of acetylated proteins, including acetylated histone H4, on the cestode laboratory model Mesocestoides corti (2). Here, we present the activity profile of a series of HDAC-inhibitors (HDACi) against on viability of M. corti larvae, using a worm tracker device for high-throughput screening in parallel with microscopy observation. We evaluated 40 compounds, comprising HDACi against class I, II and III HDACs. The commercial compounds Entinostat (a HDACi against class I HDACs) and Mz25 (a HDACi against class III HDACs), and two structure-based novel inhibitors designed against the HDAC8 from Schistosoma mansoni (3) were the most potent HDACi. These compounds produced a decrease of 100% in the viability and alterations on the tegument and morphology of M. corti at concentrations of 20 and 50 µM ($p<0.001$). Entinostat produced a 100 % decrease on parasitic viability, even at concentrations as low as 2 µM ($p<0.001$). These results suggest that HDAC class I and III from cestodes could be considered as putative drug targets for neglected diseases caused by cestodes. References 1. WHO. Geneva World Heal Organ. 2012:15 p. 2. Vaca HR, et al. Int J Parasitol Drugs drug Resist. 2019;9: 120-32. 3. Heimburg T, et al. J Med Chem. 2016;59(6):2423-2435.
Acknowledgements: This work was supported by CONICET, UBACyT and PICT. We thanks Drs. Manfred Juang and Wolfgang Sippl for provide us the HDAC-inhibitors. Nodo Bioinformático IMPaM-UBA-CONICET (https://bioinfo.fmed.uba.ar/).

0816 - CHARACTERIZATION OF A NEW SEROTONERGIC G-PROTEIN COUPLED RECEPTOR FROM CESTODES: NEW POTENTIAL TARGET FOR DRUGS AGAINST NEGLECTED TROPICAL DISEASES

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Echinococcus canadensis is a platyhelminth parasite that belongs to the class Cestoda and is the etiological agent of the Hydatid disease, a neglected disease that affects public health and the economy in Argentina and worldwide. Currently, the treatment for echinococcosis in humans relies on benzimidazoles. However, the emergence of resistant parasites, makes the discovery of new anthelmintic drugs an imperative need. To tackle this problem, we propose to characterize G-protein coupled receptors from cestodes as new pharmacological targets. In our previous work (1), we found that serotonergic GPCRs (5-HT GPCRs) are of major importance in cestode movement and showed distinctive pharmacology. The aim of this work was to study the function of a new 5-HT GPCR from Echinococcus canadensis. Similar sequences were also found in another cestode species. Bioinformatics analyses suggest the existence of genes encoding for 5-HT GPCRs and this information was used for the design of primers. New cDNA was synthesized using RNA extracted from protoscoleces of pig origin as a template for PCR reactions. The amplificated cDNA coding for the serotonergic gene was cloned, sequenced and finally used for transient transfections in HEK293 cells. Calcium levels were measured using a fluorescent imaging plate reader (FLIPR) assay (2). When the cell line was transfected with a gene encoding for the cestode receptor, the calcium levels increased only in the presence of serotonin but not with of other ligands like tryptamine, tyramine, octopamine, acetylcholine, histidine or dopamine. The dataset confirms the bioinformatics analyses showing that the cloned gene encodes for a new 5-HT GPCR conserved in cestodes. The cloning strategy followed by sequencing and expression in HEK cells revealed a new 5-HT GPCR. The results obtained confirm bioinformatics predictions and will be tested as a target for cestocidal drugs. References 1. Camicia F, et al. PLoS Negl Trop Dis. 2018; 12(2): e0006267. 2. Harvey JH, van Rijn RM, Whistler JL. Methods Mol Biol. 2013; 995: 43-54.
Acknowledgments: We especially thank to Dr. Gabriel Lichtenstein (IMPAM-UBA-CONICET) for the bioinformatical assistance.

0846 - DRUG DELIVERY SYSTEMS BASED ON POLYELECTROLYTE-DRUG-FATTY ACIDS TERNARY COMPLEXES FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS

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Cutaneous Leishmaniasis is the most prevalent form of Leishmaniasis in South America. A topical treatment is attractive because of its potential to reduce side effects, increase patient compliance and its affordability. Risedronate (Ris) and Eudragit E (EE) have shown to be active against some forms of Leishmania. Previous studies showed that EuE-Ris complexes are promising candidates for topical administration. Besides they showed in vivo anti-Leishmania activity. The literature also describes the antileishmania activity for medium chain fatty acids. Our hypothesis is that the physicochemical properties of a new material EE-Ris-Capric Acid (CA) would allow to obtain nanometric compounds with enhanced antileishmania properties. The specific objectives of this work were: a) characterize physicochemically homologous series of EE-Ris-CA systems obtained and to evaluate the release properties of the loaded drug. For this, EE-Ris-CA obtaining method was tuned up and the zeta potencial, particle size and drug release profile from Franz cells towards water, NaCl, end PBS were assayed. All systems evaluated resulted in translucent, homogeneous and physically stable mixtures, which was considered an indicative aspect of the formation of a salt or complex between AC and the components of the EE-Ris complex. All pHs value were compatible with topical route (pH: 5-6). When the molar proportion EE/CA was 1/1.2, the Z potential was considerably increased and there was also an increase in the proportion of 200 nm particles (PDI>0.3). These results, could be attributed to a greater exposure of the basic groups of PE in comparison to EE-Ris system, that could indicate some change in the structures that were previously formed. When Ris kinetic release from EE-Ris-CA was evaluated, an increase in the Ris release was founded in all media tested with regard to EE-Ris, that would indicate a release mechanism dependent on ionic exchange; thus, there is also reversible interaction between the components of the material. A new material containing antileishmanial drugs and excipients was obtained which would contribute to topical therapy to treat cutaneous leishmaniasis.

0858 - SCREENING AND IDENTIFICATION OF METACASPASE INHIBITORS, EVALUATION OF INHIBITION MECHANISM AND TRYPANOCIDAL ACTIVITY

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Targeting proteases is a common strategy to identify new antiparasitic agents due to their essential contribution to parasite growth and development. Metacaspases (MCAs) are cysteine proteases (Clan CD) present in fungi, protozoa and plants. These enzymes, which are associated with crucial events in protozoa parasites (i.e. cell death and cell cycle progression), are absent in the human host, thus arising as attractive drug targets. To find new MCAs inhibitors bearing trypanocidal activity, we adapted a continuous fluorescent enzymatic assay to a medium-throughput format and carried out the screening of different compounds collections, followed by the construction of dose-response curves for the most promising hits. We used MCA5 from T. brucei (TbMCA5) as a model for the identification of inhibitors from the GlaxoSmithKline HAT and CHAGAS chemical boxes; two collections grouping 404 non-cytotoxic compounds with high antiparasitic potency, drug-likeness, structural diversity and scientific novelty. We also assessed a third collection of 9 compounds from Maybridge database identified by virtual screening as potential inhibitors of the cysteine peptidase falcipain-2 (Clan CA) from Plasmodium falciparum. As a result, 4 hits from the HAT and CHAGAS boxes showed modest IC50 values in the range 79-142 µM. Remarkable, HTS01959 (Maybridge collection) resulted the most potent inhibitor with IC50 of 14.39 µM; also inhibiting other MCAs from T. brucei and T. cruzi (TbMCA2= 4.14 µM, TbMCA3= 5.04 µM and TcMCA5= 151 µM). HTS01959 behaves as a reversible, slow binding and noncompetitive inhibitor of TbMCA2, where the mechanism of action includes RedOx components. Importantly, HTS01959 displays trypanocidal activity against bloodstream forms of T. brucei and trypomastigotes forms of T. cruzi, with non-cytotoxic effect on VERO cells. Thus, HTS01959 seems to be a promissory starting point to develop more specific and potent chemical structures to target MCAs from trypanosomatids parasites.

0860 - PROTEINS INVOLVED IN DNA HOMOLOGOUS RECOMBINATION REPAIR IN TOXOPLASMA GONDII: BRCA2 AND RAD51 CHARACTERIZATION

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Toxoplasma gondii is an obligate intracellular parasite, belonging to the phylum Apicomplexa and is responsible of toxoplasmosis infection. Although there are treatments against toxoplasmosis, due to the toxicity of the drugs used there is an intensive search for new treatments against the parasite and innocuous to the host cell. There are conserved components of the homologous recombination DNA repair (HRR) pathway in T.gondii that could present unique characteristics which make them attractive therapeutic targets. Among them, a putative T. gondii BRCA2 was identified in silico because of the presence of conserved domains with the human homologous. In higher eukaryotes BRCA2 interacts with the recombinase RAD51, which is also present in T. gondii, generating an essential complex for the HRR. T. gondii BRCA2 and RAD51 genes were cloned and expressed in bacteria to obtain recombinant proteins used to produce specific mouse polyclonal antibodies. RAD51 was expressed as an entire recombinant protein, but for BRCA2 only the OB1 domain was expressed due to its high mass, near 480 kDa. The antibodies were titrated by ELISA, and used to detect their presence in T.gondii by Western blot (WB) and their subcellular localization by indirect immunoflourescence (IFA) either in normal conditions or using DNA damaging agents such as phleomycin and metylmethanesulfonate (MMS). The results showed no differences in the protein expression by WB in a DNA damage context, compared to non-treated parasites, for both proteins. When parasites were analyzed by IFA TgBRCA2 showed a spotted distribution along the whole parasite (nucleus included) in normal and DNA damage conditions. The antibodies obtained against these two important proteins will allow us to make progress in the understanding of the complex BRCA2-RAD51, which is fundamental to study the DNA repair by HRR observed in other eucaryotes.

0884 - HISTOLOGICAL DEMONSTRATION OF APOPTOSIS ACTIVATION IN THE LIVER FLUKE (FASCIOLA HEPATICA) FOLLOWING CLOSANTEL TREATMENT OF EXPERIMENTALLY-INFECTED SHEEP

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CIVETAN CONICET (1); FCV- UBA (2)

The apoptosis can be by DNA damage, cytokine expression, etc. The study of apoptosis has application for the understanding of biological events, such as the tumorogenesis, the action mechanism to drugs, etc. For the apoptosis detection in different cell lines and tissues there are methods such as TdT-mediated dUDP nick end labelling (TUNEL) or directly the immunolocalization of Caspase-3. Closantel is an antiparasitic halogenated salicilanilide for the treatment of Fasciola hepatica infestation in farm animals. Its action mechanism is not fully known. It's known decouple the oxidative phosphorylation but the probable degree of contribution of apoptosis is unknown. In this work, were used the Caspase-3 detection and TUNEL techniques to assess the probable involvement of the closantel in the generation of apoptosis in Fasciola hepatica. Fourteen lambs were infected orally with 200 metacercariae of Fasciola hepatica. At 16 weeks post-infection, 10 lambs were treated orally with closantel (10 mg/kg.). Adult flukes were recovered from the liver of individual lambs at 0 (no treated n= 4), 24 (n= 5) and 36 h (n= 5) post treatment. The flukes were processed for usual histological analysis. There were no injuries at the controls. The F. hepatica at 24 h PT showed minor damage to the posterior end of syncytium. Those of 36 h PT lost large areas of the syncytium in the posterior and dorsal end with cell depletion in testis and vitelline follicles and the oocytes appear rounded with condensed cytoplasm, indicating apoptosis. In testis, ovary and vitelline follicles, defects in closantel-treated trematodes were aligned with failure in the energy-demanding processes of mitosis and differentiation. These changes could be attributed to anthelmintic-induced blockage of intermediary metabolism and neuromuscular paralysis. This work confirms that closantel activates apoptosis being too this phenomenon its mechanism of action.

0886 - MODE OF ACTION OF THE SESQUITERPENE LACTONE EUPATORIOPICRIN ON TRYPANOSOMA CRUZI

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Eupatoriopicrin is a sesquiterpene lactone isolated from Stevia maimarensis (Asteraceae), an endemic plant species from northern Argentina. This compound has shown promising trypanocidal activity and selectivity against epimastigotes, trypomastigotes and

amastigotes of Trypanosoma cruzi. Taking into account that other sesquiterpene lactones isolated by our group exerted its tripanocidal activity by sterol biosynthesis inhibition, interacting with hemin and/or producing mitochondrial dysfunction [1] [2], the objective of this work was to assess the effect of eupatoriopicrin on these T. cruzi targets. The interaction of this sesquiterpene lactone with hemin was examined under reducing and non- reducing conditions by measuring modifications in the hemin Soret band absorption. The mitochondrial function was assessed by means of rhodamine 123 staining and by measurement of NADH-cytochrome c reductase and succinate dehydrogenase activities. Lipids profile was analyzed by TLC and ultrastructural changes were determined by transmission electron microscopy. Eupatoriopicrin did not interact with hemin, but affect the functionality of the mitochondria and the lipids profile in the parasite. TLC analysis showed that eupatoriopicrin did not affect sterol biosynthesis but led to triglyceride accumulation. Microscopy images analysis showed the presence of lipid droplets in parasite cytoplasm. Based on our results, eupatoriopicrin would exert its activity by producing mitochondrial dysfunction and by altering the lipid profile in T. cruzi.

References: 1.- Puente V., et al. Phytomedicine. 2018; 56: 10.1016/j.phymed.2018.10.015. 2.- Sulsen V., et al. PLOS ONE. 2016: 11. e0150526. 10.1371/journal.pone.0150526.

0927 - HIGH-THROUGHPUT SEROLOGICAL FOLLOW-UP OF CHAGAS DISEASE PATIENTS TREATED WITH BENZNIDAZOLE AND E1224 IN A RANDOMISED, PLACEBO-CONTROLLED TRIAL: SEARCHING FOR EARLY INDICATORS OF TREATMENT SUCCESS AND FAILURE

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During an infection, the immune system produces antibodies against pathogens. With time, the immune repertoires of infected individuals become specific to their clinical history and thus represent a rich source of diagnostic markers. Chagas Disease (CD) is caused by the protozoan parasite Trypanosoma cruzi. When developing new drugs, it would be advantageous to reduce the followup time in clinical trials. The current criteria for cure in CD are negativization of at least one of 3 independent samples by PCR or at least 2 serological tests. However, PCR assays have low sensitivity and seroconversion is slow using standard assays that measure antibodies to several antigens at once. Recently a new drug was tested for CD. In this study, fosravuconazole (E1224) was tested alongside benznidazole (BZ) and a placebo (Torrico et al 2018). E1224 displayed a transient, suppressive effect on parasite clearance, whereas BZ showed early and sustained efficacy until 12 months of follow-up. Using high-density peptide arrays displaying ~400,000 peptides derived from a large collection of recently identified T. cruzi antigens, we assessed changes in individual antibody repertoires along time for 36 patients from the 3 main study arms (BZ, E1224, placebo). The antibody repertoire of each patient was analyzed at recruitment (0), end-of-treatment (day 65) and at the end of follow-up (12 months). A total of 108 serum samples were analyzed in duplicate to assess the dynamics of the antibody immune in response to drug treatments (or placebo). A preliminary analysis of the data allowed us to identify antigens that increased or decreased their signal in treatment groups and also a number of subjects that showed more or earlier changes in their serological profile in response to treatment. We will discuss advances in our aim to find candidate prognostic markers to follow-up CD patients in clinical trials.

0946 - CHRONIC TOXOPLASMA GONDII INFECTION INDUCES BEHAVIOURAL AND COGNITIVE CHANGES: A MODEL OF STUDY

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Recently, lot of evidence has been accumulated that links T. gondii chronic infection with different pathologies including neurocognitive and behavioural conditions among others. There are no treatments able to eliminate the parasite at this stage or to reduce the adverse effects associated with the infection. Herein, we aimed to set out different tests to study the effect of chronic infection on neurocognitive and behavioural abilities. According to the literature, it is necessary to use at least two tests to evaluate the different abilities, so we selected the Open Field (OF), Hole Board (HB) and Forced Swim (FS) tests. Chronically infected and naive C57BL/6 mice were used. Infection was confirmed before the tests by serology, and brain cyst count at the end. The OFT lets the study of behavioural changes. The exploratory ability in infected mice was reduced compared to the control group (33.7 % less line crosses, less time spent in the centre of arena and reduction in two leg stands (85.5 %)). We confirmed this result with the HBT, where infected mice showed 62.8 % less nose-poked. To evaluate learning/memory abilities, nose-poked habituation task trials were done. When the test was carried out 24 h after training, the reduction in the exploratory activity was remarkably lower in infected mice (15.6 in infected vs. 69.7 % in Control group). Also, in FST infected mice showed shorter flotation times than controls (46.6 %). To study memory abilities, flotation time after training was analysed. While in controls this parameter was 36.8 % increased, infected mice showed no variation. Altogether, these data showed alterations to face new or previously experienced situations in chronically infected mice. Finally, the devices we have set out and the tests used in this work allow us to study the effects of chronic toxoplasmosis at a cognitive and behavioural level. Moreover, they can be used to study therapies aimed at improving the neurological effects in chronically infected mice.

0966 - INHIBITION MECHANISM OF TRYPANOSOMA CRUZI ARGININE KINASE BY DELPHINIDIN

Edward Augusto VALERA-VERA | Mariana R. MIRANDA | Chantal REIGADA | Melisa MARTÍNEZ-SAYÉ | Claudio PEREIRA

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The enzyme arginine kinase from Trypanosoma cruzi (TcAK) catalyzes the interconversion of arginine and phospho-arginine to maintain the ATP/ADP balance, and is involved in the energetic homeostasis of the parasite and stress response. Some polyphenolic compounds have been predicted to inhibit this enzyme activity in virtual screening and further proved to inhibit it in vitro and excreting trypanocidal activity in epimastigotes and trypomastigotes. One of those compounds, delphinidin, had activity in low micromolar concentrations, so we wanted to explore the mechanism of inhibition and produce a model of interaction between delphinidin and TcAK. To this end, TcAK activity was measured in absence or presence of different delphinidin concentrations, varying the arginine concentrations. The resulting data was fitted to the Michaelis-Menten equation to calculate the kinetical parameters Km and Vmax, resulting in a decrease of the Vmax with increasing delphinidin concentrations ($p= 0.005$), while Km had a trend to increase, but with no statistically significant difference ($p= 0.3$). The decrease in Vmax excludes a competitive mode of inhibition, and while further testing is required to reliably state the kind of inhibition, the trend of the km suggests it could be

a mixed inhibition with preference towards the TcAK-arginine complex. To produce a model of the interaction we did molecular docking simulations wit AutoDock 4.5 and AutoDock Vina. While some of the binding poses predicted by AutoDock 4.5 overlapped at some extent with the arginine binding site, both programs produced binding poses with good scores located in the ATP/ADP binding site, and poses with lowest scores that binds to a pocket away from the active site. In conclusion, we tested that delphinidin does not compete with arginine when inhibiting TcAK, and computational approaches suggest the binding of delphinidin might be occurring at the ATP/ADP binding site, so further testing of the inhibition mechanism as a function of ATP concentration is needed.

Hematología / Hematology

Chairs: Paola Lev | Mirta Schattner

0143 - ANTITUMOR ROLE OF HISTAMINE H4 RECEPTOR IN HUMAN T-CELL LYMPHOMA. THERAPEUTIC BENEFIT FOR COMBINATION THERAPY WITH HISTAMINE AND HISTONE DEACETYLASE INHIBITORS

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The discovery of the human histamine H4 receptor (H4R) has contributed to our understanding of histamine role in numerous physiological and pathological conditions, including tumor development and progression. High histamine levels have been determined in lymph nodes of patients with malignant lymphomas, but as far as we know there is no evidence of the expression and function of the H4R in this tumor type. The aims of this work were to study the expression of the H4R and to evaluate the therapeutic efficacy of histamine and H4R's ligands as a single treatment or in combination with inhibitors of the histone deacetylase (HDACi) for the treatment of T-cell lymphoma (TCL). Results demonstrate the expression of H4R isoforms at the mRNA and protein levels in 3 human aggressive TCL cell lines (OCI-Ly12 and Karpas 299, Peripheral TCL; HuT78 Sezary Syndrome). HEK293T was used as a negative control. Histamine and specific H4R agonists (VUF8430 and JNJ28610244) significantly reduced cell viability (CellTiter-Blue Assay) in a dose-dependent manner and induced cell apoptosis (Caspase-Glo 3/7 Assay) in the three cell lines ($p<0.05$, n=3 independent experiments performed in triplicates). The combined treatment with the H4R antagonist (JNJ7777120, 10 µM) reversed the effects of the H4R ligands (10 µM) on TCL. Importantly, we screened active compounds against TCL, evaluating a drug repurposing library of 384 FDA-approved compounds (1 µM) in combination with histamine (10 µM) in Hut78 cells. Histamine produced a synergistic antitumor effect, evaluated with a metabolic assay, with 18 of these compounds, including the HDACi panobinostat. Apoptosis, proliferation and oxidative stress studies confirmed the antitumoral effects of the combination. We conclude that H4R is expressed in TCL and it is involved in histamine-mediated responses. Histamine could be an attractive compound to be used as a single therapy or in combination with HDACi for the treatment of TCL.

0262 - MEGAKARYOCYTE (MK)-STROMAL CELL INTERACTIONS: EFFECT ON PROLIFERATION, PROPLATELET PRODUCTION, AND SURVIVAL

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Bone marrow stromal cells (SC) provide a proper environment for the development of hematologic lineages. They contribute to the regulation of haematopoietic stem cell growth and differentiation by producing and releasing cytokines, growth factors and extracellular matrix proteins. In order to study in vitro megakaryopoiesis and thrombopoiesis, the incorporation of the different SC would be an attractive model. Our objective was to evaluate the participation of different types of SC on megakaryopoiesis, thrombopoiesis and MK survival under in vitro culture conditions. CD34-positive progenitors obtained from umbilical cord blood were cultured in medium supplemented with thrombopoietin, with or without umbilical cord endothelial cells (HUVEC), bone marrow mesenchymal stem cells (MSC), skin fibroblasts (SF) (all human) or mouse fibroblast cell line (L929). Biological samples were obtained after informed consent and Ethics Commitee approval. The number of MKs (CD61-positive cells) and mature MKs (CD42-positive cells) were increased in the presence of MSC and HUVEC, reaching statistical significance in the latter (CD61, $p= 0.016$). In the presence of L929, the number of total and mature MKs decreased ($p<0.05$), while SF increased MK number without affecting mature MKs. Concerning thrombopoiesis, HUVEC increased the number of MKs producing proplatelets (PP) ($p<0.05$), while MSC and SF decreased PP ($p<0.005$) as evaluated by immunofluorescence staining and microscopic analysis. Ongoing experiments show that L929 inhibits PP, although additional experiments will be performed to confirm this finding. MK apoptosis was evaluated by fluorescence microscopy. Percentage of picnotic nuclei was not affected by the presence of HUVEC and L929 cells while it was decreased by MSC ($p<0.005$) and SF cells ($p<0.05$). In conclusion, SC seems to affect megakaryopoiesis, PP and MK survival in different manners depending on their lineage and source. Our findings should be considered when choosing an in vitro model to study MK physiology.

0319 - EFFECT OF HIGH DOSES OF ERYTHROPOIETIN ON ITS ANTIAPOPTOTIC FUNCTION ON DIFFERENTIATED ERYTHROID CELLS

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Recombinant human erythropoietin (Epo) has been successful in the treatment of anemia of different etiologies but high Epo doses, used to reduce the frequency of administration or to treat refractory anemia, have been linked with adverse outcomes. Previously, we observed a decreased effect of high Epo concentrations on endothelial cell migration. Here, we studied the antiapoptotic effect of Epo on erythroleukemia K562 cells exposed to TNF-a (T), after incubation with increasing concentrations of Epo (10, 100 and 400 U/mL) and erythroid differentiation with hemin. While Epo10 prevented the increase in the percentage of apoptotic nuclei observed with TNF-a, Epo100 and Epo400 did not show that protection. Considering that this effect could be related to the decreased availability of the Epo receptor, we investigated the activity of protein tyrosine phosphatase 1B (PTP1B) and the process of proteasomal degradation in this cell model. PTP1B activity, which regulates many aspects of the signaling pathways elicited by Epo upon EpoR activation, showed no significant differences between cultures with low and high Epo concentrations. Then, we used the proteasome inhibitor MG132 to evaluate if EpoR internalization could affect the antiapoptotic effect of Epo against TNF-a. In the presence of MG132, the results with high concentrations of Epo were similar to those observed with Epo10T (Hoechst staining, C 13.5 ± 0.7, T 31.5 ± 1.5*, Epo10T 22.1 ± 1.1*, Epo100T 29.4 ± 1.4*, Epo400T 29.9 ± 2.2*, Epo100TMG 21.7 ± 19, and Epo400TMG 22 ± 1.3 %, *$P<0.05$, n= 6). Based on this, it can be suggested that the kinetics of EpoR internalization/degradation, mediated by the

proteasome, could explain, at least in part, the lack of antiapoptotic effect of high concentrations of Epo. The present results give new insights that may contribute to understand Epo behavior when cells of different tissues are activated by a high concentration of the hormone, and may help to adequate therapeutic strategies.

0352 - ANALYSIS OF PLASMA FACTORS THAT INTERACT WITH THE ERYTHROCYTE AGGREGATION KINETICS IN WISTAR HYPERLIPEMIC RATS TREATED FOR 3 DAYS WITH FRACTION ENRICHED IN PROANTHOCYANIDIN OBTAINED FROM LIGARIA CUNEIFOLIA (PLC)

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In folk medicine, the infusions of Ligaria cuneifolia (Lc) are used to decrease arterial tension and the excess plasma cholesterol (Cho). In previous works we have demonstrated that the treatment with Lc as PLc produce in plasma diminution of both Cho and triglycerides (TG). Also, it has been reported that the erythrocytes aggregation (EA) correlated with plasma fibrinogen (FB) and to a lesser extent on high TG values. The of the present study was to analyze the effect of administration the PLc during 3 days to hyperlipemic rats, on both plasma TG, FB levels, and determine the EA. Adult male Wistar rats (aged 70 days) fed with: standard chow diet (Control, C: n= 5), standard chow diet added with 40 % bovine meat juice during 28 days (HFD, n= 13). At the end of this time, the animals were administered i.p. each 24 h with either physiological solution (HFD, n= 6 and Control group) and PLc 3mg/100 g body weight only in HFD (Treated T, n= 7) during 3 days. In the day fourth they were anesthesized i.p. with Ketamine and Xylazine (100mg/kg and 3mg/kg, respectively) to obtain blood samples by cardiac puncture. In serum TG and FB were determined by enzymatic methods. Into blood: AE, by optical densitometry, getting the average size (s) of the aggregates and the initial velocity (v) of the process, erythrocyte sedimentation rate. Results are expressed as medium ± SEM. FB: C: 274.6 ± 17.2, HFD: 271.4 ± 16.6 and T: 337.5 ± 24.3; TG: C: 143.8 ± 18.2, HFD: 415.0 ± 94.5*, T: 112.9 ± 9.4#; AE: C: s: 0.91 ± 0.48 v: 0.63 ± 0.53, HFD: s: 0.77 ± 0.37 v: 0.33 ± 0.23; T: s: 0.72 ± 0.27 v: 0.20 ± 0.11. (*p<0.05 vs. C. #p<0.05 vs. HFD). We have shown that FB values aren't modified in both groups of rats HFD and T. On the other hand, the increase in TG in HFD didn't lead to changes in EA. It's relevant remark that the 3-day of treatment with PLc significantly decreases TG values without producing changes in erythrocyte aggregation.

0485 - CONDITIONED MEDIA FROM ERYTHROPOIETIN-TREATED ERYTHROID CELLS SUPPRESS HEPCIDIN IN THE HEPG2 HEPATIC CELL LINE

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Erythropoiesis is a key regulator of Fe metabolism, as it demands a high supply of the metal for hemoglobin synthesis. In patients, erythropoietin (Epo) was found to decrease liver hepcidin (Hamp), thus allowing the release of stored Fe into the bloodstream. We previously reported a direct downregulation of Hamp by Epo in the human HepG2 cell line, as early as 6 h after exposure to the cytokine. However, other authors have postulated the existence of an erythroid regulator —erythroferrone (ERFE)—, which is produced by erythroblasts upon Epo stimulation. To investigate the indirect activity of Epo on Hamp expression in hepatic cells, human erythroleukemia K562 cells were treated with Epo (10 U/mL, 24 h), thoroughly washed and cultured in growth medium alone for other 24 h, obtaining conditioned media (CM). HepG2 cells were exposed to the CM (6 h), and Hamp levels were quantified by real-time PCR. CM of Epo-treated K562 cells (CM-E) significantly decreased Hamp mRNA compared to CM of control K562 cells (CM-C= 1.00; *CM-E= 0.33 ± 0.12; *P<0.05, n= 5). Since Epo directly suppresses Hamp in HepG2 cells, we studied its production by the K562 cell line. Epo mRNA was undetectable in both Control and Epo-treated K562 cells (RT-PCR). Similarly, protein levels of Epo were scarce in lysates and in CM of control and Epo-treated K562 cultures (Western blotting), thus excluding the possibility that Hamp suppression by CM is due to the release of Epo by these cells. This indirect activity of Epo led us to investigate ERFE expression in K562 cells exposed to the cytokine for different times. ERFE mRNA was detected at basal levels in this cell line, and increased 2-fold on average after 1 h of Epo exposure (n= 3). Our results suggest that Epo-treated erythroid cells release a factor that reduces Hamp levels in HepG2 cells, consistently with the expression of ERFE in the K562 cell line. Further research using this experimental model may help increase knowledge about Fe regulation.

0568 - ASYMMETRIC DIMETHYLARGININE (ADMA) INHIBITS THE EFFECT OF ERYTHROPOIETIN ON ENDOTHELIAL CELL MIGRATION IN A PROINFLAMMATORY ENVIRONMENT

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Hyperhomocysteinemia induces vascular endothelial dysfunction, an early hallmark of atherogenesis. Previously, we found a sensitizing effect of the inflammatory cytokine TNF-alpha on a promigratory action of erythropoietin (Epo), which was not observed when the molecule was modified by N-homocysteinylation with homocysteine thiolactone, the reactive derivative of homocysteine. The strong correlation found between hyperhomocysteinemia and levels of asymmetric dimethylarginine (ADMA), the amino acid formed during methylation of proteins, has been considered of interest in endothelial dysfunction. Therefore, the aim of this work was to study whether ADMA accumulation could affect the ability of different factors to induce endothelial cell migration. Wound healing assays of EA.hy926 endothelial cells stimulated by 10 % fetal bovine serum (FBS), 20 ng/mL VEGF or Epo+TNF-alpha (80 ng/mL and 30 ng/mL, respectively) were performed in the presence or absence of ADMA (30 or 100 µM). Results are expressed as a percentage of the cell migration obtained in the presence of FBS. FBS: 100; Control: 22.0 ± 2.4; ADMA: 20.1 ± 3.4; *FBS+ADMA30: 66.6 ± 3.5; **FBS+ADMA100: 43.3 ± 3.9; Epo+TNF-alpha: 56.4 ± 1.2; *Epo+TNF-alpha+ADMA30: 37.4 ± 2.9; **Epo+TNF-alpha+ADMA100: 31.1 ± 1.6; VEGF: 44.5 ± 2.9; *VEGF+ADMA30: 30.3 ± 2.1; **VEGF+ADMA100: 19.6 ± 2.8; *p<0.05 and **p<0.01 vs. respective controls, n= 4. It can be seen that the inhibition of the promigratory effects of all the factors analyzed was dependent on ADMA concentration. The results of cell viability and MTT assays confirmed that the effects of ADMA on the promigratory action of Epo+TNF-alpha and VEGF cannot be attributed to cytotoxicity or cell proliferation. These results underline the important role of homocysteine in cardiovascular pathophysiology, since increased homocysteine is involved in a direct inhibition of the enzymes responsible for ADMA degradation.

0638 - BCL2 AS A MARKER TO IDENTIFY REFRACTORY AND AT RISK OF RELAPSE DISEASE IN CLASSIC HODGKIN LYMPHOMA PATIENTS. ITS BLOKEADGE AS A POTENTIAL DIRECTED-TEHRAPY.

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Refractory and relapsed disease (RRD) is currently the challenge when treating classic Hodgkin Lymphoma (cHL) patients. There is no specific therapy rather than rescue chemotherapy which fails in 50% of the cases and associates with high toxicity. We have previously reported that the alternative NFkB pathway, mediated by Rel-B and NIK, plays a key role in cHL survival through high BCL2 expression levels. We aimed to analyze if mediators of this pathway and BCL2 could be useful as prognosis markers and would represent targetable factors in RRD. We analyzed NIK and BCL2 expression in Hodgkin Reed-Sternberg cells in the lymph node biopsies of 113 cHL naïve of therapy patients by IHQ [52 female Md age (range) 36 (6-88), 61 male 40.7 (9-78)]. The univariate analysis showed no correlation between NIK or BCL2 expression and the clinical and pathological parameters, including the PET scan indicated at the end of the first line treatment. The statistical significance was maintained in multivariate analysis (Cox Regression, p= 0.01). NIK expression did not associate with prognosis but the BCL2 expression level correlated with lack of response to conventional therapy and both early and late disease progression. The survival analysis, using the Kaplan-Meir curves, showed that patients with ≥60 % positive HRS cells had a shorter disease-free survival (DFS) [log rank test, p= 0.002] and a reduced overall survival (OS) [log rank test, p=0.02]. Human cHL cell lines that express BCL2 protein, were sensitive to venetoclax, a specific BCL2 inhibitor. The drug induced a citostatic effect with cell arrest in S-Phase. We found that the alternative NFkB pathway plays an important role in the refractory and relapsed Hodgkin disease, being BCL2 a key downstream target. BCL2 performed well as a prognosis marker identifying refractory patients and those that relapsed. We believe BCL2 directed-therapy should be explored in cHL patients that express this protein in the biopsy performed at diagnosis.

0656 - ACTIVATION OF TOLL-LIKE RECEPTORS 2 AND 4 ON CD34+ CELLS INCREASES HUMAN MEGAKARYO/THROMBOPOIESIS INDUCED BY THROMBOPOIETIN

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Platelet (PLT) toll-like receptors (TLR) 2 and 4 are key players in amplifying the host immune response. However, its role in human megakaryo/thrombopoiesis has not yet been defined. Our aim was to evaluated whether Pam3CSK4 (Pam) or lipopolysaccharide (LPS), TLR2/4 ligands respectively, modulate human megakaryocyte (MK) development and PLT production. CD34+ cells from human umbilical cord were stimulated with LPS or Pam3CSK4 with or without thrombopoietin (TPO). TLRs expression was determined by RT-PCR and flow cytometry (FC). Differentiation, maturation, PLT generation, c-MPL (TPO receptor) expression and IL-6 production was evaluated by FC and proPLTs and NF-E2 migration by confocal microscopy. CD34+ cells and MK express TLR2 and TLR4 at both RNA and protein level. Direct stimulation of CD34+ cells with LPS or Pam had no effect in cell growth. Interestingly, both TLR ligands markedly increased TPO-induced CD34+ cell proliferation, MK number and maturity, proPLT and PLT production when added at day 0 (n= 7, $p<0.05$), without increases of c-MPL surface expression. In contrast, this synergism was not observed when LPS or Pam were added 7 days after TPO addition. IL-6 release was observed upon CD34+ or MK stimulation with LPS or Pam but no with TPO and this effect was potentiated in combination with TPO (n= 4, $p<0.05$). The increased proliferation and IL-6 production induced by TPO+LPS or Pam were suppressed by TLR2/4 or IL-6 neutralizing antibodies as well as by PI3K/AKT and NF-kB inhibitors (n= 3-5, $p<0.05$). Additionally, increased proPLT and PLT production were associated with enhanced nuclear translocation of NF-E2. Finally, the supernatants of CD34+ cells stimulated with TPO+LPS induced CFU-M colonies. Our data suggest that the activation of TLR2 and TLR4 in CD34+ cells and MK in the presence of TPO might contribute to warrant PLT provision during infection episodes by an autocrine IL-6 loop triggered by PI3K/NF-kB axes.

0668 - FURTHER STUDIES ON THE PATHOGENIC MECHANISMS LEADING TO THROMBOCYTOPENIA IN SYSTEMIC LUPUS ERYTHEMATOSUS

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We previously demonstrated increased platelet apoptosis and decreased proplatelet formation (PPF) as contributing causes of thrombocytopenia in patients with systemic lupus erythematosus (SLE). Here, we broaden the study evaluating platelet desialylation and megakaryopoiesis in the presence of SLE plasma. Twenty-five SLE patients, healthy controls and healthy mothers from whom umbilical cord blood was obtained, signed the informed consent. Desialylation of normal platelets was observed in the presence of 67 % SLE samples as assessed by Ricinus communis agglutinin I and peanut agglutinin binding (flow cytometry). Although not statistically significant, desialylation was more frequent in thrombocytopenic than non thrombocytopenic patients (77 vs. 50 %). Fifty five % of SLE patients inducing desialylation also showed increased apoptosis and/or activation. To evaluate the effect of SLE plasma on megakaryopoiesis, normal CD34+ hematopoietic progenitors from cord blood were incubated with 10% SLE or control plasma for 12 days. The number of CD61+/CD42+ cells evaluated by flow cytometry was higher in the presence of SLE than control plasma (Mann-Whitney test, $p<0.05$). An increase in megakaryopoiesis and a decrease in PPF was concomitantly observed in the presence of two SLE patient samples with normal platelet count, suggesting that the increase in megakaryocyte production could counterbalance the impaired platelet production. Our results suggest that platelet clearance due to apoptosis and desialylation as well as inhibition of platelet production due to impaired thrombopoiesis could be relevant mechanisms contributing to thrombocytopenia in SLE. On the contrary, increased megakaryopoiesis could help to maintain normal platelet count in spite of deteriorated thrombopoiesis in some SLE patients.

0702 - DIFFERENCES IN PLATELET SIZE ACCORDING TO THE TYPE OF TREATMENT IN MYELOPROLIFERATIVE NEOPLASMS: THEIR EFFECT ON PROPLATELET MORPHOLOGY.

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Large platelets are more reactive and associated to thrombosis in some pathological conditions. Previously, we demonstrated increased maximum platelet diameter in patients with myeloproliferative neoplasms (MPN) treated with anagrelide (ANA), a-interferon (aIFN), or ruxolitinib (Ruxo), while decreased in those with hydroxyurea (HU). Here, we extended the study to 75 MPN patients. All subjects signed the informed consent. Measurements were performed with a software on pictures taken from May Grunwald Giemsa-stained blood smears. In this extended population, we confirmed our previous results and observed that platelets were larger in patients with JAK2V617F mutation than CalR+, both in untreated and HU treated patients, although statistical differences were reached only in the latter group (Mann Whitney test, $p<0.05$). To investigate if these drugs influence platelet size during proplatelet formation (PPF), normal CD34+ hematopoietic progenitors isolated from umbilical cord blood were cultured to obtain mature MKs. HU was added at day 5, during proliferation, while ANA, aIFN and Ruxo were added at day 12 when MKs reach maturity. At day 15 samples were fixed, permeabilized and stained with anti-tubulin-FITC to study proplatelet morphology. Using an immunoflourescent microscopy, pictures were taken and the size of tips and swellings along PPs was calculated. All drugs were tested at three different concentrations and at least in three independent experiments. ANA and aIFN induced a dose-dependent increase in tips and swelling size (Repeated measures ANOVA summary, ANA vs. control, $p= 0.0135$; aIFN vs. control, $p= 0.0464$) while Ruxo and HU did not exert morphological changes in PPF. Our results demonstrate that ANA and aIFN have a direct effect on proplatelet microtubular structure. The decrease in platelet size in patients treated with HU vs ANA and JAK2+ vs CalR+ could contribute to the lower incidence of thrombosis observed in HU treated and CalR+ patients.

0704 - POTENCIAL BENEFIT OF ERYTHROPOIETIN TO PREVENT IRON INDUCED CARDIOVASCULAR DESEASE

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The prevalence of iron overload cardiomyopathy is increasing. There is growing evidence that high iron levels are a risk factor for cardiovascular disease because can cause constriction of blood vessels.The aim was to study the erythropoietin (EPO) role to prevent iron induced cardiovascular disease studying key importer proteins in the heart in an animal model of iron overload and EPO. CF1 mice (25 ± 5 g; 3 months) were divided into groups (n= 4/group): 1) Iron-adequate (IA); 2) Iron-overload (IO) (iron saccharate; days 0, 4, 8, and 12 i.p.; 1,800 mg/kg); 3) EPO (days 17, 18, and 19) i.p.; 20,000 UI/kg); 4) Iron-overload+EPO (IO+EPO). Immunohistochemistry: anti-DMT1 (divalent metal transporter1) and ZIP14 (Zrt-Irt-like Protein14). Perl´s staining. Iron levels were measured by FeRcolor. The Protocol was approved by the CICUAE, UNS. Heart DMT1 expression was evident in IA and EPO groups and it was scarce in IO and IO+EPO conditions. However heart ZIP14 expression was evident in all conditions demonstrating that it´s expression not depends of the "iron signal". The decrease in the DMT1 expression in IO state would suggest a protective mechanism against iron excess in heart tissue, being the "iron signal" the predominant signal to decrease the biometal uptake. Iron levels in heart shows significant increase in IO respect to IA condition. Interestingly, the iron levels in IO+EPO were significantly decreased respect to IO. Consequently, abundant hemosiderin was observed in IO condition and it was scarce in IO+EPO group. Hemosiderin was absent in IA and EPO conditions. Our data showed that Iron uptake in IO would not depend on the expression of DMT1 either ZIP14. Thus, we can conclude that erythropoietin the "EPO signal" in high iron levels may have a direct positive effect on the heart. In conclusion, the interplay between EPO and key proteins of the iron cycle, such as DMT1 and ZIP14 may help to better understand the mechanisms involved in iron and erythropoiesis regulation in heart tissue.

0705 - SELECTIVE RESPONSE TO IRON AND EPO SIGNALS OF IRON CYCLE PROTEINS IN A MOUSE MODEL

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The Erythropoietin (EPO) is associated with iron mobilization.The aim was to analyze the regulatory relationship between iron and EPO studying iron key proteins in several tissues in an animal model of iron overload and EPO. CF1 mice divided into groups (n= 4/group): 1) Iron-adequate (IA); 2) Iron-overload (IO) (iron saccharate; days 0, 4, 8, and 12 i.p. ; 1,800 mg/kg) ; 3) EPO (days 17, 18, and 19) i.p. ; 20,000 UI/kg); 4) Iron overload+EPO (IO+EPO). Immunohistochemistry: anti-DMT1 (divalent metal transporter1) and ZIP14 (Zrt-Irt-like Protein14). Perl´s staining. Iron levels: Wiener kit. The Protocol was approved by CICUAE-UNS. Our data demostrated that the protective action of EPO against IO was selective in several tissues responding to different signals as follows. In lung: both DMT1 and ZIP 14 response to "EPO signal". Interestingly was observed that DMT1 localization in bronchial cells was changed being cytoplasmic in IA/IO+EPO/EPO, while it was localized in membrane cell and apical zone in IO condition. ZIP14 expression was dowregulated by "EPO signal" in bronchial cells. In spleen: both importers were downregulated by "EPO signal". Conversely, hepatic tissue responds to iron signal. Hepatic DMT1 and ZIP14 were dowregulated and upregulated, respectively. On contrary,in pancreas a selective importers response to "iron/EPO signal" was observed. In fact, DMT1 expression in Langerhans islets was downregulated by iron signal, however in acini ZIP14 was downregulated by "EPO signal". In all tissues Iron level was significant higher in the IO respect to IA and a significant decrease in IO+EPO respect to IO. The protective action of "EPO signal" against IO in all studied tissues could be explain by the reduced iron uptake in spleen, lung and pancreas. Nevertheless, the prevalence of the "iron signal" in liver may be explained by the increased hepatic iron uptake through ZIP14, thus reducing iron systemic level. Understanding the relations between these proteins will contribute to extend our knowledge in the field of iron and erythropoiesis.

0754 - EVALUATION OF TIME IN THERAPEUTIC RANGE IN ANTICOAGULATED PATIENTS WITH NON-VALVULAR ATRIAL FIBRILLATION IN THE HOSPITAL ESCUELA "EVA PERÓN" FROM GRANADERO BAIGORRIA

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ÁREA DE HEMATOLOGÍA DEL HEEP (1); SERVICIO DE CARDIOLOGÍA DEL HEEP (2); CARRERA DE ESPECIALIZACIÓN EN HEMATOLOGÍA. IUNIR. (3)

Patients with atrial fibrillation have a significant increase in the risk of ischemic and thromboembolic events. Oral anticoagulation reduces the risk of stroke. The efficacy of antagonists of vitamin K in this group of patients depends on tight control of anticoagulation in a therapeutic target range. The use of time in therapeutic range (TTR) is proposed as a quality overall indicator of anticoagulation therapy. Recent publications suggest that TTR values of at least 60 % are considered as optimal control. The aim of this study was to evaluate the TTR in anticoagulated patients with acenocoumarol with non-valvular atrial fibrillation attending the Area of haematology of the HEEP and to compare with those reported in the international literature. We classified them according to age, gender, cardioembolic risk and TTR. The TTRs were estimated annually for the period between March 1, 2014 to February 28, 2018 by two methods, the Rosendaal method (reference) and other simpler (TTR ratio). We collected and analyzed data from patients who met the inclusion criteria for the period 2014/2015 (n= 11), 2015/2016 (n= 26), 2016/2017 (n= 28) and 2017/2018 (n= 39). The average age was 68.2 years. The TTR average values calculated by the Rosendaal method for the periods 2014/2015, 2015/2016, 2016/2017 and 2017/2018 were 61.49; 56.12; 58.93 and 61.83 % respectively. The TTRs calculated by the Rosendaal method in the studied population were similar to that reported in the national and international literature. There was a poor linear correlation when comparing Rosendaal method against the TTR ratio method (r= 0,836). There were no significant differences in TTR controls according to age or cardioembolic risk.

Oncología/ Oncology V

Chairs: María Giselle Peters | Soledad Porte Alcon

0304 - INHIBITION OF NITRIC OXIDE PRODUCTION WITH L-NAME REDUCES THE NUMBER OF TREG CELLS AND IMPROVES THE IMMUNE CYTOTOXICITY IN BLADDER TUMORS

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Bladder cancer (BC) is a common malignancy of male urogenital tract. Tumors can be classified according to their invasion degree into non-invasive (NMI) and muscle invasive (MI). It has been showed that the constitutive expression of the inducible nitric oxide synthases (iNOS), producer of high levels of nitric oxide (NO), is a poor prognosis marker in human BC, associated with invasion and early recurrence. Previously, using the NMI murine BC model MB49 that express iNOS, it was demonstrated that the inhibition of NO with L-NAME reduced the immunosuppression and increased the cytotoxicity in tumor bearing mice (TBM). The aim of this study was to evaluate the immune cell profile and the anti-tumor specific cytotoxicity in the MI murine BC model using the MB49-I cell line that express higher iNOS than MB49 tumors. C57BL/6J mice were inoculated with MB49 or MB49-I cells. Spleen CD8+, NK and Treg were evaluated by flow cytometry. In vitro specific cytotoxicity was evaluated by co-culture of splenocytes from TBM and MB49 or MB49-I cell lines. MB49-I TBM showed a decrease in spleen CD8+ and NK cells ($p<0.05$) and an increase in Treg compared to normal mice ($p<0.05$), suggesting a tumor systemic immunosuppression. L-NAME treatment (0.5 g/L in drinking water) increased CD8+ and NK cells in MB49 ($p<0.05$) but not in MB49-I TBM. However, L-NAME was able to reduce spleen Treg in both MB49 and MB49-I TBM ($p<0.05$). L-NAME treatment also increased spleen specific cytotoxic activity against tumor cells in MB49 and MB49-I TBM compared to normal mice and untreated TBM ($p<0.01$ and $p<0.05$, respectively). Our results suggest that the use of L-NAME, at a concentration of 0.5 g/L, increases the cytotoxic activity of MB49 and MB49-I TBM against tumor cells, at least, by a reduction in Treg population.

0313 - USE OF LAB ON A CHIP (LOC) MICRODEVICES FOR STUDYING CANCER STEM CELLS. THE CHEMOTHERAPY RESPONSE IN AN INVASIVE BLADDER CANCER MODEL.

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Bladder cancer (BCa) is one of the most common tumors of the male urogenital tract and an important cause of death. The treatment of the invasive tumor is radical cystectomy. Currently, to improve patient's standard of living, conservative surgery followed by chemo (CT) and radiotherapy is proposed. Cancer Stem Cells (CSC) are a minority cell population associated with treatment resistance and tumor recurrence, so their quantification and identification are of special interest. Lab on a Chip (LOC) systems, have surged, in the last decade, as a powerful tool for cell individual study, with the benefit of using a low amount of biological samples. Our objective was to quantify and evaluate pluripotency and tumorigenic capacity of CSC in MB49-I, an invasive BCa cell line, post-treatment with CT agents, in macro culture conditions and in LOC systems. Doxorubicin (Doxo) or cisplatin (CisPt) treatment decreased the number of CSCs, measured as sphere formation efficiency ($p<0.05$) and decreased the remaining cells survival by 90% with cisPt and 65% with doxo ($p<0.001$). The histological study revealed that both CT generate smaller, disintegrated and eosinophilic spheres regard to control. Doxo or cisPt treatment induced an increase of pluripotential markers Oct-4, Sox2 and Nanog by qPCR ($p<0.005$ vs control). The in vivo growth of 15000 cells/mouse, derivate from spheres showed tumorigenic capacity, which could be responsible for tumor recurrence. Conclusion: The determination of the number of CSC, determinated as spheres, could be taken as a predictive marker of treatment response. The use of LOC devices provide the additional advantage of evaluating small samples, with translational possibility in patients.

0321 - RELEVANCE OF NITRIC OXIDE IN GLIOBLASTOMA THERAPY

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Glioblastoma (GBM) is the most common primary brain tumor, and within gliomas, the one with highest malignancy. Temozolomide (TMZ) treatment followed by radiotherapy is the main therapeutic strategy. Despite aggressive treatment, the clinical evolution ends in relapses with a live expectancy of less than two years. Increasing evidence associates the capacity of tumor regeneration and metastases with the presence of cancer stem cells (CSC). Furthermore, it has been suggested that nitric oxide (NO), as a consequence of the expression of inducible NO synthase (iNOS) could benefit GBM growth, progression and CSC maintenance. The objective of this study was to evaluate the role of iNOS in human GBM cell line U87, using specific pharmacological inhibitors (1400W, S-methylisothiourea (SMT)) alone and in combination with TMZ. Cell viability was evaluated by MTS assay after 5 days of treatment. Number of CSC was determined as sphere forming efficiency (SFE), in low attachment conditions supplemented with B27 in absence of fetal serum bovine. In 2D SMT and 1400 W (10 and 50 µM) did not affect U87 viability. TMZ

(500 µM) alone or combined with iNOS inhibitors (50 µM) reduced 50 % cell viability ($p<0.05$). Regarding CSC, it was observed that iNOS inhibitors (25 µm) decreased 50 % SFE ($p<0.01$). TMZ alone (100, 250 µM) was able to inhibited 25 and 63 % SFE respectively ($p<0.01$), while the effect was even higher when 250 µM TMZ was combined with 25 µM SMT, reducing 78 % the SFE ($p<0.001$). These results shows that inhibition of NO through pharmacological iNOS inhibitors could be a potential therapeutic target in GBM since reduced U87 cell growth in 2D and affected stem cell compartment, described as responsible of tumor recurrence. These results suggest that NO inhibitors could be considered useful to be combined with conventional therapy in order to reduce disease relapses. Even though, more investigation in this field is needed.

0379 - DRUG REPURPOSING OF ß-BLOCKER PROPRANOLOL IN OSTEOSARCOMA: PRECLINICAL ANTITUMOR EFFICACY ON 2D/3D CELL GROWTH, CHEMOTAXIS AND XENOGRAFT PROGRESSION, ALONE OR IN COMBINATION WITH STANDARD-OF-CARE CHEMOTHERAPY

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Osteosarcoma (OS), a bone cancer which primarily affects adolescents and young adults, is considered a clinical challenge due to its aggressiveness, rapid progression and limited response to standard of care therapies (SoC) such as cisplatin and methotrexate. Propranolol (PPN) is a non-selective ß-adrenergic receptor (ß-AR) antagonist originally used in the treatment of diverse heart diseases. Given that ß-AR signalling regulates many cellular processes involved in the initiation and progression of cancer, multiple efforts have been made to repurpose PPN in indications such as breast cancer, melanoma and angiosarcoma. Considering the unsatisfied clinical needs of OS, the objective of this work was to evaluate the in vitro/in vivo antitumoral activity of PPN as a monotherapy and/or in combination with SoC chemotherapy, in highly aggressive MG-63 and U-2OS human OS cells. PPN blocked promitogenic ß-AR activation by catecholamines in OS cells and drastically reduced clonogenic growth, chemotaxis and proliferation of exponentially-growing OS cells (IC50= 45 µM; $p<0.001$). Furthermore, 3D tumor spheroid growth was completely inhibited by PPN after a 7-day treatment. Synergistic cytostatic effects (CI <1) were observed after combining PPN (10 and 50 µM) with different optimal and suboptimal concentrations of cisplatin or methotrexate for 72 h. In animals bearing growing OS s.c. xenografts sustained treatment during 4 weeks with PPN (10 mg/kg i.p. daily), alone or in addition to cisplatin (suboptimal dose of 2 mg/kg i.p., three times per week), markedly abrogated tumor progression, exhibiting modulation of local tumor aggressiveness and reducing tumor growth rates by 25 or 75 %, respectively ($p<0.01$). Cisplatin treatment alone failed to inhibit OS xenograft growth. Conclusions. PPN showed a robust antitumoral activity alone or in combination with SoC chemotherapy in different OS preclinical models.

0406 - C-TERMINAL-TRUNCATED AND FULL LENGTH HEMEOXYGENASE-1 EXERT OPPOSITE BEHAVIOR OF HEAD AND NECK CANCER CELLS

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We previously reported that heme oxygenase-1 (HO-1) protein is up-regulated in human HNSCC samples and that it is localized in the cytoplasmic and nuclear compartments. We also reported that high expression of HO-1 mRNA is associated with worst survival and that pharmacological activation of HO-1 by hemin increases viability of HN13 cells. However, how full length (FL-HO1) and C-terminal truncated (t-HO1) HO-1 affects HNSCC remains elusive. In this study, we aim to elucidate if such forms of HO-1 impacts on head and neck cancer cells behavior. We established the FL-HO1 and t-HO1 overexpressing HN13 cells. We evaluated cell viability by crystal violet method, cell cycle progression by propidium iodide staining and flow cytometry, and cell migration by wound healing assay. In addition to our previous results using hemin, we found that 80 µM hemin increased cell number in S- ($p<0.001$) and G2/M ($p<0.001$) phases and diminished cell number in Go/G1 phase ($p<0.001$) at 72h. We also found that hemin delayed cell migration ($p<0.01$) respect to control. On the contrary, at same conditions, hemin failed to increase cell viability ($p>0.05$) neither alters cell cycle progression ($p>0.05$) in the normal keratinocyte cell line, HaCaT. By a genetic approach, we found that FL-HO1 HN13 cells have a higher growth rate ($p<0.001$) than its control and cell cycle progression is as similar as ($p<0,001$ vs control) it was observed with hemin treatment. However, FL-HO1 failed to alter migratory capacity ($p>0.05$). We also found that t-HO1 expression impaired HN13 cell viability ($p<0.01$ vs. FL-HO1 HN13) and induces a Go/G1 arrest ($p<0.01$) and a diminished cell number in SubGo ($p<0.01$) and S- ($p<0.05$) phases. Also, we found that t-HO1 expression delayed cell migration ($p<0.001$) respect to FL-HO1 HN13. In conclusion, our results show that head and neck cancer cells survival, cell cycle progression and migration capacity depends on predominant HO-1 form.

0407 - NOVEL CALCITRIOL ANALOGUES EM1 AND UVB1 AGAINST AGGRESSIVE BREAST CANCER CELLS AS A MONOTHERAPY OR IN COMBINATION WITH PACLITAXEL.

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Despite chemotherapy remaining as a primary therapeutic option for aggressive breast cancer (BC), its effectiveness is limited by intrinsic or acquired resistance and associated adverse effects. Therefore, new therapeutic strategies are needed. Previously, we demonstrated that the calcitriol analogue EM1 decreases the viability, migration and invasion of the 4T1 triple-negative BC (TNBC) cells. Additionally, we reported that UVB1, another calcitriol analogue synthetized by our group, reduces the viability of cells derived from the TNBC - Patient-Derived Xenografts (PDX). Hence, the aim of the present study was to continue evaluating the antitumoral effects of the calcitriol analogues EM1 and UVB1 on aggressive BC cells, alone or in combination with low concentrations of Paclitaxel (PTX). We found a synergistic effect by combining EM1 or UVB1 with non-effective PTX concentrations on viability of 4T1 cells. The resulting Combination Index values of Chou & Talalay method were 0.80059 and 0.13491 for EM1-PTX and UVB1-PTX combinations, respectively. In addition of our previous result on 4T1 cell migration, EM1 displayed antimigratory effects on MDA-MB-231 TNBC cell line ($p<0.001$). In contrast, UVB1 had no effect on these cells. However, interestingly, the

combination of the analogues with non-effective concentrations of PTX over 4T1 cell migration displayed a better effect than drugs alone (EM1-PTX: p<0.05; UVB1-PTX: p<0.001). Finally, a pilot in vivo assay was conducted to test the sensitivity of the TNBC-PDX410 to UVB1. A reduction in in vivo tumor volume was detected after 18 days of UVB1 treatment at 40 µg/kg of body weight administrated three times a week (p<0.05). Altogether, these results suggest the potential use of these vitamin D analogues as antitumor agents, alone or as a complement to conventional chemotherapy.

0408 - ANTITUMORAL EFFECTS OF PLEUROTUS OSTREATUS I-FRACTION IN BREAST CANCER

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Mushrooms are considered as "small pharmaceutical factories" producing hundreds of bioactive compounds, many of which have shown to exert antitumoral activity in different types of cancer. Argentina has a high mushrooms diversity and important scientific-technological development applied to its cultivation. However, the antitumoral phytotherapeutic potential of edible mushrooms cultivated in our country has not yet been considered. In this context, the purpose of the current study is to determine the antitumoral activity in breast cancer of Pleurotus ostreatus I-Fraction, an extract of water-soluble polysaccharides obtained from fruiting body, initially evaluating its potential immuno-independent antitumoral activity. To achieve the proposed objective, we employed a murine mammary adenocarcinoma 4T1 cells line and performed cell viability assays by colorimetric assay with crystal violet, cell cycle analysis by flow cytometry, and wound healing assay. We found that P. ostreatus I-Fraction at concentration from 2.5 mg/mL and ranging from 1.0 to 2.5 mg/mL decreased the viability of 4T1 cells in a concentration-dependent manner, at 24 hours and 48 hours respectively (p<0.001). These results also demonstrate a time-dependent effect of I-Fraction on 4T1 cells viability. In addition, P. ostreatus I-Fraction (2.5 mg/mL, 48 h) increased the number of 4T1 cells in the subG0/G1 phase (I-Fraction= 9.05 vs. vehicle= 2.3 %, p <0.001) and decreased those in the G0/G1 phase, compared to vehicle (I-Fraction= 42.3 vs. vehicle= 48.77 %, p <0.001). These results suggest that I-Fraction decreases 4T1 cell viability through an induction in cell death, without affecting cell cycle progression. By another hand, we found that I-Fraction decreased migratory capability of 4T1 cells at 13 h of treatment, compared to vehicle (p <0.01). In conclusion, these results demonstrate the antitumor activity of Pleurotus ostreatus I-Fraction on breast cancer cells.

0409 - P300 INVOLVEMENT IN METASTATIC PROCESS OF TRIPLE NEGATIVE BREAST CANCER

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Triple negative breast cancer (TNBC) are a heterogeneous group of tumors which lack specific molecular targets. Therefore, it is necessary to investigate potential tumor markers for this subtype of BC. Recent studies indicate that p300 has a pro-metastatic role in BC and we have previously shown that inhibition of p300 decreases cellular migration and invasion in a TNBC cell line. Therefore, in this work we aimed to analyze the expression and localization of p300 and its association with markers of tumor progression and clinic-pathological parameters in human TNBC. Also, we investigated the molecular mechanisms through which p300 inhibition impaired the processes previously mentioned. In TNBC biopsies (n= 45), we found that higher levels of cytoplasmic p300 correlates with lower tumor stages and a better overall patient survival (IHC, p<0.05). In TNBC (MDA-MB-231) and hormone-independent BC (LM3) cell lines, the genetic silencing of p300 induced an increase in the levels of membrane E-cadherin, a decrease of nuclear ß-catenin and in the number of stress fibers compared with the control (IF, p<0.05). In a mouse xenograft model of MDA-MB-231 we found an increase in E-cadherin and a decrease in nuclear ß-catenin expression (IHC, p<0.05) in the tumors of animals injected with VV59, a specific pharmacological inhibitor of p300. Also, in such group of mice, we found a significant reduction in the number of lung metastases respect to control group (p<0.05). Altogether these results demonstrate an antitumor role for p300 inhibition or cytoplasmic translocation in TNBC.

0415 - TUMOR MICROENVIRONMENT: EFFECT OF METRONOMIC CHEMOTHERAPY WITH CYCLOPHOSPHAMIDE (CY) AND LOSARTAN (LOS) ON M-406 MURINE MAMMARY ADENOCARCINOMA

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Metronomic chemotherapy (MCT) refers to the chronic, equally spaced, delivery of low doses of chemotherapeutic drugs, without extended interruptions. Drug repositioning (DR) in oncology refers to the use of drugs originally formulated for other indications that showed antitumor potential. CY is an alkylating drug with toxic action on proliferating cells. LOS is an antagonist of angiotensin II receptor, used to treat hypertension. Tumor microenvironment is constituted by genetically stable cells that surround and feed the tumor, favoring sustained growth, invasion and metastasis. It was previously shown that MCT with CY+LOS inhibited M-406 growth and increased mice survival without general toxic effects. Our objective was to study the effect of metronomic CY+LOS treatment, on M-406 tumor microenvironment. CBi female mice were challenged s.c. with M-406 (day 0) and distributed on day 6, into 4 groups (n= 6-7/group). GI: Control, non-treated; GII: Treated with CY 25 mg/kg/day in the drinking water; GIII: Treated with LOS 150 mg/kg/day in the drinking water; GIV: Treated as GII+GIII. Mice weight and tumor volume were determined 3 times/week. When tumors reached the exponential growth phase, mice were euthanized, tumors excised and prepared for immunohistochemical analysis. Foxp3+ cells/field decreased significantly in GIV compared to GI (p<0.05), without showing changes in CD4+ and CD8+ lymphocytes among groups. There was a significant decrease of Ki67+ cells in GIV with respect to GI (p<0.05) while no modifications in apoptosis (TUNEL) were evinced. Collagen and a-SMA levels decreased in GIII and GIV, without reaching statistical significance. HIF1a+ cells/field decreased in GII and GIV groups compared to GI (p<0.05). In conclusion: the stimulation of the immune system, the inhibition of tumor cells proliferation and the decrease in markers of cancer associated fibroblast may be, at least in part, responsible for the therapeutic effect achieved by MCT with Cy + LOS.

0421 - MITOCHONDRIAL-DERIVED PEPTIDE HUMANIN AS A CYTOPROTECTIVE FACTOR IN BREAST CANCER CELLS

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We and others have previously shown that the mitochondrial-derived peptide Humanin (HN) exerts cytoprotection in normal and tumoral cells. HN can be secreted and bind to membrane receptors. Two HN receptors have been identified: (i) a trimeric receptor composed by the ciliary neurotrophic factor receptor (CNTFR), the IL27R (WSX-1) and the 130 kDa glycoprotein (gp130), and (ii) the formyl peptide receptor-like 1 (FPRL-1 or FPR2). We have previously observed that exogenous HN facilitates tumor progression and chemoresistance in experimental triple negative breast cancer (TNBC). Here we performed bioinformatics analysis of transcriptomic databases to assess the expression of mRNA of HN and its receptors in breast cancer specimens. We found expression of HN mRNA in normal human breast tissue and breast tumors specimens of all types, i.e. luminal A, luminal B, HER2+, and TNBC. HN receptors mRNA were also detected in human breast tumors. While the expression of the trimeric receptor subunits was similar in all subtypes of breast tumors, FPR2 expression was highest in TNBC (*p<0.05, ANOVA follow by Tukey test). When we assessed the effect of HN on the response of HER2+ breast tumor cells, we found that HN (20 µM) inhibited the cytotoxic effect of Doxorubicin (50 µM), as assessed by MTT (ANOVA, *p<0.05) and TUNEL (x^2 test *p<0.05). We next evaluated the effect of HN on the secretion of immunosuppressive interleukin-10 (IL-10) and the production of angiogenic factors in HER2+ and TNBC cells. HN (10 µM) decreased IL-10 release from LM3 cells. Conditioned media of LM3 and 4T1 cells that were incubated with HN (10 µM) inhibited the proliferation of endothelial cells EA.hy926 (ELISA BrdU, *p<0.05, ANOVA).Our results suggest that the protumoral action of HN may result from a direct cytoprotective action on breast tumor cells rather than being mediated by an effect on the tumor immunosuppressive phenotype or proangiogenic capacity.

0425 - EFFECT ON THE TUMOR MICROENVIRONMENT OF METRONOMIC CHEMOTHERAPY WITH CYCLOPHOSPHAMIDE (CY) AND LOSARTAN (LOS) IN A MURINE MODEL OF MAMMARY ADENOCARCINOMA

Cintia KAUFMAN | Mónica Carolina GRILLO | Maria Virginia BAGLIONI | Antonela DEL GIÚDICE | Viviana Rosa ROZADOS | Olga Graciela SCHAROVSKY | María Jose RICO | Leandro E MAINETTI

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Metronomics, refers to all anticancer treatment regimens combining metronomic chemotherapy (chronic, low dose, no extended rest periods drug administration) and drug repositioning (the use of drugs originally formulated to other indications that showed antitumor effect). CY (drug with cytotoxic action on proliferating cells) and LOS (angiotensin II receptor antagonist, used to treat hypertension) were used in a metronomics schedule. We have demonstrated that the administration of metronomic CY+LOS in the triple negative M-234p mammary adenocarcinoma tumor model, inhibited tumor growth, increasing the survival rate without toxicity. Our aim was to study the effect of MCT with CY+LOS in M-234p tumor microenvironment. Female BALB/c mice were challenged s.c. with M-234p (day 0) and on day 6 divided in 4 groups (n= 6-10/group). GI: Control, with no further treatment; GII: Treated with CY 25 mg/kg/day in the drinking water; GIII: Treated with LOS 200 mg/kg/day in the drinking water; GIV: Treated as GII+GIII. Mice were weighted and tumor volume measured 3 times/week. When tumors were exponentially growing, they were excised and used for immunohistochemistry and flow cytometry. GII and GIV exhibited a decreased number of HIF1a+ cells vs. GI (p<0.001). Stromal a-SMA (smooth muscle actin) and intratumor collagen area were smaller in GIV than in GI (p<0.05). At day 31, flow cytometry of tumor samples showed no differences in the number of CD4+, CD8+, IL-17+ and Foxp3+ cells among groups. At day 42, GI and GIII mice had already been euthanized and tumor volume in GIV was lower than in GII (p<0.01) and the number of tumor IL-17+ cells was higher and Foxp3+ cells lower in GIV than in GII (p<0.05, p<0.001, respectively). We conclude that the antitumor effect of MCT with CY+LOS could be, in part, a result of tumor microenvironment modifications such as the decrease in hypoxia, intratumor collagen and cancer associated fibroblasts, and the stimulation of the antitumor immune response.

0426 - METFORMIN (M) AND PROPRANOLOL (P) ACT ON DIFFERENT STEPS OF THE METASTATIC CASCADE OF A TRIPLE NEGATIVE MURINE MAMMARY ADENOCARCINOMA

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IGE, FACULTAD DE CIENCIAS MÉDICAS, UNR (1); IDICER-CONICET (2)

Drug repositioning in oncology refers to the application of drugs designed for other uses that showed antitumor effect. Metformin is an antidiabetic drug and propranolol is a ß-blocker. We have reported that M+P treatment reduced tumor growth and lung metastases development in two triple negative mammary tumor models. Also, we found that M+P combination showed a potential adjuvant effect in both in vivo models. To further deepen our studies, we aimed to explore the effect of M+P on different events of the metastatic cascade in triple negative 4T1 mouse tumor model. We extracted blood from BALB/c mice bearing tumors in the exponential growth phase of: C (control) and M+P [treated with M (2g/l) + P (25mg/l)] groups. A lower percentage of epithelial cells was found in M+P group (flow cytometry, p= 0.06). To characterize survival and extravasation of circulating tumor cells, mice were i.v inoculated with $5x10^4$ green tracker labeled cells and treated as C or M+P. After 2 days, M+P group showed lower percentage of circulating tumor cells (p<0.05). Lungs of mice treated with M+P had fewer green cells than C (confocal microscopy, p<0.05). Also, mice were challenged i.v. with $5x10^4$ cells and randomly treated as C or M+P. After 30 days mice were euthanized, lungs excised, metastatic foci measured and then, used for histological (HE staining) and immunohistochemical (Ki67) analysis. The percentage of Ki67+ cells was lower in M+P than in C metastases (p<0.05); likewise, total metastatic burden decreased in M+P group (p<0.05). Furthermore, preliminary studies suggest that M+P treatment could affect the expression of some markers (E-cadherin; SNAIL) of Epithelial Mesenchymal Transition (EMT). Altogether, our studies indicate that M+P treatment affects different steps that lead to the development of metastasis, suggesting a putative use of this drug combination as adjuvant therapy. Further studies on EMT markers should be addressed to understand in depth the antimetastatic effect of M+P.

0432 - STUDY OF THE ANTINEOPLASTIC ACTIVITY OF PRODUCTS DERIVED FROM CELLULOSE-CONTAINING MATERIALS

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Breast cancer is a major public health problem being the second cause of cancer related death in developed countries. In order to propose new treatment alternatives, we have explored the effect of Levoglucosenone and its derivatives in preclinical settings. Levoglucosenone results from pyrolytic treatment of cellulose-containing materials, and it has been used for the synthesis of

compounds with different biological activities but its usefulness in oncology remains unexplored. Here, we have evaluated the effect of levoglucosenone (compound 1) and is derivatives (compounds 2, 3 and 4) on human (MCF7) and murine (LM3) mammary tumor cell lines. All compounds showed a strong antiproliferative effect, with an inhibitory concentration 50 (IC50) of 50 and 12 µM for MCF7 and LM3 respectively. Since through flow cytometry we observed only a slight increase in the sub-G0 fraction of the cell cycle, we decide to study the mechanisms involved in the reduced cell number observed. First, we analyzed the effect on senescence (b-gal assay) and autophagy (Beclin I and LC3 expression). No modulation of both processes could be detected even after 72 h retreatment. Using the fluorophore (TMRM) we observed that the compounds induce the loss of mitochondrial membrane potential which could lead to the intrinsic apoptosis pathway. Moreover, as an energetic compensatory process, a 2-fold increase of glucose consumption and lactate production was detected ($p<0.05$ ANOVA). Finally, in vivo studies employing LM3 cells were performed to evaluate whether the pre-treatment with the compounds has an effect on lung colonization. Compounds 1 and 2 highly reduced lung metastasis, [median (range): 2,5 (0-10) and 7 (3-8) respectively]. While compounds 3 and 4 had no effect as compared to control treatment [median (range): 58 (12-70), $p<0.05$ Kruskal-Wallis test]. Based on our results, we believe that our compounds could become in the future an important alternative for breast cancer management.

0444 - ENHANCEMENT OF IN VITRO TUMOR CELL SENSITIVITY IN AN ANAPLASTIC THYROID CANCER CELL LINE BY VALPROIC ACID

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Histone deacetylase inhibitors (HDACi) have emerged recently as promising anticancer agents targeting histone and nonhistone proteins. Many studies show as well that HDACi are effective radiosensitizers in various types of malignancies. Anaplastic thyroid cancer (ATC) is a rare and aggressive malignancy. Radiotherapy (RT) is one of the main modalities of treatment for ATC. RT either as altered fractionation or in combination with chemotherapy has an important role in achieving local control. Our purpose was to study the combined effect of the HDACi, valproic acid (VA), and ionizing radiation in an anaplastic thyroid cancer cell line (8505c). Cell viability was evaluated by MTT assay. Cells were pretreated with 1 mM VA and gamma irradiated at different doses. Radiation response was analyzed by clonogenic assay. Cell cycle and cell death were measured 24 and 48 h after irradiation. The microARN (miRNA) expression profile was also evaluated. VA radiosensitized cancer cells as evidenced by the reduction of survival fraction at 2 Gy from 58.4 ± 2.1 to 37.9 ± 2.6 in the treated cells ($p<0.001$). A G2/M phase cell cycle arrest was observed in all the irradiated cells (2 Gy) 24 h after the combined treatment ($p<0.05$). On the other hand, VA treatment increased apoptotic cell death 24 h after irradiation ($p<0.01$). We found 31 differentially expressed miRNAs between the irradiated cells and those pretreated with VA and irradiated. Among these, miR-27a-3p (fold change: -3.95, p= 0.0082), miR-26a-5p (fold change: -2.12, p= 0.0065) and miR-486-5p (fold change: 3.14, p= 5.24E-05) were associated with genes linked to pathways like DNA damage, cell cycle and cell death. Our findings suggest that VA can enhance the radiosensitivity of ATC cells. This effect could be mediated by differentially expressed miRNAs.

0445 - ALL-TRANS RETINOIC ACID AND LAPATINIB COMBINED TREATMENT IMPAIR BREAST CANCER STEM CELLS GROWTH

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Cancer stem cells (CSC) are resistant to both chemo and radiotherapies and are also considered as metastasis seed. Previously, we observed that CSC derived from HER2 negative breast cancer cells overexpress HER2. In order to validate a novel therapeutic strategy targeting the CSC component, we have analyzed the effect of all trans retinoic acid (ATRA) and Lapatinib (Lp) treatments on growth and cell cycle distribution of primary mammospheres derived from 4T1, HCC-70 and MCF-7 cell lines, that do not overexpress HER2. Mammospheres were treated for 96 h with Lp 1 µM (4T1 and HCC-70 cells) or 5 µM (MCF-7), combined or not with ATRA 1 µM. In triple negative cell lines (HCC70 and 4T1), ATRA and Lp combined treatments strongly reduced mammosphere growth as compared to each treatment alone (determined evaluating mammospheres diameter, number and morphology under microscopy). Moreover, the combined treatment induced cell cycle arrest at the G0/G1 phase, after 48 h of treatment in 4T1 mammospheres, analyzed by flow cytometry. On the other hand, in the HER2 negative cell line (MCF7), no effect was observed on mammospheres growth under different treatments. However, ATRA and Lp combined treatment induced cell cycle arrest at the G0/G1 phase, at longer times (72 h). In the present work we have demonstrated that ATRA and Lp combined treatment can successfully reduce breast CSC growth and induce cell cycle arrest providing in vitro evidences for the potential use of this combined therapy in HER2 negative breast cancer.

0451 - COMBINED ATTENUATION OF ANTI-APOPTOTIC BCL-2 FAMILY MEMBERS BCL-XL AND MCL-1 ACTIVITIES DISTINCTIVELY SENSITIZE PATIENT-DERIVED GLIOMA STEM CELLS TO CONVENTIONAL CHEMOTHERAPY IN A CELL LINE SPECIFIC MANNER.

Mariana Belén VERA | Germán Ignacio NOGUEIRAS | Olivia MORRIS HANON | Gustavo Emilio SEVLEVER | María Elida SCASSA | Guillermo Agustín VIDELA RICHARDSON

FLENI-CONICET

High-grade gliomas are the most prevalent and malignant primary brain tumors. They display a hierarchical arrangement with a minor subpopulation of self-renewing, highly tumorigenic glioma stem cells (GSCs) that contribute to tumor initiation and therapeutic resistance. This resistance is often due to altered expression of Bcl-2 family members. Antagonism of anti-apoptotic Bcl-2 members through BH3-mimetics is emerging as a novel anti-cancer therapy. BH3-mimetics imitate pro-apoptotic BH3-only proteins resulting in initiation of apoptosis. Recently, we found that the expression levels of the pro-apoptotic BH3-only Noxa determine the sensibility of patient-derived GSCs to BH3 mimetics. We observed that cells displaying low levels of Noxa are less sensitive to BH3 mimetics targeting Bcl-xL (ABT-263 or WEHI-539). Noxa preferentially neutralizes anti-apoptotic Mcl-1, thus we evaluated the implication of this pro-survival factor in the sensibility of GSCs to combined therapies that include Bcl-xL inhibitors. Firstly, we determined the expression of the anti-apoptotic members: Bcl-2, Bcl-xL, Bcl-w, Bfl-1 and Mcl-1 in 5 GSCs lines and found that each cell line exhibit a similar anti-apoptotic molecular signature, with Bcl-xL showing the highest expression followed by Mcl-1. As Mcl-1 inhibits apoptosis by interacting with BH3-only proteins like Bim, Bid, Puma and Noxa, we also determined the expression of these BH3-only. Using siRNA-mediated gene silencing, we studied the contribution of Mcl-1 in GSCs survival after exposure to the antineoplastic agents such as temozolamide, lomustine and vincristine. Propidium iodide staining revealed that downregulation of Mcl-1 exacerbated cell death, in a cell line-specific manner, only when chemotherapy was accompanied by Bcl-xL inhibition. These results were further confirmed when Mcl-1 inhibitor A1210477 was used. Together, our

findings suggest that Bcl-xL and Mcl-1 provide duplicate safeguard measures in maintaining glioma CSCs integrity.

0455 - ANALYSIS OF THE EFFECT OF TWO NOVEL PROPARGYLAMINES ON HUMAN TRIPLE NEGATIVE BREAST CANCER CELLS

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The lack of effective therapies for some types of cancer, the development of drug resistance and the severe side-effects of the chemotherapeutic agents reduce the clinical efficacy of a large variety of anticancer agents. Thus, there is always a constant need to develop alternative or synergistic anticancer drugs. Previously, we demonstrated the antitumoral activity and apoptotic effect of two propargylamines, MMA06 (06) and MMA4210f3 (4210) in MDA-MB-231 cells. Our aim was to analyse their mechanism of action and evaluate their combined action with Mafosfamide (MAF). The effect of 06 and 4210 (5-100 µM) on colony formation was analyzed using MDA-MB-231 human breast cancer cells. Both compounds decreased the number of colonies being the effect of 06 better than 4210 at concentrations higher than 30 µM ($p<0.05$); they also reduced the colonies sizes ($p<0.05$). In addition, the compounds tested at 10 and 30 µM, diminished cell migration evaluated by the wound-healing assay ($p<0.05$). Then, we studied the combined action of MAF (2.5-20 µM) + 06 or 4210 (300 and 500 µM) on MDA-MB-231 and MC3T3-E1 normal pre-osteoblast murine cell line. The combined treatment produced a higher decrease in MDA-MB-231 cells viability, with respect to cells treated with MAF ($p<0.05$). On normal cells, 06 (500 µM) and 4210 (300-500 µM) moved partially backwards the decrease of cell viability induced by MAF (5 and 10 µM)($p<0.05$).Also, these propargylamines showed a high selectivity index (IC50 for normal cells/IC50 for cancer cells): 3.38 and 1.91 for 06 and 4210, respectively. In conclusion, 06 and 4210 are able to decrease the formation of colonies and the motility of tumor cells. Interestingly, these new propargylamines are, not only endowed of an antitumor capacity, but also able to protect normal cells of the cytotoxic action of a chemotherapeutic drug, a result that warrants the study in in vivo tumor models.

0456 - CHARACTERIZING THE ROLE OF VAV2 IN MELANOMA.

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Skin cancer is one of the most common of all human cancers. The most dangerous form of this type of cancer is melanoma, which is associated with an increasing incidence in the population. Vav proteins are guanosine nucleotide exchange factors (GEFs) of the Rho GTPase family. As GEFs, they modulate processes highly associated to the development of cancer and metastasis, mostly through their GTPase regulatory function, but their involvement in melanoma is yet to be elucidated. In our work we explored the role of Vav2, a member of Vav family proteins, in events associated to melanoma development. By shRNA techniques and the use of mammalian expression vectors, we were able to modulate Vav2 expression in the mouse derived melanoma cell line B16-F0. By MTT and cell-counting based methods we observed that decreased levels of Vav2 lead to proliferation defects ($p<0.01$) while both, increased levels of Vav2 or the expression of a catalytically active version of this protein, promoted proliferation of melanoma cells ($p<0.05$ and $p<0.01$, respectively). Accordingly, by Western blot (WB) analysis, we observed that Vav2 was required for the activation of Erk after proliferative stimuli. By fluorescent staining and confocal microscopy, we noted that Vav2 modulation promoted actin cytoskeleton changes in melanoma cells. Wound healing assays demonstrated that decreased expression of Vav2 lead to migratory defects ($p<0.01$), while catalytic activation of this protein promoted a strong migratory phenotype ($p<0.001$). Surprisingly, by WB we noticed that Vav2 in melanoma cells was required to maintain the expression of epithelial markers like E-cadherin and B-catenin. Altogether, our data indicate a putative role for Vav2 in the control of several processes associated to melanoma growth and the development of metastasis.

0457 - PHOTODYNAMIC THERAPY IN MELANOMA: ENDOCYTIC UPTAKE OF A CATIONIC ZN(II) PHTHALOCYANINE AND ROLE OF MELANOSOMES

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Melanoma is an aggressive form of skin carcinoma, highly resistant to traditional therapies. Photodynamic therapy (PDT) is an alternative treatment modality, which combines a photosensitizer, visible light and molecular oxygen to produce reactive oxygen species that selectively destroy target cells. We have previously demonstrated that the cationic zinc (II) phthalocyanine Pc13 is a potent photosensitizer that localizes in mitochondria and lysosomes and promotes melanoma cell death after irradiation. In order to elucidate the mechanism underlying Pc13 phototoxicity, we first studied the internalization pathway of this phthalocyanine. Incubation of B16F0 cells at 4°C significantly reduced Pc13 uptake, suggesting the participation of an endocytic mechanism. When cells were preincubated with specific inhibitors of dynamin (Dynasore) or caveolae (Nystatin), cell death was partially prevented after PDT, as determined by hexosaminidase method. Similar results were obtained when cells were transfected with dominant negative mutants of dynamin and caveolin. Conversely, blockage of clathrin pathway did not affect Pc13 phototoxicity. Since melanosomes are lysosome-related organelles, we further examined Pc13 localization in these vesicles. By confocal microscopy we demonstrated that Pc13 targets melanosomes and damage in these structures was observed by TEM 1 h after irradiation. Therefore, the role of melanin in Pc13 phototoxicity was studied employing phenylthiourea (PTU), a melanin synthesis inhibitor. While short preincubation with PTU (3 h) produced a 23 % increase in cell viability after PDT, longer periods of inhibition (48 h) led to cell depigmentation and enhanced cell death. In conclusion, we demonstrated that Pc13 is internalized by a dynamin and caveolin-dependent endocytic mechanism. Melanosomes represent one of the primary sites of photodamage. Modulation of PDT sensitivity by inhibition of melanogenesis could have clinical relevance for melanoma treatment.

0459 - EXPLORING THE ROLE OF PARKINSON DISEASE-RELATED PROTEINS IN PROSTATE CANCER.

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Synucleins are small proteins expressed primarily in neural tissue and in certain tumors. Alpha-synuclein (aS) was recently connected to melanoma development and gamma-synuclein (gS) was associated to a wide range of cancer types. Both proteins are implicated in the development of Parkinson disease (PD). Interestingly, recent studies have shown a direct association between PD and the development of melanoma and prostate cancer. Our goal in this work was to explore the putative role aS and gS could have in the development of prostate cancer. We analyzed the expression of aS and gS in human prostate cancer cell lines (PC3, DU145 and LNCaP). By Western blot (WB) and immunocytochemistry we observed that both proteins were expressed in these cell lines, and by differential lysis experiments with detergents we determined that aS was preferentially present at the cytosol, while gS was detected mainly at the membrane fraction of PC3 cells. By standard shRNA techniques and the use of expression vectors, we modulated aS and gS expression in PC3 cells ($p<0.01$ for both proteins by WB). MTT-based proliferation assays demonstrated no effect of the expression of these proteins on cell proliferation ($p>0.05$). Nevertheless, by wound healing assays, we observed that PC3 cells overexpressing gS showed a more migratory behaviour than control cells ($p<0.01$). To analyze if prostate cancer cells were able to incorporate exogenous added aS as it is known for neuronal cells, we incubated PC3 with different aggregation species of this protein. By WB we observed that PC3 were able to incorporate both monomeric and aggregated aS ($P<0.01$). Indeed, by MTT-based studies, we realized that aS was not toxic for prostate cancer cells at the same concentrations compromising viability of a neuroblastoma cell line (SH-SY5Y; $p<0.05$). Altogether our results indicate a putative role for aS and gS proteins in prostate cancer. Further studies should be addressed to confirm and complement our observations.

0460 - IN VITRO AND IN VIVO ANTIMETASTATIC EFFECT OF A PENICILLIN DERIVATIVE IN MURINE MELANOMA CELLS.

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In a previous work, we demonstrated that TAP7f, an antitumor penicillin derivative formed by penicillin linked to the dipeptide Leu-Phe through a triazole group, inhibited cell adhesion, migration and invasion of highly metastatic B16F10 melanoma cells. TAP7f also exhibited antiangiogenic properties and reduced the expression levels of ß-catenin and metalloproteinases (MMPs) -2 and -9. To further explore TAP7f mechanism of action, we investigated by RT-qPCR whether the decrease in protein levels was caused by the inhibition of mRNA expression. Results showed a reduction in mRNA levels of both MMP-2 (58 ± 18 %) and -9 (46 ± 6 %), after 18 h of treatment with a 10 µM concentration of TAP7f. Under the same experimental conditions, TAP7f significantly reduced the mRNA levels of ß-catenin downstream targets cyclin-D1 (36 ± 9 %) and c-Myc (63 ± 18 %). Since increased amounts of integrin alfaV beta3 have been related to a higher metastatic potential of melanoma cells, we next studied the effect of TAP7f on integrin alfaV beta3 expression levels. RT-qPCR assays showed that TAP7f (10 µM) downregulated the mRNA levels of integrin alfaV (42 ± 8.3 %) and beta3 (37 ± 14 %). By flow cytometry, we demonstrated that TAP7f induced a decrease in the membrane expression of both integrin subunits. Based on results obtained in vitro, we explored TAP7f effect in an experimental lung metastasis model. C57BL/6J mice injected intravenously with B16F10 cells pretreated with 10 µM of TAP7f exhibited a ~50% reduction of lung nodules ($p<0.01$). Hematoxilin-eosin staining of lung tissue sections showed 1-2 tumor islands/lung section for treated mice and 5-6 tumor islands/lung section for control. In conclusion, our results revealed that TAP7f inhibited molecular signals related to a metastatic process and reduced melanoma lung metastasis in vivo. These findings provide new evidence for the development of TAP7f as a potential agent for the treatment of melanoma.

0461 - IN VITRO AND IN VIVO SYNERGISTIC ANTITUMOR EFFECT OF THAPSIGARGIN AND A PENICILLIN DERIVATIVE IN MURINE MELANOMA CELLS

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The triazolyl peptidyl penicillins (TAPs) are novel hybrids compounds having in their structure a penicillanic core linked to a peptide portion via a triazole group. In a previous study, we showed that the derivative containing the dipeptide Leu-Phe (TAP7f) activates an endoplamic reticulum (ER) stress response and induces apoptosis of murine B16-F0 melanoma cells. In this work, we first demonstrated the contribution of ER stress to the antitumor effect of TAP7f. When cell viability was determined after pre-incubating cells with a specific siRNA for CHOP, a protein involved in ER stress signaling, cell growth increased from 25 ± 2% to 40 ± 1% ($p<0.05$). Based on the usefulness of combined therapies for cancer treatment, we decided to investigate the in vitro effect of TAP7f with thapsigargin, a well-known ER stress activator. The simultaneous incubation of different concentrations of both compounds showed a higher inhibition of cell growth with respect to the effect of each individual agent. The quantitative analysis of dose-effect curves obtained by using the Compusyn software rendered combination indexes between 0.2-0.6, indicating synergism. We further evaluated the in vivo efficacy of TAP7f/thapsigargin in a mouse melanoma model. B16-F0 cells ($1x10^5$) were injected subcutaneously in the right flank of each mouse. 10-12 days after cell inoculation, mice were treated with vehicle, TAP7f (4 mg/kg), thapsigargin (0.3 mg/kg) or both compounds for 8 days via i.p. A significant reduction of tumor volume (60 %, $p<0.05$) was obtained only for the combined treatment, whereas tumors from mice receiving just TAP7f or thapsigargin were similar to control mice. In conclusion, our results revealed the in vitro and in vivo efficacy of the combination of TAP7f with an ER stress activator, suggesting that this therapy might be considered a promising tool for the treatment of malignant diseases.

0466 - HO-1 INTERACTORS INVOLVED IN THE COLONIZATION OF THE BONE NICHE: ROLE OF ANXA2 IN PROSTATE TUMORIGENESIS.

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Currently our view of cancer includes the tumor microenvironment. The dialogue between the tumor cells and its microenvironment is determinant of the characteristics of the tumor progression. Heme Oxygenase 1 (HO-1) is critical for cellular homeostasis. In prostate cancer (PCa), HO-1 may have a role

beyond its enzymatic activity. Using a proteomics approach, we found annexin2 (ANXA2) among HO-1 interacting proteins. The aim of this study was to analyze the relevance of ANXA2/HO-1 in PCa and bone metastasis. Using a transwell co-culture system we found that ANXA2 mRNA levels were significantly upregulated ($p<0.01$) in PC3 cells and down-regulated ($p<0.01$) in the pre-osteoclastic Raw264.7 cell line, when cells were co-cultured compared with cells grown alone. Immunofluorescence analysis shown a clear re-localization of ANXA2 in Raw264.7 from the membrane to the cytosol compartment under co-culture conditions, validated by a decrease of Ca^{2+} concentration in the conditioned medium ($p<0.05$). Pre-treatment of tumor cells with hemin, a specific inducer of HO-1, impaired both, ANXA2 translocation and the decrease in Ca^{2+} concentration. These results showcase HO-1 modulatory effect on the interaction between PCa and bone cells. To assess and validate the clinical significance of ANXA2 we performed a bioinformatics analysis using public database repositories. We identified low expression of ANXA2, strongly associated with poor prognosis across different PCa datasets. Multivariate analyses displayed high significant correlation with poor prognosis independent from Gleason grade (HR: 0.45; p= 0.006). For the Ross-adamas dataset (n= 280) low expression of HO-1 also correlated with poor prognosis. The expression correlation for ANXA2/HO1 was significant and positive and these genes appear to behave in a dependent manner. Thus, ANXA2/HO1 rises as a critical axis in PCa.

0467 - PRO-TUMOR ACTIONS OF SOLUBLE GUANYLYL CYCLASE ALPHA 1 SUBUNIT OVEREXPRESSION IN ECC-1 HUMAN ENDOMETRIAL CANCER CELL LINE

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Soluble guanylyl cyclase (sGC) is a heterodimeric enzyme constituted by two subunits, alpha1 and beta1 and is expressed in all cell types. sGC alpha1 subunit protein levels was shown to be moderate to high in most biopsies from human hormone-dependent malignant tumors. Previous results from our lab have shown that sGC alpha1 subunit knock-down significantly reduced cell proliferation, survival, and migration in estrogen-responsive and -unresponsive tumor cell lines. The aim of the present study was to investigate whether sGC alpha1 overexpression affects tumor progression in a human endometrial cell line (ECC-1). sGC alpha1 was overexpressed by using an adenoviral vector carrying the complete sequence of sGC alpha1 (sGCalpha1-myc) or empty virus as control. Two multiplicity of infection (MOI) were chosen: 100 and 250. Six h after virus infection, cells were incubated with complete media for 48 h. Cell cycle was studied by flow cytometry. Mitosis and apoptosis were assessed by nuclear morphology (Hoechst staining). Migration was determined through scratch motility assay. sGC alpha1 overexpression reduced the percentage of subG0/G1 DNA content (% of cells respect to control (C), C: 100 ± 0.39; MOI 100: 59.51 ± 3.04*; MOI 250: 73.72 ± 6.83*, $p<0.05$ vs. C) accompanied by an increase in the percentage of cells in S phase (C: 100 ± 12.72, MOI 100: 151.67 ± 13.82*; MOI 250: 129.32 ± 8.54*, $p<0.05$ vs. C). Nuclear morphology analysis confirmed that sGC alpha1 overexpression augmented mitotic index (MI as % of control, MOI 100: 117.4 %, MOI 250: 163.4 %*, $p<0.05$). Additionally, sGC alpha1 overexpression increased cell migration measured by wound closure (% of open wound as % of C; C: 100 ± 1; MOI 100: 62.63 ± 0.52***; MOI 250: 42.92 ± 2.79***, $p<0.001$ vs. C). Our results show that sGC alpha1 promotes cell proliferation and migration in ECC-1 human endometrial carcinoma cell line. This evidence together with previous results from our lab underlines sGC alpha1 as a relevant factor in tumor biology.

0474 - WHOLE EXOME SEQUENCING ANALYSIS OF CONSTITUTIONAL DNA IN ARGENTINEAN RETINOBLASTOMA PATIENTS

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Retinoblastoma (RB) is a pediatric tumor of the developing retina, caused by mutations in RB1 tumor suppressor gene. RB1 molecular alterations are small mutations in 80 % of cases and gross deletions/duplications in 20 %. In hereditary RB, identification of mutations is essential for genetic assessment. The purpose of this work was to detect small mutations in RB1 by whole exome sequencing (WES) in 7 Argentinean RB patients. WES was performed in leukocytes DNA from 2 familial and 1 sporadic bilateral and 4 early unilateral RB cases. The pathogenicity of the identified variants was determined according to: its presence in RB1 mutation database; its absence in sequence consortiums (ExAC and GnomAD); and predictive softwares. All pathogenic mutations were corroborated by Sanger Sequencing. Gross mutations were screened by MLPA. We have found an average of 13 sequence variants in RB1, 5 of them were present in all patients. We identified the disease-causing mutations in 2/7 cases. In one bilateral familial case, a nonsense mutation (g.70286C>A) in exon 12 was detected, the patient´s affected father showed the same mutation. The sporadic bilateral RB presented a substitution (g.5550G>A) in the last nucleotide of exon 2, according to previous reports this variant leads to aberrant splicing. In an unilateral RB patient, a likely benign inframe deletion was observed. The 4 unilateral and the other familial bilateral RB patients did not show pathogenic small mutations nor gross molecular alterations in RB1. Thus, screening was enlarged to genes associated with RB1 pathways and other tumors, but no deleterious variant was identified. We can conclude that WES was able to find constitutional mutations in RB1 gene, allowing confirmation of diagnosis and genetic assessment. Further studies must be performed in patients with no causative mutation found. Finally, the importance of this work relies on the fact that is the first one applying WES for RB molecular diagnosis in Argentina.

0482 - ESTABLISHMENT OF MAGEC2-KNOCKOUT CELLS THROUGH CRISPR/CAS9 TECHNOLOGY

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MageC2 is a member of the melanoma antigen gene (MAGE) family, specifically expressed in a wide variety of human cancers and associated with a non-favourable clinical course. It has been reported that MageC2 oncoprotein downregulates p53 and activates STAT3 transcription factors. Recently, we identified the Ras oncogene as responsible for MageC2 stability increase and phosphorylation through the MEK/ERK pathway. Then, our hypothesis is that Ras oncogene could regulate p53 and STAT3 by enhancing MageC2 protein levels. In order to analyze the functional consequences of endogenous MageC2 expression, we established a MageC2 knockout (KO) cell line through the CRISPR/Cas9 system in human melanoma A375 cells. All the obtained clones were probed for MageC2 protein expression by Western blot. Three clones were selected and sequenced for indel detection (KO1, gRNA1; KO2, gRNA1 and KO3, gRNA2). To validate the A375 MageC2 KO (A375-C2KO) clone behaviour, we quantified the mRNA levels of genes regulated by p53 or STAT3. Analysis of RT-qPCR data carried out in three pairs of biological replicates in A375-C2KO cells indicated enhanced transcription of p53 targets (p21waf1 and bax, $p<0.05$) and reduced levels of STAT3 targets (ccl2 and mmp2,

p<0.05) when compared to A375 WT cells, as expected. These results indicate that A375-C2KO cells recapitulate the signalling behaviour reported by overexpression and RNA interference approaches, and are therefore suitable as biological model to investigate the role of endogenous MageC2 expression in cancer cells.

0487 - CHARACTERIZATION OF VEMURAFENIB-RESISTANT MELANOMA CELL LINES

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In the last years, the incidence of melanoma, the most aggressive skin cancer, has increased rapidly. Approximately half of melanoma patients display the V600E mutation in the BRAF protein, which stimulates ERK activation and promotes proliferation. The FDA approves the use of 4 therapeutic agents that reduce ERK activity: i) Vemurafenib (VEM) and Dabrafenib (BRAF inhibitors) and ii) Trametinib and Cobimetinib (MEK inhibitors). Indeed, VEM is approved by ANMAT in Argentina. Unfortunately, the durability of the response is limited and the tumors quickly become resistant. The aim of this work was to generate and characterize VEM-resistant melanoma cell lines as tools to study possible resistance mechanisms in tumor cells. VEM-resistant cell variants were obtained by continuous exposure of original parental cells to increasing concentrations (0.01 – 1 µM) of VEM during 3 months. Inhibition of ERK signaling was analyzed by western blot and viability was determined by MTT assay. In addition, cell migration was evaluated in a modified Boyden Chamber. As assessed by inverted microscopy, Lu1205 melanoma-resistant cells exhibit a bigger volume and more prolongations and lamellipodia than their sensitive parents. In agreement, VEM-resistant Lu1205 cells showed greater migratory capacity than their parents under normal culture conditions. Moreover, in the presence of VEM (1 µM), levels of phospho-ERK were higher in resistant cells. On the other hand, no changes were detected in phospho-Akt levels, indicating that VEM only affects ERK signaling. In conclusion, the VEM-resistant melanoma cells obtained in our laboratory constitute a good model to study possible mechanisms involved in the development of resistance to BRAF inhibitors. Moreover, it will be useful to develop new inhibitors that may improve the response and durability of current therapies against melanoma, and potentially diminish resistance occurrence.

0491 - HUMAN ADIPOSE TISSUE FROM KIDNEY TUMOR REGULATES EPITHELIAL-MESENCHYMAL TRANSITION OF TUMOR AND NON TUMOR RENAL EPITHELIAL CELLS

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In tumor development and maintenance of a cancerous phenotype, bidirectional communication between epithelial cells and stromal environment is necessary. We recently demonstrated that human adipose tissue from kidney tumor (hRAT), showed a differential protein expression profile respect to adipose tissue from normal kidney (hRAN). In the present work, we evaluated: 1) hRAT vs. hRAN adipocytes size using Image J program. In tumor (786-O, ACHN, Caki-1) and non tumor (HK-2) renal epithelial cells incubated for 2 or 24 hs with hRAN-, hRAT, or control-CMs we evaluated: 2) Epithelial-mesenchymal transition (EMT) markers: vimentin, desmin, N-cadherin and 3) pRB/RB and ciclin D1. The tissue explants were obtained from patients with tumor kidney (hRAT, n=14) and kidney donors (hRAN, n=13). The CMs of hRAN and hRAT were collected 24 hs post incubation and the cells were treated. The expression of vimentin, desmin, N-cadherin, pRB/RB and cyclin D1 was quantified by Western blot. Statistical differences among the groups were evaluated by one-way ANOVA with Tukey´s post hoc tests. The hRAT adipocytes showed a significantly minor size compared to hRAN adipocytes (p<0.001). After incubation with hRAT-CMs, vimentin, desmin and N-cadherin were significant increased (p<0.05) in HK-2 and 786-O cell lines vs. hRAN- or control-CMs. Meanwhile, in 786-O and ACHN incubated for 2 hs with hRAT-CMs vs. hRAN- or control-CMs (p<0.05); pRB/RB was decreased (p<0.05), and cyclin D1 increased (p<0.05). In conclusion, human adipose tissue from kidney tumor stimulates adipocyte lipolysis surrounding the tumor, regulates the EMT transition and stimulates mitosis in tumor renal epithelial cells.

Metabolismo y Nutrición / Metabolism and Nutrition III

Chairs: Eleonora Pagano | Esteban Repetto

0052 - EPICARDIAL ADIPOSE TISSUE IN CORONARY ARTERY DISEASE: THE ROLE OF LIPOPROTEIN LIPASE AND FATTY ACID BINDING PROTEIN-4

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INFIBIOC. FAC. FARMACIA Y BIOQUÍMICA. UNIVERSIDAD DE BUENOS AIRES (1); INBIRS, CONICET, FAC. MEDICINA-UBA (2); DEPARTMENT OF CLINICAL LABORATORY MEDICINE, GUNMA UNIVERSITY (3); HOSPITAL DE CLINICAS, FAC MEDICINA UBA (4)

Epicardial adipose tissue (EAT) is a visceral AT surrounding myocardium and coronary arteries, which increase is related to coronary artery disease (CAD). EAT lipoprotein lipase (LPL) would be partly responsible for its increase through lipoproteins catabolism, supplying fatty acids to the tissue. In this context, Fatty Acid Binding Protein-4 (FABP4) would contribute to CAD risk. As it is known, gene and protein expression not necessarily represent the final behavior of an enzyme, and tissue behavior is not always reflected in circulation, so our aim was to evaluate LPL gene, protein and activity levels in EAT, and its serum concentration. We also assessed FABP4 and VLDL Receptor (VLDLR) tissue expression. In EAT and subcutaneous AT (SAT) from patients undergoing coronary artery bypass graft (CAD, n= 51) or valve replacement (No CAD, n= 28), LPL mRNA, protein and activity were evaluated by RT-qPCR, Western blot and a radiometric assay, respectively. In serum, LPL levels (ELISA) and metabolic profile were assessed. Tissue protein levels of VLDLR and FABP4 were evaluated by Western blot. The study was approved by the Ethic Committee of the Hospital de Clínicas. CAD patients presented higher insulin-resistance markers than No CAD (p= 0.001). Serum LPL levels were decreased in CAD patients (p= 0.03), while in EAT no differences were found in LPL mRNA or protein levels between groups; LPL activity was increased in EAT from CAD patients (p<0.001). No differences were found in EAT VLDLR expression between groups, but its levels were higher in EAT than SAT in CAD (p= 0.001). Finally, FABP4 levels were higher in EAT from CAD compared to No CAD (p= 0.006) and in EAT compared to SAT in CAD group (p= 0.03). Conclusion: This is the first time that LPL mRNA, protein and activity are evaluated in EAT. Our results suggest that the higher LPL activity in EAT from CAD patients together with increased levels of FABP4 would partly contribute to the increase in its volume.

0175 - GLP-1 AGONISTS AND INSULIN RESISTANCE: EFFECTS OF LIRAGLUTIDE ON ADIPOSE TISSUE REMODELING.

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UNIVERSIDAD DE BUENOS AIRES. FAC DE FARMACIA Y BIOQUÍMICA. INFIBIOC. DPTO BIOQUÍMICA CLÍNICA (1); INMUNOLOGÍA, GENÉTICA Y METABOLISMO (INIGEM). FACULTAD DE FARMACIA Y BIOQUÍMICA, HOSPITAL DE CLÍNIC (2); DEPARTAMENTO DE BIOQUIMICA HUMANA, FACULTAD DE MEDICINA UBA (3); INBIRS, CONICET, FAC. MEDICINA-UBA (4); UNIVERSIDAD DE BUENOS AIRES. FACULTAD DE ODONTOLOGÍA. CÁTEDRA DE BIOQUÍMICA GENERAL Y BUCAL. (5)

Insulin-resistance (IR) is characterized by adipose tissue (AT) expansion associated with hypoxia and dysfunction. Liraglutide, a glucagon-like peptide type 1 (GLP-1) agonist, has emerged for the management of IR, however it is unknown the direct effects on expanded AT. The aim of present study was to evaluate the role of liraglutide on AT remodeling in an animal model of IR. Male Wistar rats (180-200 g) were divided into 2 groups: Control (C, n=v8) fed with standard diet, and sucrose rich diet group (SRD, n=v8) fed with standard diet and sucrose 30v% in drinking water during 15 weeks. Then, both groups were subdivided according to the subcutaneous administration of liraglutide (L, 0.6 mg/kg/day) for 5 weeks. The study was approved by the Ethic Committee-FFYB (UBA). Serum glucose, total cholesterol (t-chol) and triglycerides (TG) were measured pre and post- L administration. Visceral AT (perirrenal, intestinal and epididymal) was removed and weighed. In epididymal AT (EAT) histological characteristics (adipocyte area and adipocyte and vascular density), mitochondrial density by electronic transmission microscopy and uncoupling protein-1 (UCP-1) and hypoxia-inducible factor alpha 1 (HIF-1α) mRNA levels were evaluated. As expected, SRD presented higher visceral AT mass ($p<0.05$), TG and glucose levels ($p<0.05$) than C. In SRD+L group, a significant decrease in body weight ($p<0.01$), EAT mass ($p<0.01$), TG ($p= 0.045$) and glucose ($p= 0.05$) levels compared to SRD was observed. Moreover, SRD+L presented lower adipocyte area ($p= 0.05$) and higher adipocyte ($p= 0.05$) and vascular density ($p<0.001$) than SRD. UCP-1 RNAm levels were increased in SRD+L compared to SRD ($p<0.05$) in accordance with an increase in mitochondrial density. HIF-1α RNAm levels were decreased in SRD group compared to C ($p<0.001$) and L administration did not modify HIF-1α levels in SRD+L group. In this IR model, liraglutide decreases body weight and circulating IR parameters, as well as it would favor vascularization and AT browning, improving AT functionality.

0181 - EFFECT ON THE INTAKE OF BREAD MALTED FLOUR RYE ON LIPID PROFILE IN AN EXPERIMENTAL MODEL IN GROWING WISTAR RAT.

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Rye is the second most important winter forage cereal in Argentina. The whole grain can be malted and the flour obtained from malting rye increases the amount of soluble fibers that have functional properties. Bread made with this flour, can be a product that could help to maintain a healthy diet. The aim of this study was to evaluate the intake of a bread made with malted flour rye on lipid profile during 60 days, in a rat model. A total of 32 male Wistar rats recently weaned (8/group) were fed with a control diet prepared according to the American Institute of Nutrition Diet (C), and semisynthetic diets prepared with white bread (WB), malted flour rye (MR), and bread made with malted flour rye (MRB). At the end of the study rats were anesthetized and total cholesterol (TC) and high density lipoprotein cholesterol (HDL-c) were measured in serum. Non-HDL cholesterol (Non-HDL-c) was calculated as a marker of atherogenic lipoproteins and TC/HDLc ratio as a risk index. After sacrifice, the cecum from each animal was excised, split open, and the pH of the cecal content was measured. The results showed that the cecal content of MR and MRB presented a lower pH than WB and C (6.29 ± 0.16 vs. 6.67 ± 0.05 vs. 7.15 ± 0.29 vs. 7.13 ± 0.13; respectively $p<0.0001$). WB showed higher values of TC than MR, MRB and C (118 ± 18 vs. 75 ± 8 vs. 71 ± 13 vs. 62 ± 9 mg/dL respectively; $p<0.0001$). HDLc values were higher in MRB than in C, WB and MR groups (53 ± 11 vs. 46 ± 8 vs. 41 ± 11 vs. 37 ± 7 mg/dl respectively; $p= 0.03$). Non-HDLc levels were lower in C and MRB than MR and WB groups (17 ± 2 vs. 18 ± 3 vs. 38 ± 4 vs. 81 ± 12 mg/dl respectively; $p<0.0001$) as well as TC/HDLc ratio (1.3 ± 0.1 vs. 1.4 ± 0.1 vs. 2.0 ± 0.2 vs. 3.2 ± 1.1 respectively; $p<0.0001$). Bread made with rye malted flour showed a prebiotic effect improving lipid profile, compared to white bread, considering the design of beaked goods healthier than those made only with wheat flour.
Financed by UBACyT Nº 20020170100148BA.

0330 - INHIBITION OF ALPHA-GLUCOSIDASE AND PANCREATIC LIPASE BY A PLANT EXTRACT OF DRYMIS ANDINA: IN VITRO AND IN VIVO STUDIES

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CÁTEDRA DE FISICOQUÍMICA, FACULTAD DE FARMACIA Y BIOQUIMICA, UBA (1); FACULTAD DE CIENCIAS NATURALES Y DE LA SALUD, UNIVERSIDAD NAC DE LA PATAGONIA SUR SAN JUAN BOSCO (2); FUNDACIÓN MIGUEL LILLO, FACULTAD DE CIENCIAS NATURALES, UNIVERSIDAD NACIONAL DE TUCUMÁN (3); CÁTEDRA DE FARMACOLOGÍA, FACULTAD DE FARMACIA Y BIOQUÍMICA, UBA (4)

There is a renewed momentum backed by the WHO to discover newer, cheaper and better anti-obesity agents derived from plants. In this work we analyzed an aqueous extract from a native plant, Drymis andina as inhibitor of digestive enzymes, which is a promising mechanism of action on this topic. Aqueous extract (5 %) of D. andina was investigated in terms of phenolics compounds, flavonoids, coumarins, alkaloids, saponins, tannins, mucilage, sugars and proteins. In vitro lipase and alpha-glucosidase inhibition was measured by colorimetric methods. Acute toxicity studies and in vivo inhibition of enzymes assays were carried out on C57BL6 mice. Acarbose and orlistat were used as positive controls. The lyophilized extract of D. andina mainly showed a high phenolic compounds content (total phenol content 302 ± 1 mg/g, total flavonoid content 45.1 ± 0.5 mg/g, hydroxycinnamic acids 174 ± 17 mg/g, o-dihydroxy-phenols 71 ± 1 mg/g). In the in vitro assays, the IC50 were 0.10 ± 0.03 mg/mL and 2.04 ± 0.08 mg/mL for alpha-glucosidase and lipase, respectively. No toxic effect was evident for the extract in the acute toxicity study by using the dose of 2 g/kg body weight. For oral maltose tolerance test, doses of 45, 90, and 180 mg/kg decreased significantly the postprandial glycemia in 34, 38 and 68 %, respectively. For oral lipid tolerance test, dose of 180 mg/kg decreased significantly the postprandial triglyceridemia in 78 %. In summary, the aqueous extract of D. andina did not show acute toxicity and was effective as inhibitor of alpha-glucosidase and lipase both in vitro and in vivo. The introduction of D. andina as infusion before the consumption of refined carbohydrates and/or fat rich food could be beneficial not only to prevent fat

deposition but also to reduce the negative impact on metabolism promoted by high levels of postprandial glucose and triglycerides.
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0353 - HYPERCHOLESTEROLEMIC DIET IN PERIODONTAL HEALTH: EFFECT ON OXIDATIVE STATUS AND ALVEOLAR BONE

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Hypercholesterolemia (HC) is a risk factor for CVD. Periodontitis (P) is an inflammatory disease that might affect the teeth supporting tissues and induce reactive species (RS) production in periodontal tissue. Cholesterol-rich diets decrease bone health. The objective of this study was to assess the implications of an hypercholesterolemic diet on gingival oxidative status and the alveolar bone loss in rats with and without P. Methods: Wistar rats (24) were assigned into 2 groups: control (C) fed pellets, and high-cholesterol diet (HCD). After 3 weeks, 6 rats per group were euthanized (C0, HCD0) and 6 animals per group were subjected to ligature-induced P and 72 h later these rats (C72, HCD72) were euthanized. Blood was drawn for lipid profile. X rays were used for periodontal bone support measurements (PBS, %). Mandibles were processed with Hematoxiline&Eosine. In interradicular bone, periodontal ligament height (hPL) was measured. In gingival tissue homogenate were determined the oxidation rate of 2',7' dichlorofluorescein diacetate (DCFH-DA) (spectrofluorimetrycally) and catalase (CAT) content (enzymatic method). Results (mean± SD): HCD0 and HCD72 presented HC ($p<0.01$). In gingival tissue, HCD increased DCFH-DA oxidation rate as compared to controls (HCD0: 13.9 ± 0.2, C0: 3.3 ± 0.2 u.a./min.mg prot respectively; $p<0.001$); and P produced increases in the dye oxidation rate (HCD72: 23.2 ± 0.2, C72: 10.0 ± 0.5 u.a./min.mg prot respectively; $p<0.001$). CAT content increased by HCD as compared to control fed rats ($p<0.001$), but remained unchanged by P. In alveolar bone, PBS% decreased with both HCD and P ($p<0.01$), and hPL was increased in HCD72 rats as compared to controls (471 ± 56 and 325 ± 91 µm, $p<0.003$). HC and P are involved in dynamic events associated with RS production. The generation of oxidative stress would increase cellular damage and HC influenced gingival and alveolar homeostasis and may induce the progression of P by increasing alveolar bone resorption.

0488 - DEVELOPMENT OF A NEW MURINE MODEL OF METABOLIC SYNDROME AFTER FEEDING WITH HIGH-FAT DIET IN FEMALE CBI MICE.

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LABORATORIO DE FISIOLOGÍA METABÓLICA, FACULTAD DE CIENCIAS MÉDICAS, UNIVERSIDAD NACIONAL DE ROSARIO (1); INSTITUTO DE GENÉTICA EXPERIMENTAL. FACULTAD DE CIENCIAS MÉDICAS. UNIVERSIDAD NACIONAL DE ROSARIO (2)

It has been well established that obesity and overweight are major risk factors for several diseases, such as metabolic syndrome (MetS) and cancer. The MetS is characterized by insulin resistance, dyslipidemia and central obesity. Our aim was to generate an obese female model as a research tool. CBi is an inbred mouse strain generated in the Institute of Experimental Genetics, School of Medical Sciences, UNR. Eight weeks mice were randomly separated into two groups. Control group fed with a standard diet and HFD group with a high fat diet containing 40 % of bovine fat (n= 6/group). At week 20 (10 weeks earlier than in others models), body weight gain was higher in HFD (g; mean±SEM; 130 ± 4.5) respect to Control (47 ±2.2, $p<0.0001$). Insulin resistance, evaluated by an insulin tolerance test, showed a worse response in HFD with a lower maximum glucose clearance (%; 11 ± 2.1) than Control (20 ± 3.8, $p<0.05$).The retroperitoneal HFD vs Control (6 ± 0.7 vs. 2 ± 0.5), mesenteric (1.3 ± 0.1 vs. 0.7 ± 0.1) and ovarian fat pads (1.3 ± 0.2 vs. 0.5 ± 0.1), expressed as percentage of body weight, were higher in HFD ($p<0.01$) and are taken as an index of central obesity. HFD showed hyperglycemia: HFD vs. Control; mg/dl (139 ± 5.5 vs. 78 ± 6.7, $p<0.0001$); hypercholesterolemia: 142 ± 9.6 vs. 112 ± 3.0 ($p<0.05$) and hypertriglyceridemia: 123 ± 2.6 vs. 110 ± 4.7 ($p<0.05$). Vaginal cytology was evaluated immediately after collection as an unstained, wet mount preparation, to document the stages of the estrous cycle as an index of the functional status of the hypothalamic–pituitary–ovarian axis. We conclude: 1) CBi female mice fed with HFD diet developed different features of MetS at a shorter time length than others reported models; 2) Mice in different stages of the estrous cycle showed homogeneity in their biochemical parameters, suggesting lack of influence on them; 3) Interestingly, the cell types observed during the stages of the estrous cycle were altered in the HFD group respect to control.

0500 - EFFECT OF (—)-EPICATECHIN ON CARDIAC AND SKELETAL MUSCLE FROM HIGH FAT FED MICE: A COMPARATIVE STUDY

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It was shown that diet supplementation with (—)-epicatechin (EC) ameliorates changes observed insulin-triggered signaling cascades, in adipose and liver tissues of high-fat fed mice. Because muscle is the main tissue involved in glucose homeostasis, the relationship between insulin resistance and heart and skeletal muscle is under discussion. The objective of this study was to evaluate the effects of EC on cardiac and skeletal muscle mitochondrial function from high-fat fed mice. Male C57BL6 mice were fed for 15 w with diets containing 10 % total calories from fat (C), 60 % total calories from fat (HF), C diet supplemented with EC to provide 20 mg EC/kg body (CE), and HF diet plus same amount of EC (HFE). Mitochondrial respiratory complexes I-III, II-III, and IV activities were higher in cardiac (27, 24 and 27%) and skeletal muscle (28, 43 and 43%) from HF compared to C mice. EC supplementation prevented the increase observed in skeletal muscle mitochondrial respiratory parameters in high-fat fed mice. This effect was not observed in mitochondria from cardiac muscle. Tfam and PGC-1alpha expressions were similar among groups, suggesting that de novo synthesis of mitochondria was not altered with treatments. The expression of the uncoupling protein UCP3 was higher in HF and HFE respect to C and CE groups in cardiac and skeletal muscle. Insulin receptor protein levels were similar in all groups, in both tissues. CONCLUSION: Although the mitochondrial dysfunction in response to HF diet was observed in cardiac and skeletal muscle of high-fat fed mice, EC protective effects were restricted to skeletal muscle.
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0512 - DOES MICROSOMAL TRIGLYCERIDES TRANSFER PROTEIN CONTRIBUTE TO INTRA INTESTINAL FAT IN DYSBIOSIS MODEL?

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In previous studies we associated high fat and carbohydrates diet (HFHC) with altered gut microbiota. Chylomicrons (CM) production related to microsomal triglycerides transfer protein (MTP), in charge of lipoprotein assembly, has not been well studied in this context. Cell intestinal fat (CIF) deposits could also be present in dysbiosis impairing the synthesis/secretion of CM. The aim of this study was to evaluate MTP in relation to CM characteristics and with eventual CIF content, in a HFHC diet animal model. Twelve male Wistar rats (180-200 g) were fed with standard diet (Control, n= 6) or standard diet plus 40 % fat + 15 % sucrose (HFHC, n= 6) throughout 14 weeks. Glucose, free fatty acids (FFA), lipoprotein profile and lipopolysaccharide (LPS), as altered gut microbiota marker, were measured in sera. Lipid composition was measured in isolated CM (ultracentrifugation d<0.95 g/ml). In intestinal tissue, MTP was assessed by western blott and fat content by Folch extraction followed by gravimetric measurement. Visceral adipose tissue (VAT) was evaluated removing and weighting epididymal adipose tissue. Compared to Control, HFHC showed higher LPS levels ($p<0.01$), TG ($p<0.001$), non HDL-chol levels ($p= 0.04$), TG/HDL-chol ($p<0.001$), FFA ($p<0.05$) and VAT ($p<0.01$). CM composition showed higher triglycerides (TG) levels (CM-TG: 181 ± 43 vs. 38 ± 8 mg/dL, $p<0.001$). CIF was increased in HFHC (0.62 ± 0.07 vs. 0.51 ± 0.08 g/g, $p<0.05$); even though no differences in MTP expression were observed ($p= 0.40$), MTP directly correlated with CM-TG ($r= 0.53$; $p<0.01$), and CIF ($r= 0.50$; $p<0.05$). Conclusion: this study confirmed a dysbiosis state and an altered lipid profile compatible to insulin resistance. CMs are TG over-enriched, probably promoted by MTP, which may contribute to the atherogenic profile. Paradoxically, MTP would induce intracellular fat deposit that could exert intestinal pro-inflammatory actions.

0567 - AQUAPORIN 7: MODULATOR OF CARDIAC ENERGY METABOLISM IN THE METABOLIC SYNDROME DURING AGE PROGRESSION

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It has been shown that dysfunction of some type of aquaporin (AQP) can be related with metabolic diseases. In addition, advancing on age could be associated with the change in density or functions of different isoforms of AQPs. AQP7 is a glycerol transporter through the plasma membrane, mainly in adipocytes and cardiac tissue. However, in the heart, the role of this channel in metabolic syndrome (MS) is little known. The objective of this study was to investigate changes in cardiac AQP7 in the experimental model of fructose-induced MS during age progression. Male Sprague Dawley rats were used according to the following groups: C1: 1-month old rats, F1: 1-month old rats + fructose treatment for 8 weeks; C6: 6-month old rats, F6: 1-month old rats + fructose; C12: 12-months old rats, F12: 12-month old rats + fructose. The weight gain of the animals of group F1 and F6 was greater than that of group C1 and C6 from the sixth and fifth week of treatment respectively. There was no difference in this parameter in the 12-month animals. SBP and plasma triglycerides increased in F1, F6 and F12 and a Triglyceride/C-HDL ratio greater than 3 was obtained in these groups. Basal glycemia only increased in F12 compared to C12. It was observed that the advancing age induced an increase in the expression of AQP7 in heart. The SM induced by fructose overload decreased AQP7 levels in 12-month-old animals. Our findings propose that changes in cardiac protein levels of AQP7 could be involved in metabolic and hemodynamic alterations associated to fructose treatment along age progression. Supported by UBACyT 20020170100087BA (AB) and UBACyT 20020170100586BA (MG) grants.

0748 - DEVELOPMENT AND VALIDATION OF A LC-MS/MS METHOD TO QUANTIFY PLASMA 3-NITROTYROSINE IN LACTATING DAIRY COWS AS A BIOMARKER OF OXIDATIVE STRESS

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During the transition period cows experience increases of metabolic demand and tissue oxygen requirements leading to an oxidative stress. The 3-nitrotyrosine (3-NT) is thought to be a relatively specific biomarker of oxidative damage mediated by peroxynitrite. The aim of this study was to develop a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantification of 3-NT in plasma samples from dairy cows. An ultra-fast liquid chromatography system (LC-20AD, Shimadzu) consisting of a binary pump equipped with autosampler SIL-20ACXR (Shimadzu) was used. Chromatographic separation was performed with a Shimadzu Shim-pack GIST C18 column (100 mm x 4.0 mm inner diameter, 3.0 µm particle size). Detection and quantitation were achieved by using a QTrap 3200 triple quadrupole mass spectrometer (AB Sciex) equipped with an electrospray ionization source interface. The analytical technique was validated in plasma in accordance with the International Conference on Harmonisation (ICH) guideline and linearity, accuracy and precision, recovery and matrix effect and stability were considered. After that, blood from six grazing dairy cows of a commercial dairy farm was sampled at -28, -14, 4, 14, 28 and 60 days relative to parturition and plasma 3-NT was evaluated in those samples. Method showed linearity ($r > 0.992$) over the working range (0.5 to 15 ng/mL). Accuracy (< 15 %) and precision (% CV < 15) were according to the international criteria. The 3-NT showed long-term stability (-80 °C) for plasma and under freeze-thaw cycles. The recovery values achieved at different spiked 3-NT levels were within the range of 70-110 % and matrix effect calculated was < 20 %. Plasma 3-NT concentrations increased from days 28 prepartum to day 28 postpartum ($p<0.05$). A rapid and accurate LC-MS/MS method for 3-NT quantification in plasma samples from lactating dairy cows was successfully developed, suggesting to be a useful tool for early diagnosis of metabolic disorders.

Farmacología / Pharmacology II

Chairs: Roberto Diez | Alexis Mejías Delamano

0031 - NUTRITIONAL ANALYSIS OF LEAVES OBTAINED FROM PLANTS OF THE AQUIFOLIACEAE FAMILY: HEALTH IMPLICATIONS.

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Plants and fruits have been used as a source of bioactive compounds for thousands of years. Several recent studies have examined the phytochemical content of the many plants under different growth and processing conditions. For that, the objective of this study was to analyzed the composition of plant leaf (Magnoliophyta: Aquifoliaceae family), the effect of production and processing has on its composition, and focus on others compounds that have bioactive properties. Thus, leaf samples were collected in an experiment conducted under agronomic control. After drying the samples was dried and ground down to around to ensure sample uniformity. Later, subsamples are then taken of the original sample, and each nutrient is measured separately using different chemistry techniques. The Laboratory used approved chemical, enzymatic, volumetric, and gravimetric techniques to measure core nutrients, minerals (ICP) of samples. In addition, yeasts and mold were counted (CFU/gr). The results showed that no significant differences in the qualities of the extracts were noticed regarding the production and processing methods (ANOVA). The concentration of the selected compounds was: protein (22.07 % of DM), fat (0.28 % of DM), ash (7.95 of DM), lignin (25.24 of DM), and starch (1.78 of DM). Also, contains significant amounts of total minerals, whose values range from 0.2 to 3,329 ppm. Yeast and mold count was very low. These compounds were chosen because of their importance in the chemical characterization of plants and their importance in maintaining the health of the human body. While there is a need for more research on the identification of bioactive ingredients, the evidence seems to show that the plant studied is a botanical product with a variety of compounds that can be applied for human health use.

0142 - OPPOSITE ACTIONS OF PROGESTERONE (PG) AND MEDROXYPROGESTERONE ACETATE (MPA) IN BONE AND VASCULAR CELLS

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INSTITUTO DE CIENCIAS BIOLÓGICAS Y BIOMÉDICAS DEL SUR (UNS-CONICET)

Due to the fact that the incidence of osteoporosis and cardiovascular diseases increases in postmenopausal women, hormone replacement therapy including natural or synthetic progestins such as MPA emerged as a therapeutic option. Vascular calcification developed in atherosclerotic plaque is partly due to the osteogenic transdifferentiation of vascular smooth muscle cells (VSMC). Using murine primary cultures of calvarial osteoblasts (OB) and aortic VSMC, we investigated the effect of Pg and MPA on osteoblastic differentiation markers. In order to induce osteoblastic differentiation, VSMC were cultured for 21 days in osteogenic medium (4 mM $CaCl_2$ and 10 mM glycerophosphate). When VSMC were cultured in osteogenic medium (VSMC-OB), treatment with 100 nM Pg for 21 days induced a significant reduction in ALP activity (25 % below control, $p<0.02$). Ca^{2+} values were also significantly reduced after treatment with Pg (100 nM) (29 % below control, $p<0.02$). Long treatment of VSMC with the synthetic progestin (100 nM) also showed a significant reduction in ALP activity (239.9 ± 21.0 vs. 172.7 ± 13.5 $x10^3$ IU/mg protein, control vs. MPA, $p<0.02$), as well as in the extracellular Ca^{2+} deposition (365.1 ± 38.2 vs. 207.2 ± 22.2 µg/mg protein, control vs. MPA, $p<0.02$). In contrast, in OB cells, treatment with Pg (100 nM) induced a significant increase in ALP activity and matrix Ca^{2+} levels (ALP: 111 % above control, $p<0.02$; Ca^{2+}: 26 % above control, $p<0.02$). MPA (100 nM) induced similar results (ALP: 300% above control, $p<0.001$; Ca^{2+}: 51 % above control, $p<0.02$). The mechanism of action of Pg and MPA on both cells involves the participation of Pg receptor (PgR), since pre-treatment of OB and VSMC-OB with RU486, a PgR antagonist, completely reversed the hormonal action. In conclusion, although Pg and MPA exert opposite effects on OB and VSMC-OB, both progestogens would exhibit a potential beneficial effect by inhibiting vascular calcification and promoting osteoblastic differentiation.

0205 - CLARITHROMYCIN:N-ACETYLCYSTEINE CO-AMORPHOUS COMBINATION AS ALTERNATIVE TO IMPROVE SOLUBILITY AND ANTIBIOFILM ACTIVITY

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Clarithromycin (CLM) is a semisynthetic 14-member macrolide exhibiting a broad in vitro antibacterial spectrum and a variety of clinically diagnosed infections. It is a water-insoluble base (pKa= 8.8) and with pH-dependent solubility. One approach that has been explored to overcome the problem of poor aqueous solubility is by using a pharmacologically relevant combination of two drugs in the preparation of co-amorphous systems. In this work, CLM:N-acetylcysteine (NAC) system was prepared with the aim of improving the solubility and antibiofilm activity of CLM. The NAC was selected due to its well-known mucolytic activity and security profile. The CLM:NAC solid systems were prepared by physical mixing (PM) and freeze drying (FD) methods and were characterized by infrared spectroscopy, thermal analysis, scanning electron microscopy and X-ray diffraction. The characterization of the complexes in solution was performed by phase solubility analysis (PSA). The antimicrobial activity was evaluated by determining the minimum inhibitory concentration (MIC) and fractional inhibitory concentration (FIC) and antibiofilm activity by the XTT assay. The results obtained by the different solid-state techniques confirmed the formation of an amorphous solid in the system obtained by FD, while the PM showed a behavior similar to the pure components. The PSA showed a significant increase in the aqueous solubility of CLM in the co-amorphous system. Synergism was also observed between both drugs against Staphylococcus aureus. In addition, the antibiofilm activity against this bacterium was significantly increased. The binary combination CLM:NAC had a higher solubility than the pure drug. In addition, the antimicrobial activity of CLM against S. aureus was increased it both, in planktonic state and when it was forming biofilms. Accordingly, it can be concluded that this combination is potentially useful for application in a future pharmaceutical formulation.

0221 - ABIETANE DITERPENOIDS ISOLATED FROM LEPECHINIA MEYENII (WALP.) EPLING: STUDY OF MECHANISM OF ACTION.

Macarena FUNES CHABAN (1) | Martina HRAST(2) | Mariana Belen JORAY(1) | Lucija PETERLIN MAŠIC(2) | Danijel KIKELJ(2) | María Cecilia CARPINELLA(1)

IRNASUS-CONICET. FACULTAD DE CIENCIAS QUÍMICAS. UNIVERSIDAD CATÓLICA DE CÓRDOBA. (1); FACULTY OF PHARMACY, UNIVERSITY OF LJUBLJANA (2)

Nowadays, the management of bacterial infections is a great challenge of therapeutics mainly due to the development of resistant strains against several antibiotics. Staphylococcus aureus is one of the most prevalent pathogens worldwide. In this context, methicillin-susceptible S. aureus (MSSA) and methicillin-resistant S. aureus (MRSA) represent a serious public health threat. This scenario results in the urgent need to find novel and safe compounds to develop antibiotics to control infections caused by bacteria. Peptidoglycan is the major component of the bacterial cell wall and its function is to provide shape and rigidity, protect against osmotic changes and plays an important role in the process of cell division, so its inhibition is considered an important target in antibacterial therapy, mainly of the enzymes involved in the cytoplasmic stage of the biosynthesis such as MurA-F. Since prehistory mankind has turned to nature to treat several diseases

and plants have been used by various civilizations and cultures in folk medicine. The bioguided fractionation of the medicinal plant Lepechinia meyenii led to the isolation of carnosol (1), rosmanol (2) and carnosic acid (3) as active principles effective mainly against MRSA and MSSA strains. Studies of mechanisms of action in inhibition of MurA and MurF enzymes from Escherichia coli and S. aureus were carried out using the colorimetric malachite green method in which orthophosphate generated during reaction is measured. Compounds 1-3 were able to inhibit MurA from S. aureus and E. coli showing IC50 values of 1.1-5.7-12 and 37-74-27 µM, respectively. However, none of the diterpenes isolated was able to inhibit MurF from S. aureus and E. coli. The results obtained showed that compounds 1-3 exert their antibacterial activity by the inhibition of MurA enzyme.

0222 - NOVEL LXR/ER MODULATOR AS A POLYPHARMACOLOGICAL AGENT AGAINST BREAST CANCER

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Breast Cancer (BC) is a complex disorder due to multiple genes deregulation, which prompts a robust phenotype. The pharmacological paradigm of "magic bullets", targeting individual chemoreceptors, fails as redundant functions are activated and alternative compensatory signaling routes sustain the tumor phenotype, leading to immune escape, chemoresistance and metastasis. In this way, polypharmacology arises as a new paradigm: designing multifunctional drugs that, selectively, interact with several molecular targets. This approach would tackle several signaling and metabolic routes at the same time, with one drug, leading to new and more effective treatment against BC. Our hypothesis is that a dual antagonist of both, Estrogen Receptor (ER) and Liver X Receptor (LXR), would inhibit the ER canonical survival routes, but also would inhibit lipogenesis and Warburg effect through LXR antagonism. These two are key metabolic pathways that drive cancer progression, growth, survival, immune evasion, resistance to treatment and disease recurrence. In this sense, we performed a screening of different natural and synthetic oxysterols where Compound 1 emerged as a promising compound. Our unpublished findings proved it is a dual ERalpha/ERbeta; antagonist at micro molar concentration, and a dual LXRalpha;/LXRbeta; inverse agonist at micro molar concentration, in reporter gene assays. 1 effectively inhibits proliferation and migration on the ER+ BC cell line MCF7 and the ER- BC cell line MDA-mb-231. Moreover, it inhibits migration of the human vascular endothelial cell line EA.hy926, by suppressing the NFkB signaling pathway, suggesting antiangiogenic activity. Furthermore, 1 is a natural product, easily obtained in high yields and high degree of purity from a wild plant. We present our preliminary studies on 1, with the final intention of validating the dual ER/LXR modulation as a target for polypharmacological agents anti-BC.

0230 - ANXIOLITYC EFFECTS OF SCHINUS LENTISCIFOLIUS MARCHAN (ANACARDACEAE) EXTRACTS ON MICE AND CHROMATOGRAPHIC PROFILE BY HPLC

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Schinus lentiscifolius (Anacardiacea) is a South American plant and it is used in folk medicine as digestive. However, there are no scientific reports that support its pharmacological effects. We previously showed that infusion (SchW) and tincture (SchT) of Schinus lentiscifolius reduced exploratory behaviour of mice in the open field without changing the spontaneous locomotion. Now, we studied the anxiolityc effects of SchW and SchT by test of novelty supressed fooding test, and the chromatographic profile of SchT. Mice were divided in 9 groups for the following treatments administered by i.p. injections: saline solution (SF, n= 10), ethanolic vehicle (Vh, n= 8), 0.3 mg/kg diazepam (Dzp, a well-known benzodiazepine as positive control, n= 12), 12 and 40 mg/kg SchT (n= 8-8), 200 and 400 mg/kg SchW (n= 8-8), 40 mg/kg SchT and 400 mg/kg SchW 5 min after receiving 0.5 mg/kg flumazenil (Flz) (n= 8-8). Latencies to begin eating (tlc, second) and home-cage food consumption (fhc, mg) were recorded on novelty supressed fooding test. Dzp and 400 mg/kg SchW significantly reduced tlc (62.9 ± 6.6* and 69.0 ± 8.6* respectively, vs. 127.4 ± 10.3sec for SF, *p<0.05). In the same way, 40 mg/Kg SchT significantly reduce tlc (72.5 ± 8.3* vs. 127.4 ± 10.5 sec of Vh, *p<0.05). There were no changes in fhc. Flz (antagonist of the benzodiazepine receptor) significantly reversed the effects of SchW (400 mg/kg) and SchT (40 mg/kg) (102.7 ± 10.7* vs. 69 ± 8.6 and 226 ± 21.2* vs. 72.5 ± 8.3 sec respectively, *p<0.05). Cromatographic profiles by HPLC were obtained with C18 column with tetrahydrofurane:methanol:water (15:5:85 v/v) as mobile-phase and UV-detection at 336 nm. The system well separated 6 peaks. Two flavonoids were identified in the SchT: rutine and isoquercetine. Results suggested: a) SchW at 400 mg/kg and SchT at 40 mg/kg showed anxiolytic effects by a benzodiazepine-like mechanism b) SchT contain at least 6 compounds, two of them are flavonoids: rutine and isoquercetine. Supported by UNLP 2019-2020 grant.

0239 - PREVENTION OF POSTISCHEMIC CARDIAC STUNNING BY MEDICINAL PLANTS WITH PHYTOESTROGENS: ENERGETICAL CONSEQUENCES AND MECHANISMS

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In previous works we showed that genistein, the main soy isoflavone, was cardioprotective in young males (YM) and aged females, due to PKC and mKATP channels activation (SAFE 2018). Then our hypothesis was that medicinal plants with phytoestrogens could also prevent ischemic dysfunction. Soy isoflavones are used in menopausic symptoms and maca rooth, Lepydium meyenii, is used to increase energy, but their cardiac effects were not studied. Now we evaluated whether oral soy isoflavones (ISOF, 100 mg/kg) or MACA powder (1 g/kg/day) during 7 days prevent cardiac dysfunction of ischemia/reperfusion (I/R) in rats, the gender influence and mechanisms. By RP-HPLC-DAD, the genistein/daidzein content was 78/1.4 % in MACA and 51/23% in ISOF. From untreated (C) or treated rats, isolated hearts were perfused in a calorimeter and exposed to 30 min I/45 min R. Contractile pressure (P, mmHg), diastolic contracture (LVEDP) and total heat rate (Ht, mW/g) were measured. ISOF improved the postischemic contractile recovery (PICR) from 14.5 ± 2.4 % of initial P (C) to 47 ± 9 %* in YM and from 23 ± 6 % (C) to 67.8 ± 12.2 %* in young females (YF) (*p<0.05 vs. C) at 45 min R. Similar changes appeared in muscle economy (P/Ht), and no changes in LVEDP. MACA also increased PICR in YM (to 37.4 ± 7.4 %*) and YF (to 52.3 ± 8.0 %*) as well as P/Ht, but increased LVEDP. The soy isoflavone

daidzein 5 mg/kg i.p. (DAZ) improved PICR more in YM (to 99.5 ± 19.7 %*) than in YF (to 49.5 ± 11.2 %). Perfusion of 5-HD (mKATP blocker) reduced both, PICR (to 43.2 ± 12.6 %*) and P/Ht (from 98.0 ± 13.4 to 62.3 ± 19 %*) in DAZ-treated YM hearts with high LVEDP. Contrarily in MACA-treated YM rats the PICR was not significantly changed by 5-HD nor chelerythrine (PKC inhibitor) (40.4 ± 20 % and 52 ± 11 %, respectively). Results suggest that a) ISOF, MACA and DAZ are cardioprotectives in I/R; b) DAZ cardioprotection depends on mKATP channels activation; c) MACA cardioprotection did not depend on PKC or mKATP channels activation.
Supported by UNLP-X-795 and UNPSJB PI grants.

0267 - GASTROPROTECTIVE EFFECT OF CAPPARIS ATAMISQUEA LEAVES: ROLE OF NITRIC OXIDE, PROSTAGLANDINS AND SULFHYDRYLS

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INSIBIO (UNT-CONICET)

Gastric ulcers are caused by an imbalance between protective and aggressive factors on the mucosa. Current pharmacological treatment of peptic ulcer focuses on the inhibition of the acid secretion and eradication of Helicobacter pylori. However, enhanced cytoprotection was found to be an interesting therapeutic approach. Atamisqui, Capparis atamisquea Kuntze, is an autochthonous plant from northwestern Argentina with many beneficial properties. Our previously studies showed that 5 n% infusion (I) and 10 % hidroalcoholic extract (E) from the atamisqui leaves present a significant gastroprotective effect. For the assessment of the participation of nitric oxide (NO), prostaglandins (PG) and non-protein sulfhydryl compounds (NP-SH) the ethanol-induced ulceration model was developed in Wistar rats (n= 6 animals/group). The inhibitors L-nitroargininemethyl ester (L-NAME) (70 mg/kg, intraperitoneally) and indomethacin (10 mg/kg, intraperitoneally) were used to evaluate the participation of NO and PG respectively in the gastroprotection. To determine the intervention of the NP-SH in the gastroprotective effect, the blocker N-methylmaleimide (NEM) (10 mg/kg, intraperitoneally) was used. The experimental groups were ulcer control group (NaCl 0.9 %), positive control group (sucralfate 100 mg/kg, orally) and two treated groups (I 150 mg/kg and E 150 mg/kg, both orally). The ulceration area in the stomachs of each group was determined. Pretreatment with indomethacin and L-NAME but not with NEM significantly blocked the atamisqui extracts gastroprotection. This indicates that the gastroprotective effect of both atamisqui leaf extracts is mediated in part by the PG and NO synthesis but not by NP-SH production in gastric mucosa. More studies will be necessary to define the active compound/s and the molecular mechanisms involved in this effect.

0329 - A NOVEL INHIBITOR OF C. ELEGANS GLUTAMATE-ACTIVATED CHLORIDE CHANNEL WITH POTENTIAL ANTHELMINTIC ACTIVITY

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INIBIBB-CONICET, DEPTO. BIOLOGÍA, BIOQUÍMICA Y FARMACIA-UNS (1); INSTITUTO DE QUÍMICA DEL SUR INQUISUR UNS-CONICET (2)

Nematode parasitoses cause mortality and morbidity in humans and considerable losses in livestock, domestic animals and food crops. The acquisition of resistance to current anthelmintic drugs has prompted the search for new compounds. The free-living nematode Caenorhabditis elegans has emerged as a valuable platform for anthelmintic drug discovery. We have previously synthetized a small library of oxygenated tricyclic compounds and tested anthelmintic actions by measuring rapid effects on C. elegans. Exposure to dibenzo[b,e]oxepin-11(6H)-one (C1a) induced paralysis of C. elegans. We here sought to identify its target site and mechanism of action. Given that Cys-loop receptors are involved in worm locomotion and are targets of classical antiparasitic drugs, we tested the effects of C1a on several C. elegans mutant strains lacking these receptors. We found that a mutant strain that lacks the invertebrate glutamate-gated chloride-selective channel (GluClR), which is the target of the widely used antiparasitic ivermectin, is resistant to C1a. Thus, the paralysis assays revealed that GluClR is the main drug target of C1a. To unravel the molecular mechanism underlying the paralyzing action, we expressed in mammalian cells GluClα and β subunits to form GluClRs and evaluated the effects of C1a by electrophysiological whole-cell recordings. Glutamate elicited macroscopic currents from cells expressing GluClα/β heteromeric receptors whereas C1a was not capable of eliciting responses, thus indicating that it is not an agonist of GluClRs and that its mechanism differs from that of ivermectin. We found that C1a acts as an inhibitor of glutamate-responses: Preincubation of the cell with C1a produced a statistically significant decrease of the decay time constant and total charge and a slight decrease of the peak of currents elicited by glutamate. We here propose C1a as a novel compound or scaffold with promising antiparasitic activity mediated through inhibition of GluClRs.

0399 - ACTIVATION AND MODULATION OF THE CAENORHABDITIS ELEGANS SEROTONIN-GATED CHLORIDE CHANNEL

Noelia RODRIGUEZ ARAUJO | Jeremías CORRADI | Cecilia BOUZAT

INIBIBB-CONICET, DEPTO. BIOLOGÍA, BIOQUÍMICA Y FARMACIA-UNS

Serotonin-gated ion channels (5-HT3) belong to the family of Cys-loop receptors, which are pentameric proteins that mediate fast synaptic transmission. In mammals, 5-HT3 are non-selective cationic channels that can be found as homomers (5-HT3A) or heteromers. The free-leaving nematode Caenorhabditis elegans is a model for the study of the nervous system and for antiparasitic drug discovery. As parasitic nematodes, C. elegans contains a homomeric serotonin-gated chloride channel, MOD-1, that modulates locomotory behavior. The absence of this receptor in vertebrates, converts MOD-1 into a potential antiparasitic drug target. We expressed MOD-1 in mammalian cells and explored by patch-clamp recordings its activation and modulation properties. Dose-response curves revealed an EC50 for 5-HT activation of about 1 µM, which is in the same range as that of human 5-HT3A receptors. The analysis of whole-cell currents determined that MOD-1 channels do not show rectification, desensitize slowly in the presence of 5-HT, and recover from desensitization with a time constant of about 1 s. In contrast to their actions at mammalian 5-HT3 receptors, 5-hydroxyindol and thymol do not potentiate MOD-1 currents. The antiparasitic drug ivermectin (IVM), which acts as activator or potentiator of different Cys-loop receptors, neither activates nor potentiates MOD-1 but pre-exposure to IVM inhibits MOD-1 currents. To gain further insights into the molecular function of the native MOD-1, we sought to identify serotonin-activated chloride channels from C. elegans neurons expressing MOD-1 and compared to MOD-1 channels heterologously expressed in mammalian cells. The understanding of the molecular pharmacology of MOD-1 contributes to our knowledge of the Cys-loop receptor family and to its potential as a novel drug target for anthelmintic therapy

0424 - POLYMERIC NANOPARTICLES ENHANCE THE PHOTOTOXICITY OF MONOBROMINATED AZURE B AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

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Phenothiazine are commonly used photosensitizer (PS) due to their low toxicity, high binding affinity for both Gram-positive and Gram-negative bacteria. In order to optimize the properties of these dyes, monobrominated derivative of Azure B (AzBBr) was synthesized. Although halogenation increased the singlet oxygen quantum yield of this PS, also increased the lipophilicity, favored aggregation and affected its phototoxic efficiency. The vehiculization of this PS in different Polyacrylamide Nanoparticles (PAA-NP) was employed to overcome these disadvantages. In this work we evaluated the photodynamic efficacy of AzBBr, free and loaded in two PAA-NP (NIPA and BIS, according to its components), against Gram-positive and Gram-negative bacteria. The inactivation of Staphylococcus aureus sensitive and resistant to methicillin (MSSA and MRSA), Pseudomonas aeruginosa and Escherichia coli was tested in bacterial suspensions. Different concentrations of the PS (7.5-250 µM) and light doses (7.6-15.1 J/cm^2) were applied in the treatment. The results showed that AzBBr, free and loaded in PAA-NP, were not toxic and caused significant photodynamic inactivation of all bacteria studied. S. aureus was the most sensitive bacterium to photodynamic treatment, evidencing a similar behavior in the inactivation of MSSA and MRSA. After 15 min of irradiation, the PAA-NP produced a reduction greater than 3 Log CFU/mL. Regarding Gram-negative bacteria, AzBBr loaded in NIPA-NP eradicated P. aeruginosa and caused a drop greater than 3 Log CFU/mL of E. coli. On the other hand, the BIS-NP enhanced the phototoxic activity of the PS, reaching a drop of 3 Log CFU/mL of P. aeruginosa and 2 Log CFU/mL of E. coli. In conclusion, the employ of PAA-NP in the vehiculization of AzBBr increased the photodynamic efficacy of PS against Gram-positive and Gram-negative bacteria. Particularly NIPA-NP is a promising alternative for the use of AzBBr in the treatment of infections caused by these microorganisms.

0494 - EDUCATIONAL INTERVENTION TENDING TO AVOID MEDICATION ERRORS IN A SOCIAL SECURITY INSTITUTE OF CORRIENTES.

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UNIVERSIDAD NACIONAL DEL NORDESTE

Objective: To improve prescriptive behavior in medical professionals of a Social Security Institute in Corrientes. A quasi-experimental study was conducted, without a control group. All application forms for extended treatment plans in a Social Service Institute of Corrientes were analyzed during a period of six months before and after an educational intervention during the year 2018-2019. The variables analyzed were: gender, age, diagnoses, prescribed medications, medication errors. To describe the types of errors the taxonomy of Otero Lopez was used. Were analyzed 600 application forms and 293 (49 %) prescription errors were observed during the prescription phase. Seventy seven percent (n= 150) of the patients were male, average age 52 years (range 5-91 years). The most frequent error detected before the intervention was prescription of erroneous medication (99 %) grouped as follows: a) inappropriate medications: meloxicam + glucosamine (5), ranitidine + domperidone (7), ergotamine + ibuprofen + caffeine (6), bromazepam + clebopride + simethicone (2), trimebutin + pancreatin + simethicone (7), denosumab (3), fexubostat (2); omega 3 (10), deproteinized extract of calf blood (2), donepecil (4), memantine (3); b) unnecessary medication: aspirin (15), rosuvastatin (9), omeprazole (8). Post-intervention results: only 4 errors were observed in the 600 application forms: inappropriate medications meloxicam + glucosamine (1), memantine (1), pancreatin + simethicone + trimebutin (1); unnecessary medication: aspirin (2). Through educational intervention an improvement in the prescriptive behavior was observed, especially those medications considered inappropriate, improving patient safety and quality of care.

0612 - CLASSIC HISTAMINE H1 RECEPTOR INVERSE AGONISTS ACTIVATE ERK1/2 PATHWAY AND MODULATE THE TRANSCRIPTION OF INFLAMMATORY GENES

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ININFA, UBA-CONICET (1); IBYME-CONICET (2)

Recently, we showed that widely used histamine H1 receptor (H1R) ligands that exert therapeutic actions by blocking the effects of histamine (HA), display positive efficacy concerning receptor desensitization and/or internalization. Now we aimed to investigate whether these processes affect the modulation of pro-inflammatory genes and its relationship with the activation of other signaling pathways independent from G protein. While 3 h (long term) exposure to anihistamines decreased expression of pro-inflamatory genes, when we exposed A549 cells to HA, chlorpheniramine (CHLOR) or diphenhydramine (DIPH) for 10 min (short term) and ligands were removed, ciclooxigenase 2 (COX-2) and interleukin 8 (IL-8) mRNA levels were increased after 2h 50 min (among 40 and 100 % for all ligands, $p<0.05$). Consistently, ERK1/2 phosphorylation levels were increased by HA (373 ± 102 %), CHLOR (95 ± 30 %) and DIPH (56 ± 16 %), $p<0.05$, indicating that they display positive efficacy towards this signaling pathway that has been described to be involved in regulation of both genes. When A549 cells were pre-exposed for 3 h with these ligands and after 1 h recovery, were stimulated with HA for 10 min, we found lower COX-2 mRNA levels compared to those observed without pretreatment (HA 29.5 ± 0.5 %, CHLOR 40.5 ± 16.5 and DIPH 34 ± 8.8 % of reduction, $p<0.05$). We also found lower IL-8 mRNA levels in CHLOR and DIPH pretreated samples (both around 20 % of reduction, $p<0.05$) although no differences were observed in HA pretreated cells. Thus, although short term exposure to antihistamines increase pro-inflammatory genes expression, a prolonged exposure with these ligands diminished it and impaired the increase induced by HA indicating that their anti-inflammatory effects continue despite the ligands being removed. In all, these findings reinforce the biased nature of these ligands and claim for a correct classification, providing evidence for a more rational and safe use of antihistamines.

0630 - THE ANTIALLODYNIC EFFECTS OF INTRATHECALLY APPLIED IMT504 ARE RELATED TO MODULATION OF GLIAL/MICROGLIAL RESPONSES AND OF THE EXPRESSION OF INFLAMMATORY FACTORS IN RATS WITH HINDPAW INFLAMMATION

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INSTITUTO DE INVESTIGACIONES EN MEDICINA TRASLACIONAL, CONICET-UNIVERSIDAD AUSTRAL (1); HOSPITAL UNIVERSITARIO AUSTRAL (2); LABORATORIO DE NEUROBIOLOGÍA, FACULTAD DE QUÍMICA Y BIOLOGÍA, UNIVERSIDAD DE SANTIAGO DE CHILE (3)

Chronic immune diseases, pathogenic infection, or tissue injury are common medical conditions, often leading to the development of chronic inflammatory pain which is unfortunately difficult to treat and often unresponsive to conventional therapies. We recently showed that the oligodeoxynucleotide IMT504 has remarkable antiallodynic and anti-inflammatory effects upon systemic administration in rats undergoing unilateral hindpaw chronic inflammation. In this study, we addressed if IMT504 intrathecal (i.t.) delivery is capable of modulating mechanical allodynia and its underlying mechanisms of action in the spinal cord. Male Sprague-Dawley rats with complete Freund´s adjuvant (CFA)-induced

unilateral hindpaw inflammation, received an acute i.t. injection of IMT504 (2 µg/µl; 10 µl). C-reflex, wind-up and mechanical hyperalgesia were recorded during 72 h after injection. Spinal cords were processed for immunofluorescence or western blot analysis for markers of activated glia and microglia such as fibrillary acidic protein (GFAP) and integrin αM (OX42), toll-like receptor 4 (TLR-4) and NF-B p65 subunit. Intrathecal IMT504 induced a clear reduction in mechanical hyperalgesia starting 1 h and lasting 48 h after administration, in association with parallel progressive reductions in C-reflex and wind-up responses. Furthermore, IMT504 significantly downregulated the expression of GFAP, OX42, TLR4 and NF-B. Altogether, we show that i.t. IMT504 efficiently eliminates inflammatory mechanical hyperalgesia for at least 24 h, in association with a depression in spinal sensitization and reductions in the activation of glia, microglia, and the NF-B and TLR-4 pathways. The exact mechanisms, by which these different events relate to explain the antihyperalgesic effects of IMT504, remain to be demonstrated. However, it could be hypothesized that the net effect of IMT504 are reductions in the synthesis of spinal pro-inflammatory mediators.

0670 - DUAL MODULATION OF GLUCOCORTICOID RECEPTOR ACTIVITY BY HISTAMINE H2 RECEPTOR SIGNALING. INVOLVEMENT OF RAP, ERK AND CAMP.

Valeria Nora TORRALBA AGU (1) | Agustina Soledad KELLY(1) | Natalia Cristina FERNANDEZ(1) | Carlos Alberto DAVIO(1) | Carina Claudia SHAYO(2) | Carlos Daniel ZAPPIA(1) | Federico MONCZOR(1)

ININFA, UBA-CONICET (1); INSTITUTO DE BIOLOGÍA Y MEDICINA EXPERIMENTAL (IBYME-CONICET) (2)

There are reports describing the interaction between membrane G-protein coupled receptor signaling and glucocorticoid receptor (GR) transcriptional activity. We have already reported that the signaling of the G alpha s-coupled histamine H2 receptor (H2r) increased GR transcriptional activity. The aim of the present work was to study the molecular mechanisms of this effect. HEK293 cells were transfected with plasmids coding to H2r, GR and a GR-driven reporter gene TAT3-Luc. While pretreatment with 10 µM amthamine, an H2r agonist which augments cAMP levels, increased dexamethasone (dex)-induced GR activity in a 50 % ($p<0.05$), raising cAMP levels with 25 µM forskolin reduced dex-induced GR activity in a 30 % ($p<0.05$). This discrepancy indicates that H2r regulation of GR activity is not strictly mediated by cAMP pathway, suggesting the involvement of other signaling partners. It has been described that H2r activation with amthamine also triggers ERK1/2 phosphorylation. In fact, treatment with the MEK inhibitor UO126 prevented amthamine potentiation of GR activity, pointing to ERK as a relevant player in the potentiation effect. Moreover, pretreatment with 10 µM of the H2r inverse agonists, famotidine and ranitidine, both of which decrease cAMP levels and increase ERK phosphorylation, boosted dex-induced GR activity to almost the quadruple. Trying to elucidate the role of other signaling proteins, cells were transfected with Rap-GAP, an inactivator of the small G-protein Rap. In this system, amthamine also lost its potentiating effect. The whole of our results points to a dual parallel regulation of the GR transcriptional activity: an inhibitory effect mediated by cAMP and an enhancing effect mediated by Rap and ERK proteins. Considering the co-expression of H2r and GR in several physiological systems and the widespread use of their ligands, the interaction described herein could have an impact on glucocorticoid based therapy and grants further research.

0810 - TREATMENT OF IRRADIATED MICE WITH ORAL RADIOPROTECTOR ATTENUATES INSULT.

Veronica MARTINEZ MARIGNAC (1) | Leonel MONDRAGON(2) | Lucia CERVANTES(3) | Gloria OERTLIN(1) | Fernanda CANTERO(3) | Jose Luis FAVANT(1)

IBIOGEM, CICYTTP (CONICET, ENTRE RIOS PROVINCE AND UADER) (1); RIVER PLATE ADVENTIST UNIVERSITY- UAP (2); NATIONAL UNIVERSITY OF ENTRE RÍOS, UNER, ENGINEERING FACULTY. (3)

Ionizing radiation directly affects DNA structure by inducing primarily DNA double strand breaks (DSBs), and secondarily production of reactive oxygen species (ROS) that oxidize proteins, lipids, and also induce several different damages to DNA, like generation of abasic sites and single strand breaks (SSB). Consequently, all these changes induce cell death and mitotic failure. The important use of IR in X ray exams and in radiotherapy and its undesirable effects took us to validate a murine model in order to evaluate DNA damage of X Rays and characterize natural and food supplements compounds with radio mitigation properties. Essiac Genuine tea has been used widely in the homeopathy market as a popular anticancer and antioxidant tonic. Due to the reported ROS scavenging properties of Essiac formula, we evaluate DNA damaged mitigation in 50 male Balb/c mice under 25-100m Sv-Gy, which is an average effective dose received by most X Ray exams during a year of radioimaging services by its personnel. The tea formula resulted in a significant reduction of DNA damaged of mice under the formula evidenced by Comet Assay ($p<0.01$) and acridine orange assay for micronuclei and DNA fragmentation evaluation ($p<0.02$) as well as in a normalization of the complete blood count (CBC). The tea did not show any cytotoxicity at the used doses, glucose and animal weight was similar between treatments. We not only demonstrated that Essiac tea is not toxic and acts as a radioprotector of IR X rays at doses to which are exposed the X ray personnel though we also optimized a murine model for further analysis of other natural compounds and supplements (e.g. Ascorbic Acid).

0819 - ESSENTIAL OILS AS SOURCES OF POTENTIAL ANTHELMINTIC COMPOUNDS TESTED ON THE MODEL ORGANISM CAENORHABDITIS ELEGANS

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CCT-INIBIBB, DEPARTAMENTO DE BBYF, UNS-CONICET

Parasitic nematodes are of major significance as human pathogens and have important economic impact worldwide due to considerable losses in livestock and food crops. Drug treatment of nematode infections are the pillar of worm control in human and veterinary medicine. Due to the appearance of drug resistant nematodes, there is a need of developing novel drugs. As parasitic nematodes are not ideal laboratory animals, the non-parasitic nematode Caenorhabditis elegans, has emerged as a model organism for drug discovery. Essential oils (EOs) are natural products produced by aromatic plants. EOs are complex mixtures that usually contain two or three major phytochemicals, which can be terpenes and/or aromatic compounds. We used paralysis assays of wild-type and mutant C. elegans strain and electrophysiological recordings to identify EO with potential anthelmintic activities, reveal the active components, the target sites and mechanisms of action. We found that EOs belonging to six different orders produced rapid paralysis of C. elegans and we establish the half maximal effective concentration values between 0.02-1.2 percent of EOs. We also found that all EOs inhibit egg hatching. Thus, EOs can mediate both rapid and long-term anthelmintic effects. We determined that trans-cinnamaldehyde (TC), a major component of C. verum EO, produces both paralysis and egg-hatching inhibition. By testing mutant worms, we identified that muscle L-AChR and GABA receptors are EO and TC targets in vivo. Electrophysiological studies from C. elegans cultured muscle cells identified the mechanism underlying the antiparasitic effect. Thus, by modulating two receptors with key roles in worm motility, these EO emerge as novel sources of anthelmintic compounds.

0864 - UNRAVELING THE MOLECULAR MECHANISM OF DII, A NEW ANTHELMINTIC DRUG

Sebastián GIUNTI | Pamela AZCONA | Gabriela BLANCO | Gustavo SILBESTRI | Diego RAYES | María José DE ROSA

INIBIBB-CONICET, DEPTO. BIOLOGÍA, BIOQUÍMICA Y FARMACIA-UNS

Nematode parasites cause substantial morbidity to billions of people and considerable losses in livestock and food crops. The repertoire of effective anthelmintic compounds is very limited, as drug development has been delayed for decades. By using C. elegans as a model for parasitic nematodes, we previously identified a new imidazole derivative, diisopropylphenyl-imidazole (DII), as a promising candidate for anthelmintic agent. DII lethal effects rely on a previously unidentified muscle nicotinic receptor (AChR), different from the classical levamisole-sensitive AChR. This novel AChR is composed by UNC-29 (a non-alpha subunit incapable of forming homomeric receptors) and other unidentified subunits. To elucidate its stoichiometry, we performed an initial screening of strains containing null mutations in different AChR subunits. By exposing these animals to DII (600 μm), we found a null mutant in acr-23 (an alpha nicotinic subunit) that is even more resistant to DII than UNC-29 null mutants. Since the mutants used in the initial screening had not been outcrossed to the wild-type (wt), we performed this outcross four times, selecting (by genotyping) those animals that contain the deletion in acr-23. Surprisingly, these outcrossed animals are as sensitive to DII as the wt. Moreover, when we outcrossed the original mutant strain to the wt selecting by their resistance to DII, we obtained animals that contain wild-type acr-23 alleles. This strongly suggests that another mutation, different from acr-23 deletion, causes the DII resistance. The drug resistance of these mutants appears to be DII-specific, as it is as sensitive to the classic anthelmintic levamisole as the wt. We are now focused on determining the gene that underlies this DII resistant phenotype. Parasite resistance to traditional nematocidal drugs has become a global concern. Therefore, the identification of new anthelmintics with novel targets, as DII, is mandatory to circumvent this growing problem.

0955 - NEBIVOLOL AND N-ACETYLCYSTEINE IN A MODEL OF GENETIC HYPERTENSION.

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CÁTEDRA DE FARMACOLOGÍA. FACULTAD DE CIENCIAS EXACTAS. UNLP./ CONICET (1); CÁTEDRA DE FARMACOLOGÍA, FACULTAD DE FARMACIA Y BIOQUÍMICA, UBA (2)

Introduction The imbalance between reactive oxygen species and nitric oxide is implicated in the pathogenesis of hypertension. Several studies have provided evidence of the role of an impaired glutation (GSH) system in the development of hypertension. Furthermore, N-acetylcysteine (NAC) improves NO bioavailability reducing blood pressure in adult spontaneously hypertensive rats (SHR). Nebivolol is a third-generation beta-blocker that combines beta-adrenergic blocking activity with a vasodilating effect mediated by the endothelial nitric oxide (eNOS) pathway. In the current study, we aimed to examine the effect of chronic oral treatment with the combination of nebivolol and NAC on hemodynamic and histological parameters in normotensive and SHR rats. Four weeks-old female SHR, and control normotensive male Wistar Kyoto rats (WKY) were randomly assigned into 3 groups (n= 10/group): Group 1, WKY without treatment; Group 2, SHR without treatment; and Group 3 (SHR + NAC), which received 0.6 % NAC in drinking water. Two months later rats were randomly assigned into 5 groups: Group 1, WKY without treatment; Group 2, SHR without treatment; Group 3, SHR with nebivolol (15 mg/kg/day); Group 4, (SHR+NAC) which received 0.6 % NAC in drinking water, and Group 5, (SHR+NAC+NEBI), which received NAC plus NEBI. Systolic blood pressure (SBP) was measured in conscious rats by indirect tail-cuff. Furthermore, we measured the mean arterial pressure (MAP) in freely moving rats after carotid cannulation. All rats were sacrificed at the age of 16 weeks. Target organ damage at the left ventricle was evaluated by histological analysis after Sirius red staining. The SBP measured by tail-cuff was significantly reduced in group 5 (SHR+NAC+NEBI) (mmHg; SHR: 181 ± 15 vs. SHR+NAC+NEBI: 155 ± 6, $p<0.05$), although the MAP was significantly reduced by NEBI alone or in combination with NAC (mmHg; SHR+NEBI: 166 ± 7, SHR+NAC+NEBI: 175 ± 14 vs. SHR: 201 ± 10, $p<0.05$). On the other hand, the histological analysis of left ventricle shows a significant reduction on fibrosis interstitial with all the treatments (FCI%, SHR+ NEBI: 0.54 ± 0.21, SHR+NEBI+NAC: 0.84 ± 0.13, NAC: 0.49 ± 0.16 vs. SHR: 1.65 ± 0.40, $p<0.05$). The increased of the size of cardiomyocytes (μm^2, SHR: 725 ± 96, WKY: 449 ± 93, $p<0.05$) was not prevented by any treatment (μm^2, SHR+NEBI: 693 ± 154, SHR+NAC: 707 ± 107, SHR+NEBI+NAC: 575 ± 86) The combination of nebivolol plus NAC was able to prevent the hemodynamic alterations in this model, and markers of organ target damage such as left ventricle fibrosis in SHR rats. Despite nebivolol alone does not modify peripheral SBP in SHR rats, it was able to partially prevent the increase of MAP and the fibrosis on the left ventricle.

Biología celular y molecular de procesos fisiológicos y patológicos / Biology IV

Chairs: Graciela Calabrese | Evangelina Capobianco

0411 - ANALYSIS OF MRP4/ABCC4-INDUCED EPIGENETIC SIGNATURE IN PANCREATIC CANCER

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ININFA

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer with a dismal prognosis. Histone deacetylases (HDACs) and demethylases (KDMs), as well as DNA methylases (DNMTs) and demethylases (TETs), are epigenetic modulators whose activity is frequently deregulated in various cancers including PDAC. In particular, HDAC1 and HDAC2 have been shown to play an important role in the control of proliferation, apoptosis, differentiation, migration, and angiogenesis of PDAC cells. The multidrug resistance-associated protein 4 MRP4/ABCC4 is a xenobiotic transporter involved in the regulation of cAMP signaling by extrusion to the extracellular compartment. MRP4 was found highly expressed in PDAC, and its expression correlates with increased proliferation and poor prognosis. MRP4 overexpression in the PDAC cell line BxPC-3 increased proliferation, and cell inoculation in NGS mice produced xenografts with increased weight and poor differentiation compared to mock tumors. Therefore, we aimed to analyze how MRP4 overexpression collaborates in PDAC malignant epigenetic and transcriptional signature that enables tumor progression. We analyzed the expression of several epigenetic modulators in MRP4-overexpressing BxPC-3 tumors (MRP4+), compared to wild type tumors (WT) and tumors transfected with an empty vector (mock). We found increased HDAC1 and HDAC2 mRNA and protein levels, and concomitantly decreased acetylation of H3K9ac, in MRP4+ compared to WT/mock ($p<0.05$). MRP4+ tumors also showed increased mRNA expression of key enzymes involved in epigenetic control of cancer progression: Sirt1 and Kdm1a (LSD1), involved in histone deacetylation and demethylation, and Dnmt1 and Tet1, linked to aberrant methylation/demethylation patterns in DNA. These findings suggest that, in pancreatic cancer, MRP4 contributes to the establishment of an aberrant epigenetic signature and altered

transcriptional program which may drive cells towards a proliferative and undifferentiated phenotype.

0520 - RETINOID X RECEPTOR´S ACTIVATION MODULATES A CROSSTALK BETWEEN NRF2 AND NFKB PATHWAY IN RETINAL PIGMENT EPITHELIUM CELLS UPON H_2O_2 TREATMENT

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INIBIBB-CONICET, DEPTO. BIOLOGÍA, BIOQUÍMICA Y FARMACIA-UNS

Age-related macular degeneration (AMD) is the main pathology leading to blindness in adults and has no cure or effective treatment. Retinal pigment epithelial (RPE) cells have immunomodulatory properties and their degeneration contributes in AMD development. Oxidative stress is also involved in the pathogenesis of this disease. We have demonstrated that RXR activation with HX630 protects RPE (D407) cells from H_2O_2-induced apoptosis, and prevents NFkB nuclear translocation. Also, our previous results suggested RXR-PPARgamma as the main heterodimer involved. In this work we investigate the RXR-PPARgamma involvement in the mentioned protective effect and its mechanism of action. For that, D407 were treated or not with H2O2, HX630 and/or a PPARgamma specific agonist (Pioglitazone: PG). We analyzed cell viability by MTT assay and DAPI stained; and studied NFkB pathway (which modulates inflammation and apoptosis) and Nrf2 (which activates cytoprotective genes and regulates NFkB pathway) by qRT-PCR, florescence microscopy and Western-blot. PG reproduced the inhibition of NFkB translocation and the protective effect of RXR activation against oxidative damage. PG inhibited the IKBalpha phosphorylation more than HX630, while it promoted IKBalpha synthesis. When HX630 and PG were together, the inhibition of NFkB translocation was higher than the agonists alone, although, there was no synergism in apoptosis prevention. HX630 increases Nrf2 synthesis, PG does not, and both agonists together decrease NrF2 levels suggesting proteosomal degradation. As a whole, our results show that RXR and PPARgamma agonists together potentiate the anti-inflammatory response but not the antiapoptotic effects of each agonist alone on RPE cells upon oxidative stress, and suggest that both agonists are necessary together to alter the crosstalk between NrF2 and NFkB pathway.

0537 - FKBP51 PROTEIN INCREASE CELL PROLIFERATION IN HEK293 CELLS

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DEPARTAMENTO DE QUÍMICA BIOLÓGICA-IQUIBICEN, FCEN, UBA (1); IBYME-CONICET (2)

The FK506-binding protein 51 (FKBP51) was first described as regulatory factor of steroid receptors. Currently, it is regarded that it also regulates diverse transcription factors (TFs). Previously, we demonstrated that FKBP51 modulates the transcriptional activity of NF-kB, AP-1 and E2F. Because, these TFs participate dynamically in cell proliferation, our aim was to investigate the effect of FKBP51 on cell proliferation. HEK293 cells were transfected with pCI-Neo or pCI-Neo-hFKBP51 expression plasmids. After one month of selection the stable transfected clones were selected. HEK51 cells stably overexpress FKBP51 protein, in the same way, HEKpCI-Neo were selected containing the empty vector. Cell proliferation (viable cell count with Trypan blue dye), proliferation index (flow cytometric analysis of cell division using dilution of CFSE) and cell cycle distribution (flow cytometry analysis of propidium iodide DNA stained) were evaluated. Human gene expression microarrays (SurePrint G3 Human GE v2 8x60K, Agilent) were performed for HEK51 vs. HEKpCI-Neo. After 96 hs seeding, FKBP51 overexpression significantly duplicated cells proliferation of HEK51 relate to HEKwt ($p= 0.031$). As well, FKBP51 duplicated the proliferation index from of 5.72 (HEKpCI-Neo) to 11.54 folds (HEK51). Interestingly, after 24 h of starving HEK51 showed a 50.7 % of cells on S phase, while was 38.7 % for HEKpCI-Neo. Gene expression microarray showed 272 differentially expressed transcripts ($p<0.001$, BRB array tools-NIH). These genes grouped by biological function (Knowledge Data Base, Quiagen) showed that FKBP51 affected 81 molecules involved on cellular grown and proliferation. We demonstrate that FKBP51 increases proliferation of HEK293 cells. Therefore, this work suggests a relevant role for FKBP51 in cellular proliferation and deserves further thorough studies.

*T. Bender and S. De Leo contributed equally to this work. Funding: UBACyT/CONICET. Acknowledgment: Drs. C. Robello and G. Greif, Institute Pasteur, Montevideo.

0553 - ANGIOTENSIN II PROMOTES THE SUBCELLULAR DISTRIBUTION OF MTORC PATHWAY PROTEINS IN H295R HUMAN ADRENAL CELLS

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Target of rapamycin (TOR) forms two kinase complexes named mTOR complex 1 and 2 (mTORC1/2). mTORC1 regulates cellular growth by phosphorylation of a different set of components, such as p70 S6 kinase and S6 ribosomal protein (S6), among others, which promotes protein synthesis by cap-dependent translation through the eukaryotic initiation factor 4E. Canonical PI3K/Akt pathway activates mTORC1 and Akt is able to activate mTORC2. Glycogen-synthase kinase-3 (GSK-3) pathway negatively regulates mTORC1 by phosphorylation and activation of an mTOR inhibitory protein. We have previously shown that mTORC1/2 are activated in human adrenal cells by angiotensin II (Ang II) with the involvement of a key steroidogenic enzyme, acyl-CoA synthetase 4. Proteins subcellular localization is a general principle used by hormones and growth factors to endorse precise spatial and temporal control of cellular functions. mTORC1/2 are localized in distinct subcellular compartments suggested to be an important mechanism to achieve signaling specificity. Thus, the aim of this work was to analyze the subcellular distribution of some components of mTORC1/2 pathway under Ang II stimulation in H295R human adrenal cells. We performed subcellular fractionation and observed by immunoblot that Ang II promotes mitochondrial Akt phosphorylation (pAkt) in a time-dependent manner (control vs. Ang II; $***p<0.001$). pAkt was also detected in the post-mitochondrial fraction. Ang II elicits a significant increase of phospho-S6 (pS6) in lysate, mitochondrial and post-mitochondrial fractions, as fast as 30 min ($***p<0.001$). We showed that mitochondrial S6 remains phosphorylated until 6 h, in contrast with a marked post-mitochondrial S6 inactivation (mito 6 h vs post 6 h; $***p<0.001$). We could also detect mitochondrial phospho-GSK-3 in Ang II-stimulated cells. These results indicate that Ang II-mediated response promotes subcellular distribution of mTORC pathway active components, in H295R human adrenal cells.

0578 - UPDATE OF THE RESULTS OF THE STUDY OF 4 DIFFERENT GENES, IN PATIENTS WITH CLINICAL CHARACTERIZATION OF MODY TYPE DIABETES.

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MODY type diabetes is characterized by mutations in different genes. This gives it an autosomal inheritance mode with dominant characteristics. These patients differ from classical diabetics because of their pathogenesis, treatment and prognosis, although they are often incorrectly diagnosed and poorly treated with the consequent deterioration of their quality of life. In order to correctly diagnose these patients and thus be able to give them a treatment focused on the specific pathogenesis of their disease, we have carried out the study of mutations in the HNF4A, GCK, HNF1A and HNF1B genes that cause types 1, 2, 3 and 5 of MODY respectively. The studies were performed by means of Sanger sequencing of the coding regions of the HNF4A, GCK, HNF1A genes and by new generation sequencing (NGS) on the HNF1B gene. These genes have been reported as responsible for the majority of MODY cases worldwide. Of the total of 185 unrelated patients analyzed with clinical characterization of any of the subtypes, 4 MODY1, 61 MODY2, 20 MODY3 and 3 MODY5 were diagnosed. The rest could not be diagnosed and were classified as MODYX. Likewise, we have made the diagnosis in both parents when they were available and other relatives with characteristics of Diabetes given the type of inheritance. In this way, we have been able to diagnose a group of patients with a particular type of Diabetes that requires specific treatment and follow-up. In addition, genetic counseling has been made to family members, which results in an improvement in the quality of life in them and an efficiency improvement of the resources necessary for their treatment and monitoring. In some patients diagnosed with MODY, the treatment has been reoriented, advising the intervening doctors, when these patients were treated as classic diabetics, since this produces a worse prognosis due to poor glycemic control.

0596 - TESTOSTERONE INDUCES UP-REGULATION OF MITOCHONDRIAL GENE EXPRESSION IN SKELETAL MUSCLE CELLS ACCOMPANIED BY AN INCREASE OF NUCLEAR RESPIRATORY FACTOR-1 AND ITS DOWNSTREAM EFFECTORS

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The loss of muscle mass and strength with aging, sarcopenia, is a prevalent condition among the elderly, associated with skeletal muscle dysfunction, enhanced muscle cell apoptosis and mitochondrial dysfunction. We have previously demonstrated that testosterone (T) protects against H_2O_2-induced apoptosis in C2C12 muscle cells, at different levels: morphological, biochemical and molecular. However, the role of T and its receptor at mitochondrial level is not well understood. Therefore, here we investigated the impact of physiological concentration of T on mitochondrial gene expression in C2C12 skeletal muscle cells. We found that T caused a significant increase in mRNA expression of genes encoded by mitochondrial DNA, namely NADPH dehydrogenase subunit 1 (ND1) and subunit 4 (ND4), cytochrome b (CytB), and cytochrome c oxidase subunit 1 (Cox1) and subunit 2 (Cox2) in skeletal muscle cells. In addition, gene and protein expression of the nuclear respiratory factors 1 and 2 (NRF1 and NRF2) and mitochondrial transcription factors A (Tfam) and B2 (TFB2M), key regulators of mitochondrial transcription and biogenesis, were also increased after T treatment, being the main regulator of mitochondrial fusion, OPA1, incremented as well. Of relevance, the simultaneous treatment with T and the androgen receptor antagonist, Flutamide, reduced these effects. The actions of the hormone observed were totally opposite to H_2O_2-oxidative stress induced treatment, which significantly reduced mitochondrial gene expression. Computational analysis revealed that mitochondrial DNA contains specific sequences, which the androgen receptor could recognize and bind, probably taking place thus, a direct regulation of mitochondrial transcription by the receptor. These findings indicate that androgen plays an important role in mitochondrial gene expression and biogenesis in skeletal muscle.

0607 - GLOBAL DNA METHYLATION LEVELS ANALYSIS IN A SERIE OF HEMATOLOGICAL, BREAST AND COLORECTAL CANCER SAMPLES FROM ARGENTINA

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Unlike their normal counterparts, tumor cells exhibit highly variable CpG methylation levels in a large proportion of the genome, which can lead to malignant cell transformation through multiple pathways. This prompted us to assess the extent of LINE1 methylation, a surrogate marker of global DNA methylation, of samples derived from controls and cancer patients from Argentina. Preliminary DNA methylation results from selected samples were replicated in a large serie of 146 controls (blood) and various cancer types: 112 hematological cancer (HemCa), 70 colorectal cancer (CRC) and 68 breast cancer (BrCa) samples. Further, we evaluated correlation with biological, clinical and demographic features. Blood samples were available in all cases, and for solid tumors paired tumoral/non-tumoral adjacent tissues (T/N) were available too. LINE1 methylation level was analyzed by MS-MLPA method. HemCa cases showed statistically significant higher LINE1 methylation level ($p<0.001$) compared to controls (mean= 0.93 and 0.84, respectively). This variation could be a consequence of chemotherapy. Methylation status in blood (0.86) and N tissue (0.87) from BrCa cases did not differ from controls, while levels in T tissue (0.88) were significantly higher than controls ($p<0.05$). No differences between N and T tissues were found. CRC cases showed hypomethylation for LINE1 when comparing T (0.81) to blood (0.87) or N tissues (0.88), reaching statistical significance of $p<0.05$ and $p<0.001$, respectively. This is in line with reported results. We found a negative correlation between age and methylation level in controls (-0.17, p= 0.04), and BrCa T tissue (-0.33, p= 0.03). Finally, no relevant associations between global methylation and mitochondrial genome variation (copy number and ancestry) were found for controls and HemCa sample sets. LINE1 methylation analysis in samples from lung, ovarian, pancreatic and skin cancers are ongoing.

0659 - RELEVANCE OF GLUCOCORTICOID RECEPTOR IN ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) is the most common type of acute leukemia in adults, and is characterized by accumulation of immature white blood cells with aberrant proliferation and differentiation blockade. One type of AML is Acute Promyelocytic Leukemia (APL), distinguished by the expression of promyelocytic leukemia (PML)-retinoic acid receptor (RARa) chimeric protein that acts as an antagonist of wild-type RARa. Treatment of APL patients with retinoic acid (RA) promotes at least partial transcriptional de-repression, but unfortunately provides transient clinical remission. Interestingly, we observed that APL-NB4 cell treatment with RA and glucocorticoids markedly enhances cell differentiation (Dif) and potentiates expression of RARa- target genes. Thus, we hypothesize that glucocorticoid receptor (GR) localization and target gene regulation in undifferentiated (Undif) cells somehow determine its

subsequent interaction and functional effects along the myeloid differentiation program. Therefore, in our aim to elucidate GR relevance in Undif and Dif cells, we report here that a different nuclear GR distribution is observed in western blots depending on the leukemic context (AML or APL) of cells grown either in full (F) or steroid-deprived (CS) serum-containing medium for 24h. Moreover, by RT-qPCR assays we quantified TTP (Undif-F: 0.33 ± 0.20, Undif-CS: 0.39 ± 0.17, Dif-F: 1.04 ± 0.29, Dif-CS: 0.82 ± 0.10) and DUSP-1 mRNA levels (Undif-F: 0.96 ± 0.32, Undif-CS: 0.63 ± 0.14, Dif-F: 0.57 ± 0.10, Dif-CS: 0.90 ± 0.36), resulting both in induced expression levels in Dif-cells, regardless of serum conditions or in CS-serum, respectively. Furthermore, co-IP experiments reveal a novel GR-RARa complex in Undif-NB4 nuclei, which becomes enhanced in Dif-cells. Collectively these data lead us to suggest that depending on the differentiation context, steroids may present opposing regulatory effects. Further characterization of this molecular context could aid to identify attractive targets for therapeutic strategies in myeloid leukemia.

0677 - TRANSCRIPTIONAL REGULATION IN WHOLE ORGAN MAMMARY GLAND CULTURE MEDIATED BY THE LIVER X RECEPTOR

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Liver X Receptors (LXRs) belong to the nuclear receptors superfamily of ligand activated transcription factors, being the oxysterols their endogenous ligands. They play a key role in the regulation of the cholesterol homeostasis, induce the expression on genes related to the de novo synthesis of triacylglycerides, and repress pro-inflammatory factors effects. During lactation, the mammary gland is endowed with an enormous capacity to synthesize and secrete lipid in the form of triglycerides and cholesterol esters. In previous studies, we found an increase in milk-cholesterol mediated by LXR activation in vivo. According to this founding, we aimed to study direct influence of LXR activation in the mammary epithelium. Mammary gland explants from C57BL/6JFCEN pregnant mice (15 days post coitum) were differentiated in Waymouth's MB 752/1 culture medium using a lactogenic hormone mix of insulin, aldosterone, hydrocortisone and prolactin. The culture was performed in the presence of LXR agonist GW3965 [1 µM] or DMSO (vehicle). Medium and additives were added fresh every second day. Lymph nodes were removed before culture. After 5 or 9 days of culture, mammary epithelial cells (MECs) were prepared using an enrichment protocol. Total RNA was extracted and RT-qPCR was performed. Both LXRa; and LXRb; are expressed in MECs. In response of the LXR activation, there was an increase in the expression of LXR target genes SREBP1C and ABCA1, related to de novo synthesis of triacylglycerides and cholesterol transport, respectively. Also, the expression of ABCA7, another cholesterol membrane transporter, was augmented. The greatest differences in LXR dependent gene expression were found at day 5 concomitant an increase in the expression of milk protein B-casein, a marker of mammary gland lactogenic differentiation. Together these results suggest the direct relevance of LXRs in the control of lipid homeostasis and secretion into the milk during lactation.

0708 - ISOLATION OF A BASIC PHOSPHOLIPASE A2 FROM BOTHROPS DIPORUS SNAKE VENOM THAT INHIBITS CELL ADHESION OF TUMORAL CELLS

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The vast majority of snakebite envenomings in northeastern Argentina are caused by Bothrops diporus (yarará chica). In addition to its systemic effects, B. diporus venom induces prominent local tissue damage. Phospholipase A2 (PLA2) enzymes from viperid venoms are often key players in the pathogenesis of these local effects. In the present study, we isolated a basic PLA2 from B. diporus venom, and examined its potential adhesion inhibition effect on a tumoral cell line. Purification was made by a two-step procedure utilizing ion exchange (HiTrap SP XL-AKTAprime) and gel filtration chromatography (Sephadex G-75). SDS-PAGE of the isolated enzyme showed a single typical band of ~14 kDa and PLA2 activity was evidenced by the formation of hemolytic halos in agarose-erythrocyte egg yolk gels. Cytotoxic activity of the PLA2 (1.5-500 µg/mL- 3 h incubation) was determined on a murine tumoral epithelial cell line (LM3) grown in DMEM-5% FBS at 37°C-5% CO_2. Non-cytotoxic concentrations were selected for adhesion inhibition assay. Briefly, LM3 cells (3×10^4/well) were preincubated for 30 min at 37°C with PLA2 (1.2, 1.5, 2.5, 3, 5 and 10 µg/mL) or culture medium (control) and then added to 96-well plates. After 1.5 h, non-adherent cells were removed by careful washing and aspiration with PBS. Adherent cells were fixed and stained with crystal violet. The percentage of cell adhesion was determined by comparison of the absorbance readings (620 nm) with the mean absorbance of control cells (not exposed to the PLA2), considered as 100 % adhesion. Results indicate that the isolated enzyme PLA2 induces a dose-dependent inhibition of cell adhesion. Even with the lowest dose assayed (1.25 µg/mL) a 20 % of this effect was observed and with 10 µg/mL, 60 % of cells didn´t adhere to the substrate. Although more studies are needed, these findings demonstrate the therapeutic potential of this basic PLA2 isolated from B. diporus venom.

0786 - ALPHA-1ANTI-TRYPSIN (A1AT) AVOID EPITHELIAL TO MESENCHYMAL TRANSITION IN DIABETIC RETINOPATHY

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Diabetic retinopathy is a leading cause of blindness in working-age population and is regarded as a microvascular complication due to the breakdown of the endothelial barrier. The retinal pigment epithelium (RPE), which is the major component of the outer blood-retinal barrier is also the key to maintain the integrity of retinal tissues. In diabetic retinopathy, cell-cell junction complexes between RPE cells are to disassemble and the leakage can be found through paracellular space. Dissociation of cultured RPE cells leads to dedifferentiation of the cells into fibroblast-like cells through epithelial to mesenchymal transition (EMT). To maintain the integrity of epithelial cells adherens junctions are critical. NFkB, TG2, mTOR, Wnt canonical pathway mediators like GSK3ß and ß-Catenin are proteins involved in many biological processes, such as inflammation, angiogenesis, cell adhesion, migration, survival and the EMT. HIF-1a, E-Cadherin and N-Cadherin are regulated with these pathways, where HIF-1a is related with angiogenesis and E-Cadherin and N-Cadherin are related with cellular adhesion. In this work, we explore the expression of NFkB, TGM2, mTOR, GSK3ß, ß-Catenin, HIF1a, N-Cadherin and E-cadherin on ARPE-19 cells exposed to high glycemia and A1AT treatment. ARPE-19 cells were incubated 16 h with DMEM 5.5 mM glucose (Control), DMEM 5.5 mM glucose + 4.5 mg/ml A1AT (Control + A1AT), DMEM 30 mM glucose (Diabetic), DMEM 30 mM glucose + 4.5 mg/ml A1AT (Diabetic + A1AT). Cells were harvested for Western blot, for RT-qPCR or fixed for immunohistochemistry. NFkB, TGM2, AKT, pAKT, mTOR, ß-Catenin, N-Cadherin and HIF1a were diminished with A1AT treatment, while, GSK3ß and E-Cadherin were increased. Outcomes support the hypothesis that A1AT decrease integrin mediated signaling avoiding EMT. These results help to understand mechanisms involved in inflammatory processes in diabetic retinopathy and make A1AT as a suitable molecule to diabetic retinopathy treatment.

1006 - DIETARY (−)-EPICATECHIN MITIGATES TLR4-MEDIATED INFLAMMATION IN KIDNEY FROM HIGH-FAT FED MICE

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Chronic inflammation is a common feature of obesity, insulin-resistance and dyslipidemia, conditions that contribute to the development of chronic kidney disease. The flavanol (−)-epicatechin (EC) is present in the human diet. In human and animal studies EC consumption was associated with a range of health benefits. The aim of this work was to characterize the mechanisms involved in the prevention or attenuation by EC of kidney inflammation in mice fed a high-fat (HF) diet. C57BL/6J male mice were divided into 4 groups: control (C), control + 20 mg EC/kg body weight (CE), HF diet (60 % fat from lard), and HF diet + EC (HFE) for 14 w. Body weight was higher in the HF and HFE mice compared to both control groups. EC supplementation mitigated the hyperglycemia and dyslipidemia developed by HF mice. High-fat fed mice showed increased kidney cortex vacuolization that was not affected by EC. Consumption of the high-fat diet led to endotoxemia, increased expression of kidney TLR4 (73 % over control values), MyD88 (62 % over control values), and AP-1-DNA binding measured by EMSA in nuclear fractions (78 % over control values). EC supplementation prevented all these increases. Furthermore, the increase in AP-1 activity in the HF group was not induced by the mitogen-activated protein kinases ERK and JNK. HF mice showed an overexpression of TGFß-1 in kidney cortex respect to C and HFE groups. The Nrf2 pathway and the activation of NFkB and HIF-1 were not affected by any of the treatments. In this model of obesity, mice developed kidney inflammation with evidence of fibrosis, which were both mitigated by EC. Inflammation was indicated by an increased expression of TLR4, accompanied by the activation of the MyD88/AP-1 pathway, which was not observed in HFE group. In summary, dietary EC can protect the kidneys from the inflammatory damage associated with consumption of high fat diets and obesity.

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Chairs: Julieta Aisemberg | Luis Canosa

0547 - PHARMACOLOGICAL ACTIONS OF ALLOPREGNANOLONE OVER THE OVARIAN PHYSIOPATHOLOGY WITH DIFFERENT EXPERIMENTAL MODELS

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Allopregnanolone (ALLO) is a neurosteroid derived from progesterone; its actions in the CNS are well known. ALLO modulates GABA neurotransmission with antidepressant, anxiolytic, anticonvulsant and anesthetic effects. ALLO levels fluctuate under stress and throughout the menstrual cycle, pregnancy and menopause. The objective of this work was to evaluate ALLO action over the female rat ovarian physiopathology, from the global effect on the CNS to the action on the granulose cells. We designed different experimental models in order to differentiate four levels of action. After a dose/response curve we found that ALLO 6 µM, a pharmacological dose, was capable of disrupting the hypothalamus-hypophysis-ovarian axis causing significant ovarian morpho-physiological changes (augmented follicular atresia, $p<0.001$; cyst formation, $p<0.001$; corpora lutea apoptosis, $p<0.001$; and inhibited ovulation, $p<0.001$) through the central interaction with GABAAR. This dose changed the ovarian total expression of the progesterone receptor ($p<0.01$). The ex vivo culture of GMS-NOP-ovary system, proved the peripheral effect of ALLO over the ovarian steroidogenesis augmenting P4 secretion ($p<0.05$) through the adrenergic innervation. The local intra-bursal administration of ALLO affected the ovarian morpho-physiology (augmented follicular atresia, $p<0.001$ and corpora lutea diameter, $p<0.05$), through GABAAR, but failed to inhibit ovulation. Finally, the in vitro primary culture of ovarian granulose cells treated with ALLO showed a significant decrease in the PCNA antigen ($p<0.05$). ALLO at pharmacological dose is able to affect the rat female reproductive physiology. This is the first evidence of the global ALLO actions on the reproductive axis that allow us to assess the power of ALLO effects over reproductive parameters utilizing different administration routes. ALLO is a molecule with a great versatility and with a great pharmacological potential in female reproductive physiology.

0561 - HYPERTHYROIDISM ENHANCES FETAL AND PLACENTAL GROWTH AND PLACENTAL IMMUNE CELL INFILTRATION

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Thyroid dysfunctions cause reproductive disorders as fetal deaths, preterm birth and preeclampsia. Whether thyroid hormones (THs) exert any function in placental immune cells is unknown. Therefore, the aim of our work was to assess the influence of hyperthyroidism on placental immune cells as well as the impact on reproduction in pregnant rats. To this end, 10-12 weeks old Wistar rats were injected with a daily dose of T4 (0.1 or 0.25 mg/kg s.c) to induce hyperthyroidism (hyper) or vehicle in control animals. Rats were mated 8 days after starting T4 treatment and euthanized on day 19 (G19) and 20 (G20) of gestation. Placenta samples were minced to reach single cell suspension. Then, resident placental immune cells (CD45+) were analyzed by flow cytometry and mRNA content of hormone receptors by qPCR. Also, placental and fetus weights and fetus number were measured. Hyper mothers delivered more fetuses compared to controls ($p<0.001$). The offspring of hyper 0.25 mg/kg mothers weighed more in G19 and G20 ($p<0.001$). The placentas of the hyper 0.25 mg/kg mothers were heavier than controls only in G19 ($p<0.001$). Furthermore, we showed a decrease in the expression of progesterone, estrogen and ß2 thyroid receptors in hyper 0.1 mg/kg ($p<0.05$; $p<0.01$). On G19, the percentage of leukocytes was significantly higher in both hyper groups ($p<0.05$ for 0.1 mg/kg; $p<0.01$ for 0.25 mg/kg). On G20 we showed an increase in leukocyte infiltrate respect to G19 in the control ($p<0.001$) but not in the hyper group. These results suggest that T4 administration accelerates fetal development and changes the placental sensitivity to ovarian steroids by modulating their receptors expression and advances the increase in placental resident leukocytes. To our knowledge, this is the first report that shows the modulation of resident immune cells by thyroid hormones.

0623 - CYCLIC AMP EFFLUX THROUGH MRP4 REGULATES MOTILITY AND ACTIN POLYMERIZATION IN BOVINE CRYOPRESERVED SPERMATOZOA.

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Capacitation is a key process involved in the acquisition of sperm fertilizing competence. The importance of cAMP during capacitation has led us to study the role of the MRP4 transporter, multidrug resistance-associated protein 4 that extrudes cAMP from cells. We previously described that MRP4 mediates cAMP efflux in bovine spermatozoa and that extracellular cAMP activates signaling pathways associated to capacitation. Here we deepen the study of cAMP efflux in the acquisition of sperm fertilizing ability in this species using MK571, a compound that inhibits MRP4. We evaluated capacitation by LPC-induced acrosome reaction and the ability of sperm to be released from oviductal epithelia. Both events were decreased when the MRP4 inhibitor (50 µM) was added ($p<0.05$). Immunofluorescence evidenced that MRP4 is localized in the acrosomal and post-acrosomal regions, and mid-piece of the flagellum at 15 min capacitation time. At 45 min, localization was preferentially acrosomal. As a result of MRP4 localization in the flagellum, we assessed the involvement of this protein in sperm motility during capacitation. A decrease in parameters associated to sperm motility (progressive motility, VCL, STR, ALH and BCF), measured by computer assisted sperm analysis, was observed in spermatozoa incubated for 15 and 45 min with MK571 ($p<0.05$). Since actin cytoskeleton plays essential roles in the regulation of sperm motility, we studied actin polymerization (F-actin). An increase in F-actin (assessed by Alexa 488-phalloidin) was observed in sperm at 15 but not at 45 min incubation time. This increase was detected in sperm's heads as well as in their tails. When MRP4 was inhibited, F-actin decreased, in a process that was reverted with extracellular cAMP 10 nM in the flagellum but not in the head ($p<0.05$). Our results support the importance of cAMP efflux through MRP4 in sperm capacitation and suggest that this process is involved in the regulation of sperm motility.

0657 - EVALUATION OF GROWTH HORMONE RECEPTOR AND INTRACELLULAR SIGNAL TRANSDUCTION PATHWAY PROTEINS IN A FOLLICULAR PERSISTENCE MODEL IN CATTLE

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Follicular persistence is a component of cystic ovarian disease (COD) in dairy cows. Numerous events occur during folliculogenesis and an adequate supply of nutrients is necessary. Although growth hormone (GH) has been associated with follicular development, little is known about its function in ovarian diseases. The aim of this study was to evaluate the immunoexpression of GH receptor (GHR), the intracellular signal transduction pathway proteins Janus kinases (JAKs) and the signal transducers and activators of transcription (STATs) by immunohistochemistry, during follicular development in the ovary of cows with induced persistence. The experimental model was performed with an intravaginal progesterone device to get subluteal plasma concentrations of the hormone, obtaining anovulatory persistent follicles. Cows were ovariectomized at the expected day of ovulation (P0) and at 5 (P5), 10 (P10) and 15 days (P15) of persistence (n= 5). Control cows were ovariectomized in proestrous to obtain preovulatory dominant follicles (C; n= 5). In granulosa cells, the immunoexpression of GHR decreased during folliculogenesis as follicular growth progressed both in control and persistence groups. In theca interna, the expression was lower in antral follicles of C group than in antral follicles of all persistence groups ($p<0.05$). Also, in granulosa, total JAK2 was lower in all follicular categories of the C group in relation to the persistence groups ($p<0.05$). In theca, total JAK2 was lower in antral follicles of the C group respect to persistent follicles of P10 and P15 ($p<0.05$). Phosphorylated JAK2 was lower in persistent follicles as persistence days progressed. Total and phosphorylated STAT5 showed no differences between groups ($p>0.05$). In granulosa, phosphorylated STAT3 was lower in P10 compared to P0, P5 and P15 groups ($p<0.05$). These alterations in the expression of GHR and the GH signaling pathway proteins in follicular persistence may be associated with COD pathogeny.

0683 - DYNAMIC BEHAVIOR OF THE SERUM ALKALINE PHOSPHATASE LEVELS DURING PREGNANCY

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Alkaline phosphatases (AP) are a group of 4 isoenzymes classified as placental, germ cell, intestinal isoenzymes and non-tissue specific. It is documented that AP levels are elevated in in the third trimester of pregnancy. The AP placental isoform (PLAP) is in the microvilli of the syncytiotrophoblast. Although its role in the placenta is still unclear, it was reported that exosome release into maternal circulation increases in pregnancies complicated by preeclampsia and intrahepatic cholestasis. The expression of PLAP in theses exosomes is also increased. However, its clinical significance is not explored yet. The aim of this was to study the dynamic behavior of the serum AP enzyme levels in pregnant women with preeclampsia (PE) and intrahepatic cholestasis pregnancy (IC). A retrospective descriptive-quantitative study using the clinical records was carried out in 113 women who attended their pregnancy at the Hospital Posadas during 2018 and presented PE, IC, or normal pregnancies (C). Total serum AP levels (before 20th week and after 20th week of gestational age), gestational age (ga), newborn weight (nw) and sex were considered. Sixty C (ga: 38.4 ± 1.5 w; nw: 3,215 ± 639 g), 39 PE (ga: 36.2 ± 3.1 w ; nw: 2,578 ± 902 g) and 14 IC (ga: 37.0 ± 1.9 w; nw: 2,838 ± 565.9 g) were studied. No significant differences in AP levels among the groups before 20th week were found (PE: 80.7 ± 31.7 UI/L; CI: 65.5 ± 19.0 UI/L; C: 67.3 ± 12.8 UI/L); nor after 20th week (PE: 181.6 UI/L; CI: 235.1 ± 79.9 UI/L; C: 190.0 ± 83.8 UI/L). The increased AP levels found in exosomes released into the maternal circulation in preeclamptic pregnancies do not correlate with the levels of the soluble enzyme in the maternal serum. Therefore, placental stress in pregnant women complicated by PE or IC does not seem to impact the serum levels of AP, subtracting the diagnostic value of the enzyme during pregnancy.

0724 - EXPRESSION OF OCT4 AND STELLA PROTEINS IN THE INFANT AND ADOLESCENT HUMAN OVARY

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Follicular assembly yields by birth an endowment of resting, non-growing primordial follicles, which conforms the ovarian reserve (OR). Primordial follicles in the OR remain quiescent in women for years until they are lost by atresia or activated to enter a process of maturation in the so-called growing follicular pool. At puberty, quiescent primordial follicles cyclically selected for maturation undergo folliculogenesis yielding just one preovulatory follicle-enclosed dominant oocyte at each menstrual cycle. The developmental competence of oocytes is built up throughout folliculogenesis. OCT4 expression in growing oocytes is an indication that they have entered a pathway necessary to gain a developmentally competent status. STELLA is a maternal-effect

factor with a novel function, safeguards the unique oocyte epigenome by preventing aberrant de novo DNA methylation. The aim of this study was to evaluate folliculogenesis and immunoexpression of OCT4 and STELLA in the infant (n= 6, 6-12 years old) and adolescent human ovary (n= 11, 12-19 years old). They were grouped as Control group (n= 5), patients with benign pathological disease; Study group (n= 12), patients with extragonadal cancer that received (n= 5) or not chemotherapy (n= 7). All samples showed active folliculogenesis. OCT-4 was positive in all samples in some oocytes of primordial, primary and secondary follicles. STELLA was detected only in post-menarcheal patients with cancer extragonadal that did not received chemotherapy treatment, in the oocyte cytoplasm of some primordial follicles. OCT4 expression in postnatal ovary might indicate those oocytes that have entered the acquisition of full developmental competence path. In contrast, oocytes that do not express OCT4 could be either eliminated by apoptosis or recruited later during folliculogenesis. The expression of STELLA could be related to alterations in DNA methylation in cancer processes.

0750 - ORAL HEALTH AND ADVERSE PREGNANCY OUTCOME: LPS FROM PORPHYROMONAS GINGIVALIS IMPAIRS TROPHOBLAST CELL FUNCTION AND THEIR -INTERACTION WITH NEUTROPHILS

Vanesa Cinthia HAUK | Guillermina CALO | Brenda LARA | Daiana VOTA | Lucila GALLINO | Fátima MERECH | Zaira NAGUILA | Daniel PAPARINI | Rosanna RAMHORST | Claudia PÉREZ LEIRÓS

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Trophoblast cell differentiation into an invasive phenotype and trophoblast-immune cell interaction are crucial for adequate placentation and homeostasis. Porphyromonas gingivalis (Pg) is a prevalent pathogen of periodontal disease that was implicated in adverse pregnancy outcome although the mechanisms involved are still unclear. The aim of this study was to examine the effect of lipopolysaccharide from (Pg-LPS) on trophoblast cell function and trophoblast-neutrophil interaction. Human trophoblastic cell line Swan-71 was treated with Pg-LPS (10 ng/ml). Cytokine and chemokine expression was evaluated by RTqPCR and ELISA, glucose uptake by flow cytometry using the fluorescent analogue 2-NBDG and cell invasion assessed in Matrigel-covered transwells. Peripheral blood neutrophils were purified from healthy donors and cultured with conditioned media of trophoblast cells pretreated (PgLPS-CM) or not with LPS (TbCM); apoptosis was determined by fluorescence microscopy and CD11b and reactive oxygen species (ROS) were evaluated by flow cytometry. Regulatory T cell induction was evaluated after 48h of neutrophil-PBMC coculture. Pg-LPS treatment reduced trophoblast cell invasion, glucose uptake and modulated cytokine production (IL1ß; secretion X ± SE basal 11.4 ± 4.3; Pg-LPS 21.4 ± 5.4; $p<0.05$). PgLPS-CM induced neutrophil activation with higher CD11b expression and ROS synthesis (basal Neutrophil: 13,930 ± 2,830; Neutrophil+TbCM: 10,721 ± 2,298 Neutrophil+PgLPS-CM: 19,816 ± 3,543; $p<0.05$). Neutrophil exposed to TbCM favored the induction of CD4+ Foxp3+ T cell after 48 h of coculture with PBMCs. There was no Treg induction when neutrophils were preconditioned with PgLPS-CM (% CD4+FoxP3+cells PBMCs-Neutrophil: 3.8 ± 0.6; PBMCs-TbCM Neutrophil: 10.0 ± 3.4 and PBMC-PgLPS-CM Neutrophil: 5.7 ± 0.9 %; $p<0.05$). In line with this result, lower percentage of Foxp3-GFP+ Treg cells within CD4+ cells is observed in implantation sites of WT mothers treated at 6.5 gestation day with PgLPS compared with control mice. PgLPS impairs trophoblast cell function and abolishes their deactivating effect on neutrophils. This might contribute to the pathogenic mechanisms of Pg infection during placentation.

0761 - INTERPLAY BETWEEN VIP AND MTOR IN TROPHOBLAST CELL NUTRIENT UPTAKE. IMPACT ON THE TROPHOBLAST-IMMUNE INTERACTION

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The transport of nutrients by cytotrophoblast cells regulates placental metabolism throughout pregnancy. The mammalian target of Rapamycin (mTOR) functions as a placental growth signaling sensor and modulates nutrient transporters expressed on trophoblast cells (Tb). A decreased placental mTOR activity was reported in pregnancies complicated by placental insufficiency and intrauterine growth restriction. We have previously shown that the vasoactive intestinal peptide (VIP) stimulates human cytotrophoblast cell glucose and amino acid uptake along with increased GLUT1/3 and SNAT1 expression. Accordingly, a murine pregnancy model with VIP-deficient Tb cells presented reduced fetal weight at day 14.5. Here we deepened into the mechanisms of nutrient transport induced by VIP in cytotrophoblast cells focusing on the interplay between exogenous/endogenous VIP and mTOR activity. Also, based on the close regulatory interaction of cytotrophoblast cells and maternal leukocytes at the early maternal-placental interface, we explored glucose uptake by monocytes conditioned by VIP and Tb cell factors. The human Tb cell lines Swan-71/BeWo were used. VIP knocking-down was carried out with a VIP siRNA. System A activity was measured by 14C-MeAIB incorporation and VIP/mTOR expression by qRT-PCR and flow cytometry. mTOR phosphorylation was studied by western blotting. Glucose uptake in CD14+ monocytes isolated from peripheral blood was assessed by flow cytometry using the fluorescent analog 2-NBDG. VIP stimulated Na+-dependent 14C-MeAIB uptake in Tb cells (n= 4; $p<0.05$) and induced mTOR phosphorylation (n= 3; $p<0.05$). In VIP-knocked down Tb cells, mTOR mRNA and protein expression was reduced (n= 3; $p<0.05$). Finally, VIP and Tb conditioned media modulated glucose uptake by monocytes. Our findings support an interplay between endogenous/exogenous VIP and mTOR in nutrient uptake by Tb cells. A metabolic conditioning of maternal leukocytes by trophoblast cells is also proposed.

0878 - HIPPOCAMPAL HORMONE RECEPTORS EXPRESSION IN LATE PREGNANT AND LACTATING RATS: EFFECT OF MILD HYPERTHYROIDISM

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INSTITUTO DE MEDICINA Y BIOLOGÍA EXPERIMENTAL DE CUYO (IMBECU)

Thyroid disorders are associated with anxiety, depression and disturb responses to stress. The effect of stress is associated with functional changes in hippocampus (HpC) and hypothalamus by activating the hypothalamic-hypophysis-adrenal axis (HHA) and glucocorticoid release induced to stress. This response is physiologically attenuated during lactation. We found that mild hyperthyroidism (HyperT) increases stress-induced serum prolactin (PRL) and corticosterone secretion in lactating rats suggesting that HHA remains activated in lactation. To explore possible causes of this effect we studied the expression of thyroid receptors (TR), the long isoform of PRL receptor (PRLRl), members of the PRL signaling pathway, estradiol receptor (ER) and glucocorticoid receptor (GR) in HpC of Wistar female rats in different reproductive states (day 19 of gestation (G19), 2 (L2, early lactation) and 12 (L12) of lactation) in control (Co) and HyperT rats. Mild HyperT was induced with T4 (0.1 mg/kg/day, s.c.), a dose that allows the maintenance of lactation. HpC mRNA was obtained and the expression of receptors TRa1, TRa2, TRb1, TRb2, ERa, GR and PRLRl and members of the PRL signaling pathway (STAT5b, SOCS1 and SOCS3) was

determined by RT-qPCR. HpC mRNA content of TRs, STAT5b (activator of PRL signaling) and SOCS1 (supressor of PRL signaling) decreased from G19 to L12 in Co and HyperT rats ($p<0.05$). HyperT induced increases of TRa2 ($P< 0.05$ vs Co) and TRb2 in L2 ($p< 0.01$ vs. Co) without changes in others isoforms. HyperT increased STAT5b in G19 ($p<0.05$ vs. Co). ERa and PRLR mRNAs were unchanged by treatment or reproductive state while GR increased in L12 in both groups ($p<0.05$ vs. G19).These results indicate a physiological decline in HpC responsiveness to thyroid and PRL hormones in the transition from pregnancy to lactation in Co, while the increases observed in TRa2, TRb2 and STAT5b described in HyperT rats may be involved in the persistence of high HHA axis reactivity.

Endocrinología / Endocrinology III

Chairs: María Sonia Baquedano | Silvina Gutiérrez Oschmann

0066 - THE PHTALATE DEHP DOWNREGULATES THE PITUITARY ESTROGEN RECEPTOR ALPHA EXPRESSION AND IMPACT ON LACTOTROPH AND GONADOTROPH CELL PROLIFERATION

Pablo Aníbal PÉREZ | Jonathan TOLEDO | Liliana Del Valle SOSA | Ana Lucía DE PAUL | Alicia Ines TORRES | Silvina GUTIÉRREZ

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The normal functioning of the pituitary gland is the result of a balanced mechanism capable of rhythmically control the proliferation and hormonal secretion of different endocrine cell populations in response to endogenous factors such as Estrogen (E2) acting through estrogen receptors (ER). Also, it has been shown that exogenous agents as phthalates, substances used for plastics manufacture, might act as endocrine disruptors in several tissues, simulating E2 effects. The purpose of this study was to analyze the Di-2-ethylhexyl phthalate (DEHP) effect on pituitary ERa expression and its impact on cell proliferation. We used Wistar female rats prenatally treated with DEHP. At 21 days of postnatal life pituitary glands were extracted and intracellular ERa (ERa) and membrane ERa (mERa) positive cells were quantified by flow cytometry (FC). Also, primary pituitary cell cultures were stimulated with DEHP, E2 or DEHP+E2 for 72 h. The lactotroph and gonadotroph proliferation was quantified by double immunostaining (ki67+PRL or Ki67+bLH). The percentages of ERa+ and mERa+ cells were quantified by FC. ANOVA Fisher ($p<0.05$). Our results showed that DEHP prenatal exposition significantly reduced the cell number that express ERa (DEHP: 73 ± 4.6 vs. control: 86.4 ± 0.5 %) and mERa (DEHP: 5.7 ± 0.8 vs. control: 8.9 ± 0.2 %). In vitro, DEHP decreased ERa+ pituitary cells (DEHP: 79.2 ± 1.1 vs. control: 86 ± 1.2 %) and mERa+ pituitary cells (DEHP: 8.2 ± 1.3 vs. control: 11.5 ± 1.5 %). In lactotrophs, DEHP inhibited the Ki67 expression (DEHP: 21.1 ± 2.2 vs. control: 27.1 ± 2.1 %). Also, DEHP reversed the E2 proliferative effect observed in this cell type (DEHP: 25.4 ± 1.2 vs. 30.7 ± 1.9 %) In gonadotrophs, DEHP inhibited Ki67+cells (DEHP: 20.0 ± 4.2 vs. control: 28 ± 2.5%), without modifying E2 effect. These observations suggest that DEHP impacts on the pituitary gland during the development, downregulates ERa expression and affects the lactotroph and gonadotroph proliferation in adulthood.

0158 - LONG-TERM EFFECTS OF SUCROSE CONSUMPTION ON GLYCEMIA AND RAGE EXPRESSION IN JUVENILE VERSUS ADULT RATS.

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IBYME-CONICET

Excessive consumption of sucrose in early stages of development has deleterious neurobiological and behavioral effects in adulthood. Among these disturbances, difficulties in memory retrieval by early sucrose exposure were previously described. Here, we examined the glycemic levels of the animals and their response to an intraperitoneal glucose overload. We found that sucrose consumption increased the AUC (area under the curve derived from the oral glucose tolerance test) in both young and adults (two-way ANOVA, $FTreat(1,34)= 7,658$; $p= 0.0091$. Fisher's LSD post hoc test, $p= 0.047$ and $p= 0.040$, respectively) but only consumption during youth maintained this effect in the long term (two-way ANOVA, $FTreat(1,34)= 4,445$; $p= 0.0429$. Fisher's LSD post hoc test, $p= 0.020$). RAGE expression was also assessed by Western blot. Age differences were detected by two-way ANOVA in the two brain areas examined, the mPFC and the vHIP, but only in the mPFC differences by treatment were observed (two-way ANOVA, FTreat-Age$(1,20)= 2,327$; $p= 0.001$). Surprisingly, sucrose exposed animals in their youth showed decrease of RAGE while adults raised these values in the mPFC (Fisher's LSD post hoc test, $p= 0.037$ and $p= 0.002$, respectively). When all animals were pulled together, there was a negative correlation of the exploration ratio of the memory recognition test and the RAGE levels in the mPFC ($F(1,14)= 7,225$; $p= 0.0434$; $r^2= 0.59$) indicating that higher RAGE values relate with poorer novelty recognition on the final task of the memory test. Similarly, the basal plasma glucose levels also correlated with lower exploration ratio on the final recognition task ($F(1,21)= 5,744$; $p= 0.0264$; $r^2= 0.223$). When basal glycaemia was analyzed together with mPFC RAGE levels, a positive correlation was observed ($F(1,18)= 5,693$; $p= 0.0289$; $r^2= 0.251$). In summary, these results suggest that sucrose induced-hyperglycemia is detrimental for the memory a phenomenon that is related with the RAGE pathway in the mPFC.

0210 - THE KALLIKREIN-KININ SYSTEM IN THE PITUITARY GLAND IS INVOLVED IN LACTOTROPH FUNCTION AND CONTROLLED BY DOPAMINE AND ESTRADIOL

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TGFß1 is a potent inhibitor of lactotroph cell proliferation and prolactin (PRL) secretion and Tissue Kallikrein (KLK1) was described as an important activator of latent TGFß1 in vivo. The Kallikrein-Kinin System (KKS) is complex; kininogens are cleaved by KLK1, releasing kinins, which exert their effect throw its receptors B1R and B2R. Whereas B2R is constitutively expressed, B1R is inducible in pathological conditions. We have previously found that the pituitary expression of most components of the KKS, as well as local TGFß1 activity, is reduced in prolactinomas. Then we postulate that the recovery of pituitary KKS, could improve local TGFß1 activity counteracting prolactinoma development. To this end, we first deepen the study of the pituitary KKS regulation by dopamine and estradiol and the pituitary cell types expressing kinins receptors. Female mice lacking the dopamine receptor type 2 (Drd2KO, prolactinoma) vs. WT counterpart were used. 1- Double immunofluorescences were performed to assay B2R expression in different pituitary cell-types in WT females. We found that lactotrophs, somatotrophs and gonadotrophs express B2R. 2- Adult females were injected with E2 valerate (0.2 mg/kg, s.c.), cabergoline (DA agonist, 2mg/Kg, i.p.), sulpiride (DA antagonist, 5mg/kg, i.p.) or vehicle (castor oil or saline) and were sacrificed after 3 hours. Pituitary expression of KKS components was evaluated by RTqPCR. We found that E2 exerts a negative regulation of klk1, b2r and b1r expression in WT females, but this control is lost in Drd2KO. On the contrary, DA exerts a positive regulation of klk1 expression but negatively regulates b2r expression in WT females. We conclude that the positive DA-regulation exercised on the pituitary KLK1 expression is lost in the

Drd2KO pituitary, reducing KLK1 local activity, reducing TGFß1 activation, and contributing to prolactinoma development. The improvement of pituitary KKS activity could represent a novel treatment for resistant prolactinomas.

0226 - DEREGULATION OF THE PITUITARY SMAD-INDEPENDENT ACTIVIN SIGNALLING PROMOTES PROLACTINOMA DEVELOPMENT.

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Activins are known inhibitors of lactotroph function. Pit-1 is a pituitary-specific transcription factor that plays an important role in regulating PRL expression. Despite pSMAD2/3 is known as the activins-canonical intracellular signalling, it was described that activin represses Pit-1 expression in lactotroph cells in a Smad-independent mechanism. We have previously demonstrated that decreased pituitary activin expression is involved in prolactinoma development. In the present work we have studied the activin-signalling pathways involved. We used two different animal models of prolactinomas: female mice lacking dopamine type 2 receptor (Drd2-/-) and female mice overexpressing the ß subunit of the human chorionic gonadotrophin (hCGß+). We used wild-type (wt) mice as controls. Despite the reduced activin expression found in prolactinomas vs wt pituitaries, we found unexpectedly increased pSMAD3 expression (Western blot) in prolactinomas. But nevertheless, by using double immunostaining, we observed that pSMAD3 co-localizes mainly in FSH+ cells, but not in PRL+ cells. Then we focused on the activin alternative pathway involved in prolactinoma development. Our results show that wt female pituitaries present high activin expression concomitant with strong expression of p-p38 in lactotroph population (double IHC). However, activin expression is decreased in prolactinomas concomitant with decreased p-p38 expression in PRL+ cells, increased Pit-1 mRNA expression (q-RT-PCR) and tumor development. This highlights the importance of the activin inhibitory action on lactotroph function and places the activin system and the p38 MAPK pathway as new targets in the treatment of dopamine agonist resistant prolactinomas. Key words: pituitary, prolactinoma, activins, p-p38 pathway

0268 - EFFECTS OF THE CHRONIC TREATMENT WITH L-3,4-DIHYDROXYPHENYLALANINE (L-DOPA) ON THE NEUROENDOCRINE STRESS RESPONSE

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Stress evokes a complex response mediated by two systems: the hypothalamus-pituitary-adrenal (HPA) axis and the sympathetic-adreno-medullar (SAM) axis. Glucocorticoids and catecholamines secreted from the adrenal glands and sympathetic nerves are the main hormone effectors of the physiological adaptations observed during stress. Moreover, prolactin (PRL) is another pituitary hormone secreted under stressful stimuli. Catecholamines are synthesized from the hydroxylated precursor L-Dopa. This agent is also used with therapeutic purposes, e.g. in Parkinson's disease. In the present research, we studied the effects of L-Dopa on the response to a stressor. Adult male Wistar rats (300 g) were chronically treated (24 days) with LEBOCAR (L-Dopa (75 mg/day) - Carbidopa (7.5 mg/day)) orally in drinking water and stressed by immobilization during the last 9 days of treatment. We first explored the activity of the SAM axis. Circulating noradrenaline increased in rats treated with LEBOCAR ($p<0.05$; HPLC), while its content in the adrenals showed no significant alteration. Serum adrenaline (A) levels augmented by LEBOCAR treatment or stress ($p<0.05$; HPLC). Also, the adrenals from stressed animals showed higher content of A ($p<0.05$). Next, we studied the reactivity of the HPA axis. Chronically stressed rats displayed a lower ACTH secretion (ELISA) and a downregulation of POMC expression (qPCR) in the anterior pituitary ($p<0.05$). In addition, LEBOCAR treatment induced a reduction in serum ACTH and POMC levels ($p<0.05$). As expected, serum corticosterone (ELISA) peaked under chronic stress, an effect that was inhibited by treatment with LEBOCAR ($p<0.05$). Finally, pituitary PRL gene expression (qPCR) was downregulated by LEBOCAR treatment with a more pronounced effect when rats were also stressed ($p<0.05$). In summary, our results suggest that L-Dopa alters the neuroendocrine stress response by enhancing SAM axis activity and declining HPA axis reactivity and PRL expression.

0298 - SEXUAL DIMORPHISM OF THE KISSPEPTIN AND DOPAMINERGIC SYSTEMS IN THE HYPOTHALAMUS OF THE SOUTH-AMERICAN PLAINS VIZCACHA (L. MAXIMUS).

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CENTRO DE ESTUDIOS BIOMÉDICOS, BIOTECNOLÓGICOS, AMBIENTALES Y DE DIAGNÓSTICO-UNIVERSIDAD MAIMÓNIDES

Vizcacha shows the highest sexual dimorphism among rodents. It presents atypical reproductive features as natural poliovulation up to 800 oocytes per estral cycle, inhibition of follicular atresia and ovulation during pregnancy with new corpora lutea formation. Brain dimorphism occurs during the embryogenesis and such process is modulated by the hormonal environment they are exposed, which modifies their structure and neurochemical composition. Within hypothalamic structures, this process affects the anteroventral-periventricular nucleus (AVPV) and the arcuate nucleus (ARC) among others. The aim of this work was to characterize the hypothalamic dimorphic sexual nuclei in the vizcacha focusing on the neurochemistry of cellular composition. Adult non-pregnant female (FM) and male (M) vizcachas were used (n=6/group). The localization of AVPV and ARC was determined by Nissl staining, and estrogen receptor alpha (REalpha), tyroxine hydroxylase (TH) and kisspeptin (Kiss) expression were studied by immunohistochemistry. Sexual dimorphism was observed at macroscopic level, being the brain weight/body weight ratio higher in FM than in M (0.47%, t test, $p<0.05$). In addition, Nissl staining showed a significantly increased AVPV area in FM with a significantly increased number of REalpha, TH and Kiss immunoreactive neurons and immunoreactive cell area ($p<0.05$). In ARC, TH showed similar variations than AVPV, however, Kiss did not show differences between sexes. AVPV of both sexes showed colocalization of REalpha with Kiss and with TH, whereas in ARC, REalpha colocalized with Kiss. Estradiol plays an important function in the establishment of brain sexual dimorphism through the REalpha which plays a key role in the sexual dimorphism maintenance. In consequence, the expression level of REalpha should support TH and Kiss expression in the AVPV nucleus in female vizcachas. The association of REalpha with Kiss and TH would be important as part of the indirect GnRH regulation pathway.

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0311 - PROLACTIN STIMULATES FOLLICULAR STEROIDOGENESIS IN AN INDUCED HYPERPROLACTINAEMIC MODEL IN PLAINS VIZCACHA (LAGOSTOMUS MAXIMUS)

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CEBBAD, UNIVERSIDAD MAIMÓNIDES

Over the past years, our lab has been working with female vizcachas since this autochthonous rodent has atypical reproductive characteristics that position it as an unconventional animal model for endocrine studies. It has been established that prolactin (PRL) plays a key role in the corpus luteum (CL) maintenance and progesterone (P4) production during pregnancy in mice and rats. We have recently developed a model of induced hiperprolactinaemia in vizcachas by treating adult non-pregnant females with sulpiride (a potent inhibitor of D2 and D3 dopaminergic receptors) for 7 consecutive days. To investigate PRL relevance over ovarian performance, we analyzed the ovarian steroidogenic enzymes 3ß-HSD, 17ß-HSD and CYP19, and the ovarian hormone receptors PRL-R, LH-R and FSH-R in hiperprolactinaemic (SULP) and control (CTL) vizcachas. Also, we studied steroid serum levels by ELISA and performed follicular counting in hematoxilin-eosine ovarian stained sections. Circulating P4 levels did not differ between experimental groups. Yet, serum estradiol (E2) was significantly higher in SULP animals ($p<0.05$, $n=5$). Immunoexpression levels of all PRL-R, LH-R and FSH-R were higher in SULP group, specifically in secondary follicles compared to CLs, and a similar immunoreactive pattern was observed for the 3ß-HSD, 17ß-HSD and CYP19. Moreover, follicular count showed a significant increase in pre-ovulatory follicles in SULP vs CTL group. We did not register differences in the amount of secondary follicles and neither in the CLs between both groups. Our results indicate that, PRL stimulates E2, by favoring steroidogenesis in the pre-ovulatory follicles instead a direct stimulus over the luteal P4 production observed in other rodents.

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0345 - FILAMIN A MODULATES ANTERIOR PITUITARY CELL PROLIFERATION

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CENTRO DE MICROSCOPÍA ELECTRÓNICA-FACULTAD DE CIENCIAS MÉDICAS. INICSA-CONICET

Recent studies have identified Filamin A (FLNA) as a scaffold protein that plays vital roles in cellular signalling transduction. Mutations in human FLNA gene have been shown to cause developmental defects and aberrant expression has been observed in many tumours. However, its role in pituitary prolactinoma has seldom been discussed. The aim of this work was to analyse the expression levels of FLNA during pituitary tumour development and to determinate the effect on pituitary cell proliferation. To carry out this objective, female rats treated with estradiol benzoate for 20 (20d), 40 (40d) and 60 (60d) days (hyperplasic/adenomatous pituitary model) and transfected GH3 cells for FLNA overexpression (GH3F+) were used. FLNA expression was determined by Western blot (WB) and subcellular localization was visualized by fluorescence and transmission electron microscopy (TEM). Cell cycle progression was analysed by flow cytometry and ki67 index (proliferation marker) by ICQ. Statistical analysis: ANOVA-Tukey. WB analysis showed two FLNA-immunoreactive bands, at ~280 kDa (full-length) and ~90 kDa, (cleaved form). A weak expression of both isoforms was showed in control, with an increase at 40d and a decrease in latter phase (60d). Furthermore, proliferative phase analysis (S-G2/M) by flow cytometry also revealed a peak at initial stages of with a decrease in latter phases. FLNA subcellular distribution was observed to be mainly cytoplasmic in normal pituitary, whereas 40d was found in cytoplasm and also in nucleus with a specific nucleolus mark. The overexpression of FLNA in GH3 cells significantly decreased Ki67 index. Our results suggest an inhibitory role of FLNA in anterior pituitary cell proliferation as part of the mechanisms that limit cell growth. Furthermore, this conclusion is supported by FLNA nucleus and nucleolus localization at late stages of tumour development. We need further studies to elucidate the mechanistic nature of this phenomenon.

0448 - TGFB1 IMPROVE THE OCTREOTIDE INHIBITORY EFFECTS IN FUNCTIONING AND NON-FUNCTIONING PITUITARY TUMOR CELLS

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Octreotide (OCT), somatostatin analog that binds with high affinity to receptor SSTR2, is widely used to inhibit GH secretion and cell proliferation in GH-secreting adenomas. However, a significant percentage of patients are resistant to OCT. The aim was to investigate if OCT inhibitory effects are modulated by alterations on SSTR2 expression and/or interaction with TGFb1/Smad2/3 pathway. We determined the expression of SSTR2, SSTR5, TbRI and TbRII by IHC and WB in normal pituitaries (n= 6) and in 16 functioning (4 PRL, 10 GH and 2 ACTH) and 9 non-functioning adenomas (NFPA). Ethics Committee (Repis N° 37/2014). GH3 and human NFPA cells, WT and overexpressing SSTR2, were treated for 24 h with the OCT (100 nM) and/or TGFb1 (4 mg/ml). SSTRs and TbRs mRNA and protein expression were analyzed by qPCR and WB, GH and PRL secretion by WB, cell proliferation by BrdU incorporation. In vivo experiments by xenograft model with nude mice. Protocol approved by CICUAL-FCM-UNC. (t-test or one-way ANOVA-Fisher). Pituitary tumors exhibited a markedly decrease in SSTR2, SSTR5 and TbR2 expression compared to normal pituitary gland. We observed that the combination OCT/TGFb1 lead to a significant reduction in GH and PRL secretion levels compared to OCT treatment and the hSSTR2 overexpression sensitized pituitary tumor cells to the anti-secretory effect of OCT. A significant proliferative reduction in GH3 and NFPA human cells was showed after OCT/TGFb1 treatment compared to OCT alone, effects that were potentiated in hSSTR2 overexpressing cells. These responses were associated with a significant decrease of ERK1/2, AKT and Cyclin D1 proteins and an increase of Smad2/3-mediated anti-proliferative cascade. The in vivo assays showed that the cytostatic effect of OCT was improved in presence of TGFb1 after 11d of treatment. Our results demonstrated that OCT inhibitory effects on GH- and PRL-secretion and proliferation were improved in presence of TGFb1. These responses were reinforced in pituitary tumor cells with higher levels of SSTR2.

0565 - ADRENOCORTICAL RESPONSE TO SEPSIS IN INSULIN RESISTANT ANIMALS

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Adrenal insufficiency in patients with insulin resistance (IR) could affect the body's response to stressful situations. Administration of sucrose in the drinking water (SRD), to rodents, constitutes a valid model for human IR as it reproduces many of its characteristics. The aim of the present study was to analyze the HPA axis response in

SRD-treated rats subjected to a ligation and cecal puncture procedure (CLP), a very well characterized sepsis model. Adult male Wistar rats were randomly distributed in six groups: Control, SRD, Control-Sham, Control-CLP, SRD-Sham and SRD-CLP. Rats in SRD groups received 30 % sucrose for 15 weeks. IR was determined during the 10th week by an insulin tolerance test. Sham or CLP surgeries were performed during the 15th week. For the following 24 hours before sacrifice, animals were observed and body temperature was measured. Results indicate that surgery induces a significant increase in serum corticosterone levels in all groups ($p<0.0001$). Compared to Control-Sham, corticosterone levels were higher in the C-CLP, SRD-SHAM and SRD-CLP groups ($p<0.01$) while only the SRD-CLP group showed higher circulating ACTH levels ($p<0.01$). Histological examination of H&E stained tissues showed an area of greater vascularization in the adrenal cortex of the SRD-CLP group compared to others. eNOS mRNA levels, as determined by RT-qPCR were higher in the SRD-CLP group compared to others. In summary, regardless of circulating ACTH levels, SRD treatment prevented the CLP-dependent increase in corticosteronemia. Taking into account the key role of glucocorticoids in the stress response, animals presenting an attenuated glucocorticoid output during sepsis may have a worse prognosis.

0803 - OXIDATIVE DAMAGE RESPONSE ACTIVATION AND MITOCHONDRIAL DYNAMICS ALTERATIONS INDUCED BY 17ß-ESTRADIOL IN NORMAL AND TUMORAL PITUITARY CELLS

Ezequiel GRONDONA (1) | Liliana SOSA(1) | Bethania MONGI BRAGATO(1) | Ana Clara VENIER(1) | Alexandra LATINI(2) | Ana Lucía DE PAUL(1)

CENTRO DE MICROSCOPÍA ELECTRÓNICA-FACULTAD DE CIENCIAS MÉDICAS. INICSA-CONICET (1); LAB. DE BIOEN. Y ESTRÉS OXIDATIVO, CENTRO DE C. BIOLÓGICAS, UNIVERSIDAD FEDERAL DE SANTA CATARINA (2)

The relationship between cellular senescence and mitochondrial dynamic shift during the development of estrogen-induced pituitary tumours was recently described in our laboratory. Besides, it is known that energetic deficiencies and cellular damage linked to the accumulation of mitochondrial DNA mutations can be promoted by the estrogen oxidative action. In this context, we set out to evaluate in vitro the possible 17ß-estradiol (E2) pro-oxidant actions on mitochondrial dynamics and the damage response activation under pituitary tumour contexts. Pituitary tumour was induced in adult male Wistar rats by the subcutaneous implantation of estradiol benzoate capsules (30 mg) for 10 days. Control group: implanted with empty capsules. Then, glands were collected and dispersed pituitary cells were cultured and exposed to E2 (1- 10-100 nM) for 15, 30 and 60 min. The ROS production was analyzed by flow cytometry and p-Nrf2 protein expression by immunofluorescence. Mitochondrial protein expression: MFN2 and OPA-1 (mitochondrial fusion) and p-Drp1 (mitochondrial fission) was assessed by Western blot. Statistical analysis: ANOVA-Fischer ($p <0.05$). In tumoral cells, physiological doses of E2 (1 nM) promoted a significant increase in ROS levels and p-Nrf2 expression after short exposure times, while, in normal cells, 10 nM E2 induced this oxidative response after 30 min of treatment. An increase in mitochondrial protein expression levels was observed as concentration and time of exposure to E2 increased, indicating a tendency towards fusion. In pituitary tumoral cells, the E2-induced pro-oxidant environment promoted oxidative stress that was counterbalanced through the Nrf2 antioxidant pathways activation. These changes were accompanied by mitochondrial dynamics alterations, favouring the metabolic readaptation toward the fusion process. Thus, cell viability would be guaranteed by these cellular responses meeting greater energy demands, typical of tumoral contexts.

0857 - PARTICIPATION OF INHIBITORY GPCR IN NORMAL PITUITARY CELL PROLIFERATION INDUCED BY FGF2

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INICSA Y FACULTAD DE CIENCIAS MÉDICAS (CONICET-UNC)

The growth factors effects may be regulated by the inhibitory G protein-coupled receptors (GPCR-Gai) activation, thus modifying the metabolic activity of pituitary gland. Considering that intracellular communications are essential; the aim was to evaluate whether GPCR-Gai regulates the basic fibroblast growth factor (FGF2) proliferative activity in normal pituitary cell populations. Anterior pituitary cell cultures from female rats were treated with FGF2 (10 ng/mL) or somatostatin analogue (OCT, 100 mM) alone or co-incubated with or without an inhibitor of GPCR-Gai, pertussis toxin (PTX, 500 nM) or MEK inhibitor (U0126, 100 µM). Cell proliferation was analyzed by double-immunocytochemistry of BrdU and lactotroph (PRL) or somatotroph (GH); cell cycle by flow cytometry and cell death by TUNEL at 24 h. The somatostatin receptors, SSR2 and 5 were determined by WB and IF; and the ERK1/2, JNK, P38, AKT, S6, c-Jun and cell cycle regulators: cyclin D1, E1, CDK4, p21 and p27 by WB. Statistics: ANOVA-Bonferroni. The SSR2 and 5 were expressed in PRL and GH cells. The lactotroph and somatotroph cell proliferation was increased by FGF2 whereas OCT decreased the cell mitosis respect to control group. The FGF2/OCT co-incubation significantly decreased the proliferation in both cell types associated with a decrease of p-ERK, p-AKT, p-S6, c-Jun and an increase of JNK, effect that was reverted by PTX or U0126 pre-incubation. The TUNEL positive cells and p-P38 expression did not exhibited changes. In addition, the FGF2/OCT co-incubation significantly increased the G1-phase arrest, effect related to an increase in the cell cycle inhibitors p27 and p21 expression and a decrease of cyclin D1 while cyclin E1 and CDK4 did not show any significant variation. These results show that FGF2/OCT treatment induced a decreased of PRL and GH cells population associated with G1-phase arrest, modulating the proteins expression involved in the regulation of cell proliferation and cell cycle progression.

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Chairs: Carlos Pomilio | Mariela Pérez

0103 - METABOLIC IMBALANCE PROMOTES EPIGENETIC MODULATION OF RETINAL MÜLLER CELLS FUNCTION BY THE HISTONE DEACETYLASE SIRT6

Daniel STEIN(1) | Deborah TOIBER(1) | Raul MOSTOSLAVSKY(2) | **Dafne Magali SILBERMAN** (3)

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Müller glial cells (MGs), the major glial component of the retina, respond to any perceived insult by a reaction called reactive gliosis which plays a neuroprotective role but, if persists, it may lead to neurodegeneration. Understanding the modulation of the gliotic responses is essential for developing efficient therapies. High glucose (HG) concentrations can induce cellular changes that may last even if glycemic control is achieved. SIRT6, a NAD-dependent sirtuin deacylase, modulates aging, energy metabolism and neurodegeneration. In previous studies we showed that SIRT6 deficiency causes retinal neurotransmission defects, changes in expression of glycolytic genes and apoptosis. Given the importance of glucose availability for retinal function, the fact that MGs are a critical source of growth and neural factors, and the key role of SIRT6 in modulating glycolysis, we aim to analyze SIRT6 involvement in MGs function subjected to HG. Primary cultured MGs subjected to HG concentration (25 mM) showed decreased levels of SIRT6 and increased levels of H3K56 acetylation ($p<0.05$) than cells treated with low glucose (5 mM). The levels of the

neovascularization promoting factor VEGF increased in MGs under HG ($p<0.01$) and reverted to control values when SIRT6 was overexpressed. Furthermore, SIRT6 silencing resulted in the increment of VEGF levels supporting the idea of SIRT6 as a direct modulator of this factor. We also found that HG induced a decrease in the neural factor BDNF levels while this effect was reverted in SIRT6 overexpressing MGs. However, BDNF levels did not show variations in SIRT6 siRNA expressing cells compared to controls. Moreover, and in accordance to our previous findings in diabetic animals, retinas from mice with conditional deletion of SIRT6 in the CNS exhibited increased levels of VEGF and lower levels of BDNF than WT ($p<0.01$). Our results suggest that HG would induce a neurodegenerative response in MGs that would be epigenetically regulated by SIRT6.

0360 - MUSCLE TROPHISM AFTER TESTOSTERONE TREATMENT IN THE WOBBLER MOUSE, AN SPONTANEOUS MODEL FOR AMYOTROPHIC LATERAL SCLEROSIS (ALS).

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IBYME-CONICET (1); IBYME-CONICET; DEPARTAMENTO DE BIOQUÍMICA HUMANA, FACULTAD DE MEDICINA, UBA (2); DTO DE FARMACOLOGIA, FACULTAD DE MEDICINA, UBA (3); IBYME-CONICET Y DTO DE CIENCIAS FISIÓLOGICAS, FACULTAD DE MEDICINA, UBA (4)

Patients suffering amyotrophic lateral sclerosis (ALS) present muscle atrophy in upper and lower limbs and difficulties in swallowing and dysarthria in association to motoneuron degeneration. Wobbler mice (WR) are animal models of ALS showing selective loss of motoneurons, astrocytosis and microgliosis in the ventral horn of the cervical spinal cord, brain stem and motor cortex. The incidence of ALS is greater in men than in fertile women; however, it increases after menopause. Therefore, sex steroid hormones could be involved in disease susceptibility or outcome. Previously, we demonstrated that testosterone treatment to WRs reduced astrocytosis and increased glutamine synthetase immunoreactivity, an enzyme necessary for maintaining glutamate concentration in synaptic cleft. Now, we investigated the effect of testosterone treatment on myelination and muscle trophism. Treatment consisted of 10 mm silastic tubes containing crystalline testosterone s.c. for 2 months to male symptomatic WR. Non-treated WR or control received an empty silastic tube s.c. Testosterone serum levels and seminal vesicles weight were significantly higher in testosterone-treated WRs compared to empty silastic-treated WRs ($p<0.01$). Luxol fast blue (LFB) staining was used to identify myelin. We quantified the % of area stained over a threshold in white matter of the cervical cord. Significant group differences were shown by ANOVA ($F(3,16)= 29.86$, $p<0.001$) in the % of stained area for LFB in the ventro-lateral funiculus (VLF). Lower LFB staining was shown in WRs (mean ± SEM-WR: 27.56 ± 5.57 vs. control: 87.40 ± 0.92; $p<0.001$), while higher values were demonstrated after testosterone treatment (WR+testosterone: 41.94 ± 5.62 vs. WR, $p<0.05$). As regards to biceps muscle mass, one way ANOVA also indicated significant group differences ($F(3,24)= 7.55$, $p<0.01$). WRs showed biceps atrophy (WRs: 0.62 ± 0.08 mg/g body weight vs. controls: 0.86 ± 0.04, $p<0.05$). However, greater mass was shown in testosterone-treated WRs in comparison to empty silastic-treated WRs (WR+testosterone: 1.05 ± 0.08, $p<0.01$ vs. WR). No effect of testosterone treatment was found in controls. These preliminary data showed protective effects of testosterone in motoneuron degeneration.

0368 - REGION-SPECIFIC ASTROCYTES EXERT DIFFERENTIAL NEUROPROTECTION IN AN IN VITRO MODEL OF HUNTINGTON'S DISEASE

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Evidence shows that there is significant heterogeneity in astrocytes, as regards morphology, gene expression and function. In this work we investigated the action of astrocytes from two areas involved in Huntington`s disease (HD): the striatum and the cortex. We have previously demonstrated that BDNF exert protective and antioxidant effects on astrocytes treated with 3-nitropropionic acid (3-NP) a toxin widely used to model HD in vitro. First, we determined TrkB expression and ERK activation in cortical and striatal cultured primary rat astrocytes treated with BDNF (50 ng/ml) + 3-NP (15 mM) for 24 h. We showed before that cultured astrocytes do not express TrkB-full length (TrkB-FL) protein; therefore TrkB-truncated (T1) isoform expression was assessed by Western blot. In cortical and striatal astrocytes BDNF and BDNF+3-NP increased protein expression of TrkB-T1 expression ($p<0.001$). 3-NP alone did not modify protein levels of this receptor. Also, in cortical and striatal astrocytes BDNF increased levels of pERK while 3-NP per se decreased them compared to control group ($p<0.05$). In astrocytes treated with BDNF+3-NP, ERK activation was also increased ($p<0.001$). To study neuroprotection, we used cortical and striatal astrocyte conditioned medium (ACM) to treat ST14A-Q120 cells, a cell line derived from embryonic rat striatal cells which express the N-terminal fragment of mutant human Htt with a 120 glutamine region (120 CAG repeats). ACM from striatal astrocytes treated with BDNF protected ST14aA-Q120 from 3-NP induced death ($p<0.001$), whereas ACM from treated BDNF cortical astrocytes did not modify 3-NP effect on ST14A-Q120 cells. These data suggest that striatal treated astrocytes with BDNF secrete soluble neuroprotective factors. Furthermore, only ACM from 3-NP+BDNF treated striatal astrocytes increased viability of ST14A-Q120 cells compared to ACM-3-NP. A better understanding of astrocytes heterogeneity would help elucidate astrocyte functions and how their malfunction contributes to neurodegenerative diseases.

0387 - STUDY OF NEUROPROTECTIVE FACTORS MEDIATED BY OMEGA 3 AND COGNITIVE STIMULATION IN THE FACE OF MILD AMNESIC COGNITIVE IMPAIRMENT IN PATIENTS RESIDING IN THE CITY OF CÓRDOBA-ARGENTINA.

Tatiana CASTRO ZAMPARELLA | Paula ABATE | Magdalena Marcela CÁCERES | Veronica BALASZCZUK

INSTITUTO DE INVESTIGACIONES PSICOLÓGICAS IIPSI UNIDAD EJECUTORA DEL CONICET-UNC

General description: Neurodegenerative and vascular desease have a high prevelance in older adults. In Latin American countries, with low economic resources, goals in public health should be based on preventive and educational actions, promoting brain protection factors. Background shows that the diet based on food rich in essential fatty acids Omega 3 (O3) contributes to the prevention of neurological clinical conditions. Objective: To study neuroprotective factors mediated by the dietary supplement (O3) and cognitive stimulation in patients with mild amnesic cognitive impairment from the city of Córdoba, Argentina. We conducted observational case-control studies. Non probabilistic sample of adults between 60 and 80 years both sexes of the city of Córdoba, Argentina. n= 20 Pre-post evaluation of blood lipid profile, brain magnetic resonance with hippocampal volumetry and complete neurocognitive evaluation battery. Treatment for 24 weeks with O3 capsules (1,000 mg) and 24 weeks of treatment with cognitive stimulation workshops. Supplementation with O3 is expected to mitigate or protect against the progression of mild amnesic cognitive impairment in the sample of adults/older adults and even more increases this effect along with cognitive stimulation.

Discussion and conclusions: Little is known about the protective effects of O3 in Argentina. Currently, this country has a high aged population, therefore, the risk of cognitive and demential pathologies is very frequent. Hence, health trends are set to prevent and provide life´s quality for the adult population.

0840 - MODULATION OF GLIAL AUTOPHAGY BY PERIODIC DIETARY RESTRICTION AS A POTENTIAL THERAPEUTIC STRATEGY IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE.

Amal Patricio GREGOSA MERLINO | Ángeles VINUESA | Carlos Javier POMILIO | Melisa BENTIVEGNA | Jessica PRESA | Flavia Eugenia SARAVIA | Juan BEAUQUIS

IBYME-CONICET

Alzheimer's disease (AD) is the leading cause of dementia for which there are no effective treatments. Dietary restriction (DR) has been proposed as a potential therapeutic strategy for age-associated diseases. Our objectives were to analyze memory and hippocampal alterations in an animal model of familial AD, the PDAPP-J20 transgenic mouse (Tg) and to evaluate the effects of a periodic DR protocol (3 cycles of 40 % DR for 5 days and ad libitum (AL) diet for 9 days). We observed cognitive impairment, impaired adult neurogenesis and progressive amyloid beta (Aß) deposition in the hippocampus of AL-fed Tg mice. Periodic DR was associated to cognitive improvement, increased hippocampal neurogenesis, and reduced hippocampal amyloid load. Through immunofluorescence for LC3 (autophagosomes) and GFAP (astrocytes), we found that autophagy is modulated in Tg mice with a high proportion of LC3 localized in astrocytes (2-way ANOVA, $p<0.01$ for genotype effect). We also found that astrocytes contained Aß co-localizing with LC3. From these results we hypothesized that the reduction in amyloid plaques associated to DR would be partly mediated by astroglial autophagy. Therefore, we evaluated the C6 astrocyte line exposed to fibrillar beta amyloid (fAß, 2, 6 or 24 h) and previously nutrient restricted (NR; 2 % FBS) or not (10 % FBS) for 6h. Exposure to fAß for 2 h showed that LC3 was increased after bafilomycin treatment, an inhibitor of autophagosome-lysosome fusion, indicating a functional autophagy (Aß NR 0.4 ± 0.14 vs. Aß NR BAF 2.24 ± 0.38, $p<0.001$). At 6 and 24h of Aß exposure, LC3 levels rose in Aß but not in Aß+NR cells (Aß vs. Aß+NR, 6 h $p<0.001$, 24 h $p< 0.05$) and this effect was lost if bafilomycin was administered in the last 2 hours (Aß+NR vs. Aß NR BAF, 6 h $p<0.05$, 24 h $p<0.05$) suggesting that autophagy was blocked by Aß but conserved with prior NR. We conclude that NR could prevent autophagy alterations that occur in response to Aß and could be a potential therapeutic strategy in AD.

0843 - EFFECTS OF A HIGH-FAT DIET IN A MOUSE MODEL OF ALZHEIMER´S DISEASE.

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IBYME-CONICET; CÁTEDRA DE QUÍMICA, CICLO BÁSICO COMÚN, UBA

Insulin resistance and obesity, associated to the consumption of hyperlipidic diets, are considered risk factors for the development of cognitive disorders and neurodegenerative diseases such as Alzheimer's disease (AD). Insulin resistance, inflammation and cognitive dysfunction are common manifestations in the context of both neurodegenerative and metabolic pathologies. Our objective was to study the effects of the exposure to a moderately high-fat diet (HFD), from 6,5 to 8 months of age, on cognitive performance and hippocampal glial and neuronal changes in a transgenic model of AD, the PDAPP-J20 mouse. Interestingly, HFD exposure did not induce changes in body weight at the end of the protocol. Although we found that diet had no effect on glucose blood levels, transgenic mice showed decreased glycemia compared to non-transgenic groups ($p<0.05$). We found a tendency towards decreased hippocampal insulin signaling, measured by Western blot of pAkt/Akt, in HFD-treated animals, suggesting an effect of this diet on insulin sensitivity. The analysis of the open-field test showed an anxious-like behavior in transgenic mice fed a control diet and also in HFD-fed transgenic and non-transgenic mice (Distance travelled in the center of the arena= NTg 20.63 vs. Tg 12.67 %, $p<0.005$; CD 20.21 vs. HFD 13.09 % $p<0.01$), evidencing similar effects of the genotype and also of the HFD on animal behavior. Our results show that HFD induced behavioral and brain metabolic alterations in adult mice, sharing similarities with PDAPP-J20 mice that model Alzheimer's disease.

0854 - DEXAMETHASONE BLOCKS MOTOR IMPAIRMENT AND BRAIN MORPHOLOGICAL CHANGES INDUCED BY THE MIXTURE OF TAURINE AND ALCOHOL IN A MICE MODEL OF ALCOHOL HANGOVER.

Alipio PINTO | Silvia CARBONE | Jorge GOLDSTEIN | **Rodolfo Angel CUTRERA**

IFIBIO

It has been proposed that inflammatory mechanisms could be involved in alcohol hangover (AH). In previous work we demonstrated that at the beginning of AH mice treated with alcohol (OH) and taurine (TAU), the main component of energy drinks, showed behavioral and morphological changes in the brain. The aim of this work was to study if the pretreatment with dexamethasone (DEXA) could block the motor disabilities and the neural stress produced by TAU in an experimental model of AH. Mice (n= 8-12) were ip pretreated with DEXA (7.5 mg/kg) 24 h before the i.p. injection of OH (3.8 mg/kg) and/or TAU (190 mg/kg). Controls were injected with saline. The Tight rope (TR) and Hanging wire (HW) tests were used to study neuromuscular coordination and muscle tension, respectively. Another group of mice (n= 8) were perfused and the brains were subjected to immunofluorescence with an anti-GFAP antibody (glial fibrillary acidic protein) and an anti-MBP antibody (myelin basic protein) to identify reactive astrocytes and myelin sheath respectively. All the studies were performed 6 h after treatment with OH and/or TAU (beginning of AH). It was observed that DEXA blocked significantly (ANOVA-Tukey, $p<0.001$): A) In HW test, the decrease in latency to fall (sec) in OH and OH+TAU groups; B) In TR test, the loss of neuromuscular coordination (sec) in animals with OH and a tendency to improve the performance in DEXA+OH+TAU. It was also observed a significant increase ($p<0.001$) in astrocytic reaction and a significant decrease in MBP in OH, TAU and OH+TAU treated mice compared to the control ones. On the other hand, DEXA significatively achieved to block the astrocytic reaction in OH+TAU treated mice and the reduction of MBP in TAU and OH+TAU treated mice. These results suggest that an inflammatory process may be mediated by the decrease in muscle tension and morphological changes in the brain caused by combined treatment with OH and TAU evidenced at the beginning of AH in mice.

0900 - NEUROPROTECTIVE EFFECT OF FK506 AGAINST OXIDATIVE STRESS

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Immunophilins are proteins that bind immunosuppressive drugs. Those that bind cyclosporin A (CsA) are cyclophilins (ej, CyPA), and those that recognize the macrolide FK506 are FKBPs (FK506-binding proteins), a subfamily to which FKBP51 (51-kDa) and FKBP52 (52 -kDa). Both FKBPs were described in steroidal receptor heterocomplexes with Hsp90, Hsp70 and p23. FKBP51 and FKBP52 have 60 % similarity and 75 % homology. Previous laboratory studies showed that FK506 has regulatory effects on neurodifferentiation through both FKBPs, such that overexpression of FKBP52 or silencing

of FKBP51 promoted neuritogenesis. Similarly, the axonal damage of the cells was reversed with FK506, being accelerated when overexpressed to FKBP52 or by knock-down of FKBP51. This suggests that these immunophilins could have neuroprotective or neuroregenerative actions in adverse situations such as, for example, oxidative stress associated with neurodegenerative diseases, cerebrovascular accidents or neuronal overexcitation. In this study, it was analyzed whether treatments with FK506 can prevent and/or reverse the deleterious effects associated with oxidative stress of H_2O_2. Undifferentiated N2a (murine neuroblastoma) cells were incubated in DMEM/OptiMEM medium (without serum) with 1 µM FK506, observing the rapid generation of neurites. Two hundred fifty µm thick sections obtained from prefrontal cortex of male BALB/C mice (60 d) were incubated in special medium on 4 % agar. After 72 hours of tissue stabilization, the explants were incubated for 4 hours with 200 µM H_2O_2. The induction of Hsp90, Hsp70, FKBP52 and p23 was evidenced, which was prevented by pretreatment (1h) with 1 mM FK506. Regarding FKBP51, the controls showed three bands corresponding to their known phosphorylated isoforms, while explants treated with H_2O_2 showed only the least phosphorylated band. Pretreatment with FK506 protected phosphorylated isoforms, showing the same pattern of isoforms as the control. In turn, the samples treated with FK506 showed only the intermediate phosphorylated band, suggesting that this isoform (reactive with anti-P-Tyr antibodies) is favored in the mechanism of action of the drug.To show effects in vivo, relative hypoxia was generated by stereotactic injection of 2 ml 50 mM $CoCl_2$ in the prefrontal cortex of the right hemisphere, using the contralateral as a control. Chaperone expression was induced in tissue lysates obtained 24 h later, which was partially prevented by pretreatment (24 h) with 10 mg/kg FK506. The effects on motor balance were studied after 21 days (FK506 injected every 3 days) by Rotarod and open field (Anymaze), observing a better and faster recovery in mice treated with FK506. This is the first study that shows a neuroprotective effect of FK506 against oxidative stress.

0907 - INCREASED VASCULAR PERMEABILITY TO EVANS BLUE DYE IN THE HIPPOCAMPUS OF PDAPPJ20 MICE, MODEL OF ALZHEIMER'S DISEASE (AD). POTENTIAL IMPLICATION OF ER STRESS MECHANISMS.

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IBYME-CONICET (1); DEPARTAMENTO DE QUÍMICA BIOLÓGICA FCEN - UBA (2)

The blood-brain barrier (BBB) limits flux from and into the brain compartment that is essential for normal neuronal functioning and information processing. Post-mortem tissue analysis indicates BBB damage in AD. However, timing and mechanisms underlaying BBB breakdown remain elusive. Endoplasmic reticulum (ER) stress is caused by disruption of homeostatic mechanisms that cause unfolded proteins to accumulate. The ER adapts to stress by activating the unfolded protein response (UPR). Chronic ER stress is increasingly being associated to neurodegeneration. We found augmented vascular permeability in the hippocampus of 12-month-old PDAPPJ20 transgenic mice, compared to the group, assessed by Evans Blue i.v. injections and analysis of coronal brain sections (D.O.= 5,666 ± 387 vs. 12,373 ± 3,082, n= 5-6, $p<0.04$) and a higher number of microhemorrhages per μm^2 in the hilium (four fold more in transgenic mice vs. non-transgenic mice, $p<0.0001$) Human brain microvascular endothelial cells (hBMEC) proved to be a very suitable human cellline for an in vitro BBB model. We treated hBMEC with 1 µg/ul of Thapsigargin (Tg), a known inductor of ER stress. Treated cells showed increased mRNA expression of Ire1$\alpha$and PERK by RT-qPCR (two and three fold increase, n= 3, p= 0.01, respectively), evidencing UPR activation. We also employed a sealed monolayer of hBMEC on a transwell membrane, monitored by TEER. Tg exposure provoked loss of resistance and augmented permeability to Evans Blue and NaFl. In addition, Aβ1-40 peptide was able to induce changes in GRP78/BiP-a central central regulator for ER stress-in endothelial cells: we found not only increased intensity in the cytoplasmic BiP area ($p<0.05$) but a different pattern distribution, with predominance of bright dots, suggesting the implication of ER in the brain vasculature exposed to amyloid peptides. Further experiments are in progress to elucidate the role of ER stress in endothelial cells and BBB integrity in vivo and in vitro models of AD.

0935 - STX2 FROM ENTEROHEMORRHAGIC E. COLI INDUCES NF-KAPPAB ACTIVATION IN REACTIVE ASTROCYTES

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Shiga toxin 2 (Stx2) from enterohemorrhagic E. coli causes hemolytic uremic syndrome (HUS) and acute encephalopathy, which may lead to fatal outcomes in patients. When neurological symptoms are evidenced mortality rate may rise up to 40 %. The mechanism by which the encephalopathy emerges in patients with HUS is still unknown. Reactive astrogliosis is a widespread glial response to brain injury and NF-kappa B activation was related to the proinflammatory-neurodegenerative astroglial polarization. The aim of this study was to determine whether Stx2, LPS or a combination of both produce astrocyte reactivity in vitro, and whether this reactivity involves the activation of NF-kappaB pathway. Primary cortical astrocytes were obtained from P3-P5 C57 mice. Confluent astroglial cultures were incubated either with control (saline solution), LPS (50 ng/ml), Stx2 (50 or 200 ng/ml), or a combination of both toxins. GFAP expression and astroglial cell morphology was evaluated by immunocytochemistry. Reactive astrogliosis was observed following the treatment with 200 ng of Stx2 plus 50 ng of LPS in comparison to the control (0.058 ± 0.006 control vs. 0.088 ± 0.006 Stx2+LPS, measured as the number of filamentous astrocytes per total number of astrocytes). Nuclear translocation of p65 NF-kappaB subunit was measured as an index of NF-kappaB activation. The 3h treatment with 50 ng/ml LPS, 200 ng/ml Stx2, and 200 ng/ml Stx2 plus LPS showed a significantive NF-kappa B activation in primary astrocytes when compared with controls (63.19 ± 4.51, 23.77 ± 1.97, 55.30 ± 4.20, 1.33 ± 0.51 respectively; expressed as a ratio of nuclear p65 vs. total number of astrocytes). We conclude that Stx2 causes reactive gliosis in vitro and NF-kappaB activation which it may be involved in the proinflammatory astroglial polarization known to produce neurodegenerative effects.

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0942 - DIFFERENTIAL SUSCEPTIBILITY TO INFLAMMATION AND NEURODEGENERATION IN TWO MODELS OF AGED RATS

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LELOIR INSTITUTE FOUNDATION - IIBBA CONICET

Parkinson's Disease (PD) is a neurodegenerative disorder whose main feature is the neuronal loss in the substantia nigra (SN). Our group and others have demonstrated that inflammation can cause or exacerbate neuronal demise in the SN, suggesting that the modulation of inflammation could be a possible site of therapeutic intervention. Several factors can influence the development of PD. Age is the most relevant risk factor for this disease. Also, a mutation (G2019S) in the LRRK-2 gene is the most prevalent mutation in PD patients. Interestingly, aged animals and animals carrying a LRRK-2 (G2019S) mutated gene produce an increased response to

inflammatory stimuli when compared with adult animals. Therefore, we hypothesize that aged animals expressing LRRK-2 (G2019S) show an increased inflammatory response and therefore a higher risk for neurodegeneration in the SN. To test the hypothesis, aged rats (12-18 months) were inoculated in the SN with adenoviral vectors expressing LRRK-2(G2019S) or eGFP (control). 21 days later, all animals were intravenously injected with adenoviral vectors expressing Interleukin-1b (AdIL-1b), causing inflammation. In the aged LRRK-2 (G2019S)/AdIL-1b animals, a marked increase in microglia activation but no signs of neurodegeneration or motor symptoms were detected. In parallel, aged rats injected with a subtoxic dose of 6-OHDA or vehicle were challenged with peripheral AdIL-1b or an adenovirus expressing betagalactosidase. Only 6-OHDA/AdIL-1 animals showed signs of neurodegeneration (28.44 %) with 2-fold increase in microglia activation and no differences in astrogliosis. We conclude that although both models showed increased microglial activation, nigral degeneration was observed in 6-OHDA-treated but not in LRRK-2 (G2019S)-expressing aged rats, suggesting different mechanisms of nigral susceptibility to inflammation in both models. These data set the basis to identify molecules of inflammation-mediated neuronal demise in aged animals.

0949 - THE REDUCTION IN THE CONCENTRATION OF SEROTONIN IN THE CNS INDUCES AN INCREASE IN AGGRESSIVE BEHAVIOR IN RATS

Maria Belen MULLE BERNEDO | Sebastian GARCIA | Ricardo Jorge CABRERA

IMBECU

Serotoninergic system modulates, among others, appetitive and aggressive behaviors. Main raphe nucleus outputs connect to olfactory bulbs (OB). We reported that pCPA i.p. administration (tryptophan hydroxylase inhibitor) increases the offensive/aggressive related behaviors at the 3rd and sixth days after administration. However offensive/aggressive behaviors highly increased on the 6th day. The aim was to examine if the depletion of 5-HT in the OB was related to the modifications in the offensive/aggressive behavioral manifestations. For this purpose, 60-day old male Sprague-Dawley rats were divided into 1) Naïve, 2) control i.p. saline injection and 3) pCPA 300 mg/kg, i.p. All groups were evaluated on day 6th in the arena using the resident/intruder test. At the end of the experiments, animals were euthanatized by decapitation and OB were obtained. The concentration of 5-HT and 5-HIIA were measured by HPLC. All the data were expressed as mean± SEM (n=6) and analyzed by ANOVA I and Tukey post hoc test. A significant decrease ($p \leq 0.001$) of 5-HT and 5-HIIA after six days of pharmacological treatment was observed. The metabolic ratio between 5-HIIA / 5-HT showed a significant increase ($p \leq 0.001$). A significant negative correlation was observed between offensive/aggressive parameters and 5-HT concentration. We conclude that the depletion of 5-HT and 5-HIIA induced a dysfunction on the serotoninergic OB inputs and clear, direct relation with aggressive behavior in rats.

Infectología y Parasitología/ Infectology and Parasitology IV

Chairs: Fabiana Alovero | Marta Cardinal | Marisa Fernández | Karina Gómez | Alejandro Schijman

0191 - TOXOPLASMA GONDII INFECTION IN VULNERABLE POPULATIONS OF ARGENTINIAN PREGNANT WOMEN: PREVALENCE, SEROCONVERSION RISK AND CONGENITAL TOXOPLASMOSIS INCIDENCE RATE

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Toxoplasmosis is a worldwide zoonosis caused by protozoan parasite Toxoplasma gondii. In people with healthy immune system, the infection is usually asymptomatic. However, primary infection in pregnant women can cause severe congenital toxoplasmosis in the developing fetus or newborn child. The diagnosis of congenital toxoplasmosis is difficult to establish until seroconversions during pregnancy are suspected. There are at present only few epidemiology studies about calculations of congenital toxoplasmosis incidence rates based on seroconversion risk in Argentinian pregnant women. Moreover, there is no information about how changes in urban environment due to expanding poor urban areas can produce favorable conditions for toxoplasmosis transmission. In this study, through a cross-sectional retrospective study we have assessed the age-dependent prevalence of Toxoplasma gondii infection among a representative cohort of pregnant women (n= 2000), who requested prenatal screening in Ingeniero Carlos Snopek Hospital between 2013 and 2016. This Hospital is the main health public service in Alto Comedero district, located in the southern area of San Salvador de Jujuy city. This suburban area is characterized by the presence of squatting households and low-income residents. Firstly, we observed that prevalence distribution significantly increases with the age of patients. Secondly, we have checked if both variables age and prevalence could adjust to a linear regression by using binomial regression method with identity link. The test was significant ($p<0,001$) and incidence rate of seroconversion during pregnancy have been found remarkably high (7.43/1000 pregnancies). Additionally, estimated congenital toxoplasmosis incidence was 22.7/10.000 alive newborns. This value is 8.79-fold higher than the globally estimated indicator for congenital toxoplasmosis. The future perspectives of this study, also applicable to other poor urban contexts, will be presented in the poster.

0202 - IMPAIRED MIGRATION OF CD8+ T CELLS AND POOR PARASITE CONTROL IN TARGET TISSUES OF IL-10 DEFICIENT MICE DURING ACUTE TRYPANOSOMA CRUZI INFECTION.

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UNIVERSIDAD DE BUENOS AIRES (1); IMPAM (UBA-CONICET) (2)

CD8+ T cells play a critical role limiting T. cruzi multiplication in peripheral tissues. To achieve this, they need to become functionally activated in lymphoid organs and sense environmental signals produced at the sites of inflammation to migrate, target and eliminate cells infected with intracellular amastigotes. We have previously established that IL-10 deficient mice (IL-10 KO) are more susceptible to infection with T. cruzi. Our findings demonstrated that splenic CD8+ T cells from IL10 KO infected mice fail to expand and to become fully activated thus suggesting an immunostimulatory role of this cytokine during acute T. cruzi infection. We hypothesize that impairment in CD8+ T cell activation has consequences on their migration and control of parasite replication in target tissues. Here, we analyzed the effects of absence of IL-10 in hearts of T. cruzi infected mice and particularly, we analyzed histological changes, tissue infiltrating lymphocytes, parasite load as well as chemokines and chemokine receptors involved in migration of CD8+ T cells. After 21 days of infection tissue sections of hearts from IL-10 KO mice stained with hematoxylin and eosin exhibited a higher score of diffuse and perivascular infiltrates than WT mice. Correlating with the higher

tissue inflammation, mRNA expression of chemokines CXCL9 and CXCL10 was upregulated in heart tissues of WT and IL-10 KO infected mice but to a greater extent in the last group ($p<0.05$). When we analyzed CXCR3 expression of splenic CD8+T cells, they exhibited a 4 to 5-fold increase in infected WT as well as IL-10 KO mice group. Yet, the mRNA expression of CXCR3 -receptor of the aforementioned chemokines at the surface of T cells- in heart tissues was upregulated in the WT mice but not in IL-10 KO mice ($p<0.01$). Accordingly, the relative number of tissue infiltrating CD8+ T cells was higher in hearts from infected of WT mice than in IL-10 KO mice ($p<0.05$). Subsequently, parasite burden in infected hearts as measured by quantitative real time PCR was significantly lower in WT mice compared to their IL-10 KO counterparts ($p<0.05$). Together, these results show that IL-10 participates in events that lead to homing of CD8+ T cells to target tissues. Even though lack of IL-10 increases target tissue inflammation and boosters the CXL9/CXL10 chemokine gradient, CD8+ T cells from IL-10 KO increase their expression of the chemokine receptor CXCR3 at secondary lymphoid organs but fail to migrate to inflamed tissues. The lower recruitment of CD8+ T cells in heart tissues finally leads to poor parasite control of IL-10 KO mice. Further experiments are being performed to understand how IL-10 participates in the recruitment of CD8+ T cells to inflamed tissues in the context of T. cruzi infection. Recent papers have highlighted the positive role of IL-10 in driving tissue resident CD8+T cell-control of viral replication and tumor cells.

0404 - REGULATION OF T CELL RESPONSE BY B CELLS IN TRYPANOSOMA CRUZI INFECTION

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Previous studies in mice with partial (Xid) and absolute (µMT) deficiencies have shown that B cells in the context of infection with T. cruzi are able to regulate the T response. However, little is known about the mechanism by which this regulation occurs. Consequently, we aimed to evaluate the modulation by B cells induced by trypomastigotes and which are the implications in the T response. In the present work, we demonstrate that the co-culture of naive B cells (purified by CD43 negative selection) with trypomastigotes induced the proliferation of these cells and secretion of cytokines such as IL-6 and IL-10. These effects are enhanced in the presence of a CD40 agonist. On the other hand, we observed that the co-culture with trypomastigotes (CL Brener strain) increases the expression of MHC-II and the co-stimulatory molecules CD80 and CD86, all of which are essential for the B-T cell interaction. We have observed that IL-10 secretion by B220+ cells is associated with cells previously reported as regulatory phenotypes (marginal zone and T2-MZP). Subsequently, it was tested if the supernatant of B cells-trypomastigotes co-cultures is able to regulate the T cell response. For this, we cultured purified naive CD4+ cells in the presence of anti-CD3/CD28 and conditioned medium from the co-cultures. A decreased proliferation accompanied by a drop in IL-2 secretion was observed. In addition, a decrease in IFNg; secretion was observed together with an increase of IL-4 secretion indicating the regulation of the Th1/Th2 balance. Moreover, the co-culture of CD4+ cells together with trypomastigote-pretreated B cells induced a significant increase in the proportion of CD4+/Annexin-V+ cells, indicating an increase in their apoptosis rate. Therefore, our results support that T. cruzi trypomastigote interacts and educates B cells to modulate CD4+ responses by various mechanisms: inhibiting their proliferation, altering Th1/Th2 balance and inducing apoptosis.

0420 - DAILY VARIATIONS IN THE EXPRESSION OF GENES RELATED TO INSECTICIDE RESISTANCE AND MOLECULAR BASES OF THE BIOLOGICAL CLOCK IN TRIATOMA INFESTANS

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INICSA, CONICET AND CÁTEDRA DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR, FCM UNC

Triatoma infestans present rhythms in diverse biological processes. Previous studies in the expression of three cytochromes P450 genes and the NADPH cytochrome P450 reductase (CPR) gene revealed that P450 genes would be involved in the development of resistance to insecticides in T. infestans. To investigate the presence of rhythms in the expression of genes related to insecticide resistance, we explored the daily expression at mRNA level of CPR gene and a P450 gene (CYP4EM7) in fat body of groups of T. infestans maintained under light/dark cycle (LD), constant light (LL), and constant dark (DD). In LD, the CPR gene expression profile of females showed two peaks, conserved in DD and lost in LL, that suggest the CPR expression is under endogenous clock regulation. In contrast, in males was not observed a rhythmic profile in the expression of the CPR gene. In LD, the expression of CYP4EM7 gene showed daily significant variations. Females presented a peak at dawn and males showed two peaks, one at dawn and other at sunset. On the other hand, to better understand the molecular bases of the biological clock, we analysed the expression at mRNA level of the clock genes period (per) and timeless (tim) in LD, LL, and DD conditions. The per and tim genes expression in nervous tissue of adults T. infestans varies with a daily rhythm in LD, showing a significant peak at sunset. These rhythms agree with those described in Drosophila melanogaster and would promote a peak of PER and TIM protein levels at night. As expected, in LL no daily increase was detected in per and tim transcript levels. Besides, the presence of per transcript in different tissues of adult individuals and in nervous tissue of nymphs evidenced activity of peripheral clocks in adults and activity of the central clock in nymphs of T. infestans. Further studies will help to clarify the relationship between the endogenous clock and the expression of genes involved in the insecticide metabolisms.

0442 - IN VITRO RESTORATION OF POLYFUNCTIONAL CD4+ T CELLS SPECIFIC FOR TRYPANOSOMA CRUZI AFTER TREATMENT WITH IL-7 AND IL-27 IN EARLY CLINICAL STAGES OF CHRONIC CHAGAS DISEASE

Maria Ailen NATALE (1) | María Gabriela ALVAREZ(2) | Bruno LOCOCO(2) | Melisa CASTRO EIRO(1) | Gonzalo CESAR(1) | Graciela BERTOCCHI(2) | María Cecilia ALBAREDA(1) | Susana LAUCELLA(1)

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Subjects with chronic Trypanosoma cruzi infection show a decrease in the frequency and function of parasite-specific T cells over time. The cytokines IL-7 and IL-27 play important roles in the maintenance and function of T cells. In a previous work, we observed that in vitro administration of IL-7 and IL-27 restores IFN-gamma production in response to T. cruzi antigens in PBMCs of chronic Chagas disease patients who had not yet developed cardiomyopathy. Here, we assessed whether treatment of PBMCs with IL-7 and IL-27 could improve polyfunctional T. cruzi-specific T-cell responses. Multiparameter flow cytometry was performed after 16-20 h of incubation of PBMCs with T. cruzi antigens in the presence or absence of these cytokines. In vitro treatment with rhIL-7 induced a higher frequency of CD4+ T cells with three functions enriched in IL-2-producing cells. The proportion of polyfunctional T cells did not change after treatment with rhIL-27 but CD4+ T cells with one and two functions were enriched in IL-2-producing cells. We further explored the characteristics of IFN-gamma- and IL-2-co-producing T cells after in vitro treatment with IL-7 and IL-27, by measuring the expression of the anti-apoptotic

molecule Bcl-2 as well as the expression patterns of the senescence markers PD-1 and CD57. T. cruzi-responsive IFN-gamma+IL-2+CD4+ T cells showed higher levels of Bcl-2 expression in cytokine-stimulated cell-cultures, whereas there were no changes in the expression of PD-1 and CD57 markers in this subset compared with cell cultures with media alone. Our findings support that the quality of parasite-specific T-cell function can be restored after ex vivo treatment with IL-7 and IL-27 of PBMCs of patients in early clinical stages of chronic Chagas disease.

0447 - DEVELOPMENT OF A ANTIMICROBIAL AGENT WITH A PROMISING USE IN WOUND HEALING MANAGEMENT.

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UNC, FCQ, DEPARTAMENTO DE CIENCIAS FARMACÉUTICAS- UNITEFA, CONICET (1); INSTITUTO DE INVESTIGACIÓN MÉDICA MERCEDES Y MARTÍN FERREYRA (2)

In the vast world of medicine there are several fields of study of interest; one of them is wound healing. Wounding leads to exposure of subcutaneous tissue that provides a nutritious environment conductive to microbial colonization and proliferation. The presence of bacteria at the wound site down regulates the host immune response, ultimately delaying the natural wound healing process, making the total healing time unpredictable. The usefulness of silver as an antimicrobial agent has been known for a long time. It´s especially important in the topical antibacterial treatment of burn wounds. The most widely use reagents have been silver nitrate and silver sulfonamides. However, they have showed a limited clinical usefulness. The aim of this work is to develop a new water-soluble silver complex formed with triethanolamine (TEA) and silver nitrate ($AgNO_3$) for use in wound healing treatment. The antimicrobial activity of the developed silver complex was tested against S. aureus by determining its minimum inhibitory concentration (MIC). The potential cytotoxicity effect was assessed in vitro against L929 fibroblasts. Antimicrobial results showed that the silver complex displays effective antimicrobial activity comparable to MIC values of $AgNO_3$. This study sets a platform for further work for the use of the novel developed silver complex from the wound care application perspective.

0495 - EVALUATION OF THE INHIBITORY CAPACITY OF ACETONIC EXTRACTS OF ROOTS AND AERIAL PARTS OF ZINNIA PERUVIANA AGAINST MICROBIAL STRAINS OF CLINICAL IMPORTANCE

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The evolution of highly resistant bacterial strains has compromised the use of new generations of antibiotics. Medicinal plants can be a possible source of bioactive compounds. Zinnia peruviana is used as a gastroprotective and also against bacteria, parasites and fungi. The aim of this work was to know the antimicrobial activity of acetonic extracts of leaves, flowers and roots of Z. peruviana. The antimicrobial activity was tested in vitro using the microwell dilution method against Staphylococcus aureus ATCC 29213, Staphylococcus epidermidis ATCC12228, Listeria monocytogenes CLIP 74904, Escherichia coli ATCC 35218, Pseudomonas aeruginosa ATCC27853 and Candida albicans ATCC 36801. The extracts were tested in a concentration ranging from 5,000 to 9.75 µg/mL. 2,3,5-Triphenyltetrazolium chloride 0.01 % (w/v) was used as visual indicator of microbial growth. The inoculum was adjusted to concentration of 107 CFU/mL. After 24 h incubation at 37ºC, the minimal inhibitory concentration (MIC) was defined as the lowest concentration of the extract in the medium in which there no visible growth. Extracts that showed inhibitory activity were submitted to a subculture on the surface of the agar plates, in order to evaluate the minimal microbicidal concentration (MMC). All extracts tested showed antimicrobial activity. However, the roots were more active for bacteria and yeasts. The best inhibitory and microbicide activity was detected against Gram-positive bacteria (39 to 78 µg/mL). The leaf extract showed good activity against Gram-positive (MIC= 625 µg/mL). The lowest activity was detected in flowers (1,250 to 1,250 µg/mL). The high biological activity of roots of Z. peruviana marks an alternative potential for the treatment of microbial infections. Further studies on toxicity and potential use in vivo models are necessary.

0516 - TARGETING OF CHIKUNGUNYA VIRUS ENVELOPE PROTEIN AS AN ANTIVIRAL STRATEGY TO INHIBIT CELL ENTRY

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CONICET

Chikungunya virus (CHIKV) is a member of the Alphavirus genus of the Togaviridae family. It is transmitted by mosquitoes of the Aedes species and is characterized by severe polyarthralgia that may last for months. Until today, no antiviral agent or vaccine has been approved for treatment or prevention of CHIKV infection. Virus invasion of the host cells is mediated by the envelope proteins, E1 and E2. Antiviral targeting of virus envelope proteins is an effective strategy for therapeutic intervention. Our goal was to design and synthesize lead compounds that can interact with a druggable pocket at the interface of the E1-E2 heterodimer. First, we used Autodock Vina to perform a virtual screening of commercially available compounds from Maybridge and Chembridge libraries. Selected compounds were bought or synthesized, and then tested against CHIKV using a reporter virus assay. Compound CHIK-1 showed a good antiviral activity (EC50 1.5 µM) and a selectivity greater than 50. Time of drug addition assays showed that CHIK-1 exerts its antiviral activity in the early stages of the viral life cycle. As CHIK-1 has a chiral center, we analyzed the antiviral activity of R and S enantiomers. To this end, we performed an enantioselective synthesis using as intermediary enantioenriched epoxides that were prepared using a methodology described in the literature. Forty-one CHIK-1 derivatives were designed and synthesized. Seven derivatives displayed low micromolar activity using a recombinant CHIKV-GFP reporter assay. Furthermore, pharmacokinetic in vitro and solubility assay for active compounds are currently ongoing. In conclusion, we uncovered novel entry inhibitors against CHIKV by prospective docking-based virtual screening and MD optimization. Now, we are seeking the solubility and stability optimization of lead compounds.

0530 - URSOLIC ACID PROMOTES TRYPANOSOMA CRUZI CLEARANCE IN MACROPHAGES BY MULTIPLE MECHANISMS

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Trypanosoma cruzi is the etiological agent of Chagas disease, which is endemic in Latin America. It affects 8 million people in this region, and causes around 12,000 deaths per year. Infective trypomastigotes and intracellular amastigotes are the two forms of this parasite found in mammalian organisms. Ursolic acid (UA) is a natural pentacyclic triterpene compound which has been shown to reduce the peak of parasitemia in mice infected with trypomastigotes from the Y strain. UA is also an inducer of autophagy, a vesicular transport pathway required for cellular homeostasis with important hydrolytic functions. In previous

studies, we demonstrated that autophagy participates in the elimination of intracellular amastigotes by means of xenophagy, a pathway of capture and degradation of intracellular microorganisms dependent on autophagy. Due to a direct cytotoxic action of the UA on epimastigotes, the replicative stage in the insect vector was discarded, we decided to study the action of the UA in the elimination of the amastigotes of T. cruzi in the macrophages and the possible participation of the autophagy and other mechanisms in this process. To test this, we infected macrophages with T. cruzi Y strain for 24 hours, and then treated the samples for 24, 48 or 72 hours under both control and UA (10 µM) conditions, and evaluated the amount of amastigotes by indirect immunofluorescence and western blot. We also performed cell viability tests with alamar blue to study the UA cytotoxicity on mammalian cells. Xenophagy (by IFI), and ROS generation (by 2',7'-dichlorodihydrofluorescein diacetate reaction) was also tested as two possible mechanisms of action of this drug. Our data show that UA decreases the amount of amastigotes in macrophages. We also observe that UA induces the autophagy pathway, and that LC3, the marker of autophagy, is recruited around amastigotes indicating xenophagy of these parasites. Moreover, the productions of ROS after 24 hours of treatment are increased. We conclude that UA decreases the amount of intracellular amastigotes by multiple mechanisms. UA stimulates the autophagy pathway promoting parasite capture and degradation through xenophagy. On the other hand, UA stimulates the production of ROS, which is toxic for T. cruzi but, interestingly, UA not have this effect on non-infected cells. However, we do not rule out a direct action of the UA on amastigotes, which we are studying by transmission electron microscopy.

0581 - IN VITRO ANTIVIRAL ACTIVITY OF NORDIHYDROGUAYRETIC ACID AND ITS TETRAMETHYLATED DERIVATIVE ON ARBOVIRUS WITH MEDICAL-VETERINARY IMPORTANCE

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In the search for new antiviral agents from native plant species, we began studying Larrea divaricata CAV. (Zigophyllaceae) and its main metabolite: nordihydroguaiaretic acid (NDGA). The antiviral effect of NDGA and its derivatives has been reported in numerous studies, the range of viruses evaluated is wide. However, there are few reports on arboviruses. We aimed to evaluate the in vitro effect of NDGA and its tetra methylated derivative, NDGA-4-M, on arbovirus: Chikungunya (CHIKV), St. Louis encephalitis (SLEV), Bunyamwera (BUNV) and Dengue types 1 and 4 (DENV-1, DENV-4), trying to establish the stages of the viral replication cycle affected. Cytotoxicity was assessed as a measure of the host cell viability in vitro by the neutral red uptake method. The virucidal and antiviral effects were evaluated by the plaque forming units (PFU) method. To those compounds showing active, we studied their action in several stages of the viral replication cycle, by using the UFP reduction method at different treatment times. Concentration of NDGA and NDGA-4-M causing 50 % of cytotoxicity (CC50) in LLC-MK2 cells were 115.7 µM and 7.8 µM respectively. NDGA-4-M was not able to inhibit any of the viruses tested. Although NDGA was not active also on CHIKV, SLEV and DENV-4, it was active on DENV-1 and BUNV with a selectivity index of 8.4 and 5.2 respectively. NDGA also produced an inhibition greater than 3 logarithms on DENV-1 when assessing virucidal activity. When evaluating the NDGA effect at different times of the viral replication cycle, it was determined that NDGA acts during the first two hours post-internalization (p.i.) on DENV-1 infection. By contrast, it was active all the time p.i. viral of the BUNV and, to a lesser extent, when cells were pre-treated before infection. Since there is currently no specific antiviral therapy available for the effective clinical treatment of infections produced by arboviruses, these results make NDGA a promising drug to treat these infections.

0606 - ASSESSMENT OF RESISTANCE PATTERNS OF ANTIMICROBIALS WITH HIGH IMPORTANCE IN HUMAN HEALTH, IN DIAGNOSED BOVINE NEUMONIA.

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Bovine respiratory disease (BRD) is the most important illness in feedlot cattle, where animals are subjected to high pharmacological pressure with antimicrobial (ATM) drugs for prevention or treatment. Some of ATM used for prevention or treatment of BRD, are considered by OMS as very high and high importance for human health. The aim of this study was to assess the resistance frequency of Pasteurella multocida and Mannheimia haemolytica isolated from dead cattle with BRD, in order to determine the bacterial resistance. This trial involved the analysis of 41 isolated strains from 38 dead animals. Antimicrobial susceptibility testing was performed on all isolates strains using broth microdilution and a commercially available bovine/porcine panel (Sensititre®; Trek Diagnostic Systems, Cleveland, OH, USA). The frequency of resistance for the group I of antimicrobials (agents of very high importance to human health-Fluorquinolones and 3rd generation of Cephalospirines) was a 0% for the 2 bacteria assayed. However, the frequency of resistance for Category II antimicrobials (high importance to human health—aminoglycosides, macrolides and lincosamides) was variable. Thus, the resistance pattern obtained for clindamycin was 47.6 and 55 % for either P. multocida and M. haemolytica, whilst for tilosyn was 19 and 100 %, respectively. The 21.9 % of the strains analyzed did not show resistance to any class of the antimicrobials tested. Regarding the multiresistance pattern, the 26.9 % of the strains showed resistance to only one antimicrobial group while the 51.2 % of the strains showed resistance to 2-4 antimicrobials. M. haemolytica resulted the bacteria with the highest resistance levels in animals died by BRD disease. The determination of the frequency of resistance pattern is essential to rationalize the therapeutic in animals subjected to high pharmacological pressure, in order to design new treatment interventions for reducing the development of bacterial resistance.

0614 - HIGH-THROUGHPUT MUTATIONAL ANALYSIS OF TRYPANOSOMA CRUZI ANTIGENIC EPITOPES REVEAL CONSISTENT CONSERVATION OF KEY RESIDUES ACROSS HUMAN CHAGAS DISEASE POPULATIONS.

Leonel BRACCO (1) | Janine RAMSEY(2) | Melissa NOLAN(3) | Faustino TORRICO(4) | Iván MARCIPAR(5) | Jaime ALTCHEH(6) | Norival Jr KESPER(7) | Juan Carlos VILLAR(8) | Fernán AGÜERO(1)

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Chagas Disease is a major health problem for which no vaccine for public health interventions are yet available. Diagnosis is essential

for the identification of infection and ecoepidemiological surveillance. Diagnosis is routinely performed using serological methods, which require well characterized antigens. Although available tests give satisfactory results in most cases, discordant results remain as a possible cause of undetected infected patients. We have previously conducted a large-scale screening of T. cruzi linear B-cell epitopes using high-density peptide arrays, leading to the development of a new proof-of-principle multiepitope diagnostic test that has excellent diagnostic performance1. However, understanding which residues in an epitope are important for antibody binding can lead to improved reagents. To further characterize known antigens, we performed Alanine scans of 649 different proteins (881 antibody-binding peaks/epitopes, represented by 2,913 peptides). This experiment was based on replacing each amino acid residue in each peptide for an Alanine (or a Glycine if the original residue was itself an Alanine), and assessing the impact on reactivity of each modified epitope. Using this peptide array design, we have assayed 45,492 peptide variants against 108 Chagas Disease serum samples (from 6 different countries). We developed an algorithm to integrate and analyze the effect of these epitope mutations, and to visualize key residues for each antigen and sample. We identified precise residue positions in epitopes that play a fundamental role in the seroreactivity. In summary, we observe an average of ~6 key residues per epitope. As an example, Ag2/CA-2, a known antigen, displays the same 7 key residues in all reactive sera. In contrast, SAPA, Ag36, and B12 display different degrees of reactivity conservation of their key residues. This variable responses for different antigens, can be used in the design of improved antigens for diagnostic applications.

0616 - BIOACTIVITY OF OLEANOLIC ACID AND ITS ACETYLATED AND SILYLATED DERIVATIVES

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Triterpenes are compounds widely-spread in plants and an integral part of commonly consumed foods. Pentacyclic triterpenes consist of a 30-carbon skeleton formed by the cyclization of squalene exhibiting mainly antiproliferative properties. Oleanolic acid (OA) (3b-hydroxy-olean-12-en-28-oic acid) is a triterpenoid with pharmacological activity ranging from antitumor, antiviral, anti-inflammatory and others, but there are few studies on its activity against resistant infection. In this context, the antimicrobial activity was tested. Derivatives of OA were obtained by simple chemical reactions. The acetylated derivative was obtained by combining the substrate with acetic anhydride, pyridine and dimethylaminopyridine. While silylated compound was obtained with pyridine, trimethylsilyl chloride and hexamethylene diamine. The antimicrobial activity was tested using the microwell dilution method against Staphylococcus aureus, Staphylococcus epidermidis, Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa and Candida albicans. The compounds were evaluated in concentrations from 2,500 to 39 µg/mL. Triphenyltetrazolium 0.01 % was used as visual indicator of bacterial growth. After 24 h incubation at 37 ºC, the minimal inhibitory concentration (MIC) was defined as the lowest concentration of the compound in which there no visible growth. Compounds that showed inhibitory activity were submitted to a subculture on the surface of the agar plates, in order to evaluate the minimal microbicidal concentration (MMC). The absence of development indicated microbicidal activity. The best inhibitory activity was detected against Gram positive by OA, with MIC values of 78 µg/mL for Staphylococcus and <39 µg/mL for Listeria. While the derivatives obtained showed concentrations of 2,500 µg/mL or higher for all strains. Therefore, the product without modifications showed better antimicrobial activity and more studies are needed to enhance the properties for therapeutic purposes.

0620 - BIOLOGICAL EVALUATION OF NORBELLADINE ANALOGUES AGAINST PATHOGENIC BACTERIA

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4'-O-methylnorbelladine (MN) is a protoalkaloid which is the common precursor of all Amaryllidaceae alkaloids such as haemanthamine, lycorine and galantamine. The latter is primarily isolated from daffodil (Narcissus spp.), snowdrop (Galanthus spp.), and summer snowflake (Leucojum aestivum) and is currently used in the palliative treatment of Alzheimer's disease in the early stage [1]. The potential health effects of Amaryllidaceae alkaloids have been highly investigated, but there are a limited number of studies on the bioactivity of their precursors or analogs. In this study we evaluated the antibacterial activity of 2'-chloro-MN (1) and 2'-bromo-MN (2) against strains of methicillin-resistant Staphylococcus aureus ATCC 43300, Pseudomonas aeruginosa ATCC 27853 and Listeria monocytogenes CLIP 74904. Compounds 1 and 2, as hydrochloride, were synthesized by condensation of the corresponding substituted aldehydes and tyramine and further reduction with sodium borohydride. The antibacterial activity was assayed using microplate method in tripticase soya broth supplemented with 0.01 % (w/v) of 2,3,5-triphenyltetrazolium chloride as visual indicator of bacterial growth. The inoculum was adjusted to concentration of 107 CFU/mL. Minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were tested. Compound 1 and 2 showed a MIC and MBC of 250 µg/mL against methicillin-resistant S. aureus. Compound 1 was active against P. aeruginosa and L. monocytogenes with MIC/MBC= 1,000/2,000 µg/mL and 500/1000 µg/mL, respectively. Meanwhile, compound 2 showed activity against both P. aeruginosa and L. monocytogenes with the same MIC/MBC values (500/500 µg/mL). Both compounds showed bioactivity against the gram-positive and gram-negative pathogenic bacteria tested. This finding justifies the conduct of future studies of antimicrobial activity in vitro and in vivo of these compounds.

[1] M.B. Kilgore, T.M. Kutchan.Phytochem. Rev. 15 (2016) 317–337.

0632 - INVESTIGATING SERODISCORDANCE AND BORDERLINE SEROLOGY IN CHAGAS DISEASE USING HIGH-DENSITY PEPTIDE ARRAYS.

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Diagnosis of chronic Chagas Disease is currently based on serological techniques. Although available diagnostic tests give satisfactory results in most cases, there is currently no gold standard and discordant results remain a possible cause of undetected cases. Here, using state of the art high-density peptide arrays we examined the global human antibody repertoire of chronic Chagas Disease patients as well as serodiscordant and borderline serology cases. First, peptide arrays displaying 2.8 million unique peptides from the complete proteomes of T. cruzi strains CL-Brener (DTU TcVI, 19,668 proteins) and Sylvio X10 (DTU TcI, 10,832 proteins) were assayed with serum samples from infected subjects from Argentina, Bolivia, Brazil, Colombia, Mexico and the US, as well as negative samples from the same regions. This allowed us to identify the antigenic subset of T. cruzi peptides recognized by a diverse collection of sera. Next, in a second screening focusing on 400,000 peptides selected from this subset

we assayed individual serum to study 28 serodiscordant cases from Argentina and Mexico with borderline serology. These displayed a wide reactivity based on the number of positive peptides and quantification of signal. We observed almost complete lack of correlation between the quantitative values obtained in commercial ELISA (Wiener v4.0) and those obtained by the array. Hence, there is much room for improvement of current serological diagnosis. Based on the reactivity against 6 known antigens, we have separated these sera in three groups: one group of 17 sera reactive against several antigens; a second group of 8 sera reactive with fewer antigens and a group of 3 sera that were negative against most known antigens assayed. Using this information, we will shortlist novel antigens from the high-content screening to improve existing diagnostic kits. In this presentation we will revisit the concept of serodiscordance in the light of all new data arising from this screen.

0696 - MOLECULAR DETECTION OF TRYPANOSOMA CRUZI IN OCULAR TISSUE FROM PUTATIVE CORNEAL DONORS

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Infection by Trypanosoma cruzi, the etiological agent of Chagas disease, is endemic of America where 6-8 million subjects are infected (prevalence in Argentina 3.6%). Due to heart and intestines are T. cruzi target organs, seropositive individuals are excluded as donors, whereas for kidney transplant the use of both dead and living seropositive donors to negative recipients is accepted. About cornea transplants, seropositive donors are rejected even for seropositive acceptors nevertheless WHO consensus makes general indications in which infected donors can be accepted, only in extreme cases and subject to the informed consent. This general consideration is applied for safety without having been, to date, proved the parasite presence in the transplanted tissue. Herein we analyzed ocular tissues (20 corneas and corresponding sclera rings, and 7 eye muscles) from ten deceased seropositive donors (6/4 M/F, 30-74 years old) from Argentina that were admitted consecutively at Hospital Santa Lucia in Buenos Aires, Argentina. DNA extraction was carried out by means of QIAgen (DNeasy blood and tissue kit) with a previous incubation with proteinase. DNA integrity was checked by PCR amplification of the 290bp β-actin amplicon. Presence of T. cruzi DNA was analyzed by means of PCR reactions targeted to the variable region of kinetoplastid DNA (kDNA) (primers 121 and 122) and to the nuclear satellite sequence (TCZ1 and TCZ2). Considering tissue samples, 10 % of corneas (2/20), 20 % of sclera rings (4/20), and 14.3 % of eye muscles (1/7) have positive PCR findings. From patient analysis, corneas were T. cruzi positive in 20 % (2/10) of corneas, 40 % (4/10) sclera rings, and 25 % (1/4) eye muscles. Interestingly, the two donors with positive corneas also had sclera positive findings, suggesting higher parasite burden or a special tissue tropism. This is the first report of T. cruzi presence in human cornea that bring light on the use of seropositive patients as donors.

0706 - ANALYSIS OF JOINT VARIATION BETWEEN HUMAN CASES OF TEGUMENTARY LEISHMANIASIS AND SAND FLY ABUNDANCE IN A HYPER–ENDEMIC AREA OF ARGENTINA.

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Leishmaniases are a group of diseases caused by Leishmania parasites that are transmitted by sand fly female bite. In Argentina, the north of Salta province is a hyper-endemic area of Tegumentary Leishmaniasis (TL), being Oran department one of the most affected zones. To achieve deeper knowledge about the disease transmission in that region, we studied the joint variation of TL cases and sand fly abundance in two periurban sites of Oran. Sand fly captures were executed with CDC traps placed at the neighborhoods El Cedral (EC) (one night/sampling) and Taranto (TA) (three nights/sampling) across a year. Species identification of female sandflies was made by observation of spermatheca and cibarium. Also, the clinical information of patients diagnosed at Instituto de Investigaciones de Enfermedades Tropicales (IIET) since 1989 to 2018 was analyzed to determine the monthly mean of TL cases and the time of evolution of lesions. A total of 102 female sandflies were caught in EC neighborhood, while 1,434 in TA. The most abundant species was Nyssomyia neivai. The months with the highest proportion of gravid females were December and February for EC and TA neighborhoods, respectively ($p<0.05$). Regarding patient information, the male: female ratio was 6:1 with a median age of 32 years old. The time of evolution determined was one month. It was seen that the peak of patient cases took place in March for EC and in May for TA neighborhoods, namely three months later. This lag between gravidness period (high risk of infection) and peaks of TL cases may be explained due to the time of evolution (one month), plus an incubation period that seems to last two months. Considering the sex ratio and the productive age of patients, the transmission could have been mainly sylvatic during work activities. The analysis of joint variation allowed reaching a better characterization of disease transmission which is fundamental for designing and implementing prevention and control measures.

0732 - FIELD IMPLEMENTATION OF A 3D PRINTER BASED DNA EXTRACTION METHOD COUPLED TO LAMP FOR CONGENITAL CHAGAS DISEASE DIAGNOSIS

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INGEBI-CONICET (1); AI BIOSCIENCES (2); CEADES (3); FUNDACIÓN MUNDO SANO (4); FIND (5); IS-GLOBAL (6)

Congenital Chagas disease entails the transmission of Trypanosoma cruzi infection from a mother to her child. With currently available chemotherapies, the cure rate for infected children is almost 100 % if administered early upon infection. It is therefore of great relevance to diagnose newborns on time. However, the algorithm to detect congenital T. cruzi infection involves the performance of microhematocrite or micromethod at delivery or during the first months of life and a confirmatory serology at 10 months of age. In highly endemic areas where people live far away from reference centers, many infants never go back to confirm the diagnosis and receive treatment if infected. The challenge is then to implement sensitive and rapid diagnostic techniques that can be performed in minimally equipped laboratories. At present there is a prototype loop isothermal amplification molecular test available (T. cruzi-LAMP kit, Eiken, Japan), with similar sensitivity to that of real time PCR (qPCR), but easier to use. Nonetheless, highly purified DNA is needed and obtaining it is time consuming and requires equipment unavailable in endemic regions. Thus, our aim was to couple the T. cruzi-LAMP kit to a recently developed DNA extraction device based on a low cost 3D printer (named PrintrLab), and to test its use in a hospital

located in the "Gran Chaco", a highly endemic region for Chagas disease. The PrintrLab was programmed to purify DNA from whole blood-EDTA samples and to provide the incubation step for the T. cruzi-LAMP reaction. The process took about 2.5 hours to yield a result, while manual DNA extraction and subsequent qPCR normally take more than 6. Performance of the "PrintrLab-LAMP" duo was tested with blood-EDTA samples artificially contaminated with 0, 1, 2, 5, 10 and 100 parasites eq/mL and a sensitivity around 2 pararasites eq/mL was achieved. Finally, 70 clinical samples from infants born to seropositive mothers were evaluated and all the micromethod positive ones, 6 samples in total, were detected by the "PrintrLab-LAMP" approach. In conclusion, the "PrintrLab-LAMP" device showed a good sensitivity, the protocol was faster than other molecular techniques and it could be successfully used in a minimally equipped laboratory.

0764 - DEVELOPMENT OF QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION COUPLED WITH HIGH-RESOLUTION MELTING (HRM-QPCR) ANALYSIS FOR THE DIAGNOSIS OF TRYPANOSOMA EVANSI IN CANIS LUPUS FAMILIARIS

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The Trypanosomiasis caused by Trypanosoma evansi affects a wide diversity of mammals being zoonotic potential in man, with a case reported in 2005 in India. This haemoflagellate protozoan can parasitize most domestic mammals, being horses, dogs, and cattle the most affected species. Diagnostic tools for this parasitic infection are scarce, even though this trypanosomiasis can be very lethal if the animals are not treated. This work reports the development of a multi-diagnosis assay based on qPCR coupled to HRM that differentiates infections with diverse species of trypanosomatids and Leishmanias with zoonotic potential in peripheral blood samples from canines. The molecular marker selected was the Internal Transcribed Spacer (ITS1) present in the ribosomal RNA locus. This marker is highly conserved and present size variability among trypanosomes species. The results using as a template gDNA of different trypanosomatid species showed specific amplification with distinctive patterns in Melting Curves for T. evansi, T. cruzi, T. brucei, T. rangeli and different species of Leishmanias. This was confirmed in agarose gels, resulting in single or multiple bands with a size range from 250 to 480 bp. Its clinical validation was carried out on 14 peripheral blood samples from domestic canines from northeastern Argentina. The results showed positivity for infection with T. evansi in 36 % of the samples. Additionally, through this standardized technique, in one sample it could be detected infection with Leishmania infantum with low parasitemia, confirmed by sequencing and subsequent alignment of the ITS1 region with reference sequences. Therefore, molecular diagnosis of animal trypanosomiasis by HRM-qPCR represents a viable tool for wide-scale epidemiological studies, which may be used to report the true prevalence of the infection and allow implementation strategies to control these zoonotic diseases in Argentina, as well as the rest of South America, Africa, and Asia.

0831 - CHARACTERIZATION OF EXTRACELLULAR VESICLES DERIVED FROM THE INTERACTION OF TRYPANOSOMA CRUZI WITH HOST CELLS IN THE MODULATION OF IMMUNE SYSTEM

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The protozoan Trypanosoma cruzi is the etiologic agent of Chagas' disease, initially restricted to the Americas, but has spread throughout the world, reaching millions of individuals. T. cruzi has a complex biological cycle where it needs to evade the immune system and invade host cells to complete the infection. One of the most effective mechanisms in innate immune defense against pathogens is the complement system, which consists of a set of proteins that are activated in cascade and which culminates in the formation of a pore in the membrane of the microorganism, causing its lysis. T. cruzi have developed several mechanisms to escape the complement system and to invade eukaryotic cells, expressing different molecules and releasing extracellular vesicles. Extracellular vesicles (EVs) are small vesicles composed of a lipid bilayer which comprises microvesicles and exosomes, according to their size and biogenesis. Our group have shown the release of EVs during the interaction between the parasite and host cells promotes complement system inhibition and increases the invasion of metacyclic forms of T. cruzi to host cells. Here, our aim was to understand the secretion of EVs by different stages of the parasite and how these EVs could manipulate host immune system to effects the infection. Parasites from CL Brener and Dm28 strains of T. cruzi was differentiate to metacyclic forms (METAs) by a nutrient starvation process and tissue-culture derived trypomastigotes (TCT) was obtained from supernatant of infected VERO cells monolayers. To induce EVs secretion, the different stages from the parasites was exposed to THP1 cells in a relation of 5:1 (parasites:cells) for one hour at 37 °C. Subpopulations of EVs was isolated by differential centrifugation method, with large EVs (LEVs, predominantly microvesicles) obtained from a 11,000 xg centrifugation and small EVs (SEVs, predominantly exosomes) from a subsequent 100,000 xg centrifugation. The two subpopulations of EVs was differentially secreted from the parasites and had different features. Moreover, it was seen that EVs from different strains was capable of inducing a cytokine response in dendritic cells, acting as communicators during the infection and modulating the immune system. The next steps of this work is to understand if different subpopulation of EVs have different functions in the resistance and invasion of T. cruzi and to characterize the role of T. cruzi EVs in modulating the secretion of cytokines and nitric oxide by macrophages.

0868 - LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) AS A DIAGNOSTIC TOOL FOR CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis and causes skin lesions, mainly ulcers, on exposed parts of the body, leaving life-long scars and serious disability or stigma. Biopsy is widely used for the diagnosis of CL to obtain specimens for direct diagnoses (smear and culture). Molecular diagnosis is a promising alternative; although it is not well suited for adoption in laboratories with limited resources. Isothermal DNA amplification methods have the advantage of not requiring expensive equipment. The aim of this work was to use a previously reported LAMP assay to detect CL colorimetrically (Mikita et al., 2014, Rivero et al., 2017). LAMP reactions were performed using pan-Leishmania primers based on the 18S-rDNA sequences. Briefly, 5 µl of DNA extracted from cultures or biopsy specimens were subjected to amplification in reaction mixtures containing 40 pmol FIP and BIP primers, 20 pmol LF and LB primers, 5 pmol F3 and B3 primers, 1 µl (8 units) Bst DNA polymerase (New England Biolabs), the reaction buffer (20 mM Tris–HCl, 10 mM KCl, 8 mM $MgSO_4$, 10 mM $(NH4)_2SO_4$, 0.1% Tween-20), and 1.4 mM of each dNTP using a heat block for the amplification cycle. The LAMP assay was set up testing different concentrations of betaine and temperatures. Two approaches were used to confirm the amplification by using electrophoresis in agarose gel and by visual inspection after the addition of the fluorescent dye SYBR® Green (Invitrogen, S7563).

This assay was positive for DNA from Leishmania guyanensis, L. mayor, L. amazonensis, L. braziliensis and L. infantum. LAMP was negative for Toxoplasma gondii and Trypanosoma rangeli. The detection limit was 1.0×10^2 parasites/mL. This assay was positive also in 10 specimens obtained by lesion´s biopsy. The advantages of this novel tool include the speed with which the assays can be completed, the no-need of trained personnel, and the fact that it can be performed without complex and expensive laboratory equipment.

0874 - IFN-G INDUCTION BY THE TC13TUL ANTIGEN FROM TRYPANOSOMA CRUZI IN NAÏVE BALB/C MICE

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Trypanosoma cruzi, the etiological agent of Chagas disease, releases factors which modulate the host immune responses, including Tc13 antigens. Regarding innate immune responses, Tc13 antigen from Tulahuén strain, Tc13Tul, has shown to induce B cell expansion and non-specific IgM production on cultures of splenocytes from naïve BALB/c mice. To obtain further information about this role, we evaluated Tc13Tul ability to induce cytokine secretion by in vitro and in vivo stimulation. In vitro Tc13Tul stimulation of splenocytes from naïve BALB/c mice induced higher IFN-g secretion than that induced by the control protein MBP (554 ± 140 pg/ml and 35.33 ± 18.56 pg/ml, respectively). Differences in neither IL-17 nor IL-4 were detected. Tc13Tul-induced IFN-? secretion was also observed in cultured naïve splenocytes from the LPS-resistant C3H/HeJ mouse strain. In vivo administration of Tc13Tul (or MBP as control) to naïve BALB/c mice (3 daily ip doses of 1 ?g/mouse/dose) increased non-specific IgG in sera. In addition, in vitro cultured splenocytes from Tc13Tul-inoculated mice secreted a higher basal level of non-specific IgM than controls and the in vitro Tc13Tul stimulation of these cells showed an additive effect on IgM secretion. Regarding Tc13Tul-induced IFN-g secretion, in vitro cultured splenocytes from Tc13Tul-inoculated mice have no differences in basal levels respect to controls; however, when splenocytes were in vitro stimulated with Tc13Tul, cells from Tc13Tul-inoculated mice showed higher IFN-g secretion than cells from MBP-inoculated mice (950 ± 265.2 pg/ml and 147.3 ± 55.48 pg/ml, respectively). Results indicate that Tc13Tul participation in the innate immune response against T. cruzi is mainly exerted in phenomena related to the evasion of the immune system, such as non-specific Ig production. In contrast, as IFN-g is an important factor involved in T. cruzi resistance, this may be considered a Tc13Tul effect in favor to the host.

0876 - VACCINE STRATEGIES AGAINST BABESIA BIGEMINA BASED ON PRIME-BOOST IMMUNIZATIONS IN MICE WITH RECOMBINANT PROTEINS AND MODIFIED VACCINIA ANKARA VECTOR

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INTA

Babesia bigemina is an apicomplexan tick-borne parasite that infects RBC causing cattle morbidity and mortality in vast world areas. Vaccination with attenuated strains is effective but they have inherent disadvantages. Immunity to Babesia sp. requires both innate and adaptive responses including CD4+ T cells and neutralizing antibodies. The aim of this study was to evaluate prime-boost heterologous schemes in mice using immunogens that activate both humoral and cellular responses. Three recombinant proteins and two modified vaccinia virus Ankara (MVA) expressing a chimeric multi-antigen were obtained and evaluated as vaccines. The multi-antigen comprises B and T cell epitopes of the B. bigemina proteins: AMA-1, RAP-1 and TRAP-1. Epitope prediction was performed by bioinformatics using the B. bigemina genome. Mice were immunized at day 0 with recombinant proteins and at day 30 with each MVA. One group received a prime of AMA-1, RAP-1 and TRAP-1 in equal amounts and 30 days after alpha-MVA (containing epitopes of AMA-1 and RAP-1) and beta-MVA (containing epitopes of TRAP-1). A second group received a prime of AMA-1 and RAP-1 and a boost of alpha-MVA. A third group was immunized with recombinant TRAP-1 and then with beta-MVA. Two control groups received either a heterologous protein and wt-MVA or vehicle only. Serum samples were collected for antibody analysis and spleen cells were obtained at day 60 for cellular and cytokines assays. Priming with a cocktail of the 3 antigens and a boost with alpha-MVA and beta-MVA induced the highest level of specific IgG antibodies and activation of IFN+ CD4+ and CD8+ specific T cells. This group also showed a high ratio (>1) of IgG2a to IgG1 for the recombinant proteins AMA-1 and RAP-1 suggesting a strong induction of Th1-biased response. In summary, we have shown that a three-protein cocktail and both MVA used in prime-boost regimes are immunogenic for both antibodies and CD8+ /CD4+ T cells generating promising levels of B and T cell mediated immunity.

0890 - FINDING OF FASCIOLA HEPATICA IN A CAPYBARA (HYDROCHAERIS HYDROCHAERIS) IN TANDIL, PROVINCE OF BUENOS AIRES, ARGENTINA

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CIVETAN CONICET

Fasciolosis is a zoonotic parasitic disease caused by Fasciola hepatica. The life cycle of this parasite is indirect, it needs a snail of the Lymnaea family as an intermediate host to complete the cycle and so the occurrence of cases is limited to the presence of these snails. Most of the studies of this parasitic disease are in domestic animals and humans. Great economic losses are generated by this disease. Wild species are known to act as reservoirs and disseminators of the disease. The south-eastern zone of the province of Buenos Aires has been described with reference to the presence of Fasciola spp. in cattle but it is not yet known whether wild herbivores living in the zone are involved in the biological cycle of this disease. Among the wild species that have been positively reported to Fasciola hepatica the capybara, (Hydrochaeris hydrochaeris) is a poorly described species. In August of the present year, in the district of Tandil (Bs. As.), a dead capybara recently run over was found. At the macroscopic inspection the liver was apparently normal. At the magnifying glass inspection of the gall bladder, characteristics yellowish eggs were found. They were photographed with the Leica microscope and it can be seen that due to their morphology, size and location they were compatible with Fasciola hepatica eggs. PCR was performed in search of the mitochondrial gene ITS1 (species indicator) confirming that the eggs found in the capybara belonged to Fasciola hepatica. This is attractive for two main reasons; first, in the area where the animal was found there are no scientific reports of this disease or the presence of the snail, which prompts us to work on a more exhaustive search for cases. And secondly, the study of wild species as transmitters of this disease is not well studied and this ends up being a complication for producers and for the population in general, so we consider that its study is very important.

0897 - IN VITRO EVALUATION OF THE ANTIMICROBIAL EFFECT OF EXTRA VIRGIN OLIVE OIL (EVOO) AND OF THE ACTIVE COMPOUNDS HYDROXYTYLSOL AND OLEUROPEIN AGAINST HELICOBACTER PYLORI.

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Helicobacter pylori colonize the human gastric mucosa of half of the world's population. The infection can remain throughout life leading a chronic infection. Eradication therapies involve the use of a proton pump inhibitor and two antibiotics. This not always successful due to the acquired resistance to antibiotics. Natural medicine can provide new compounds that contains active principles with antimicrobial properties. The aim was to evaluate in vitro the antimicrobial effect of extra virgin olive oil (EVOO) and its main phenolic compounds Hydroxytyrosol (H) and Oleuropein (O) against H. pylori. The antibacterial activity against 2 strains of H. pylori (NCTC 11638 and HP661) was evaluated by microbroth dilution assays using Mueller Hinton broth (MHB). Fifteen µl of 1 x 10^8 CFU/ml inoculum of H. pylori strains were added to 100 µl of two-fold dilutions of EVOO, H and O. It was incubated for 24 h at 37 °C to determine the minimum concentration of the antimicrobial agent capable of inhibiting growth (MIC). Serial dilutions of Amoxycilin were used as control in the susceptibility test. Anova test was performed for statistic analysis. The antimicrobial effect of EVOO in both strains showed a MIC of 229.5 µg/ml. The compounds separately showed no effect. Interestingly, the combination of both compounds assayed by checkerboard method showed an inhibitory effect at values of H= 19.27 µg/ml+O= 2.1 µg/ml against NCTC strain, while in 661 strain, the values were H= 154.16 µg/ml+O= 8.44 µg/ml. The difference observed between both strains could be due to HP661 is resistant to levofloxacin. The compounds used in combination showed synergistic effect against H. pylori strains, this would potentiate the use of the EVOO. However, in this study the MIC of EVOO is higher than the combination of H-O, probably due to other compounds present. This study provides, for the first time, valuable information about the antimicrobial potential of EVOO against H. pylori. Further studies investigating the mechanism of action are needed in order to propose EVOO and its active compounds for antimicrobial therapy.

0904 - OMP19 AS A PROMISING ADJUVANT FOR IMMUNOTHERAPY IN ACUTE CHAGAS DISEASE.

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OMP19, a membrane protein for Brucella abortus, showed to be a good enhancer in the development of prophylactic immunity. Although experimental vaccines are able to reduce parasitic load, they allow parasites to survive within the host, modulating and evading the immune response. Immunotherapy attempts to modify this response, presenting the antigens in a different way and counteracting parasitic evasion strategies. We study the combination of OMP19 with parasite antigens (Ag) as an immunotherapy for Chagas disease. Groups of six female C3H mice were infected with a lethal dose of trypomastigotes of the RA strain of T. cruzi and treated with: 1) Ag+OMP19, 2) Ag, 3) OMP19, 4) control (PBS) at the 1st and 7th d.p.i or 5) benznidazole (Bnz) during 14 consecutive days. During the acute phase of the infection we observed a significant reduction of parasitemia in group 1 vs groups 2, 3 and 4. Control mice (4) lost more than 50 % of their body mass, conducing to a mortality of 66 %, while group 1 had a 100 % survival. Animals treated with Ag+OMP19 had a significantly in vivo (DTH) and in vitro (proliferation) cellular immune response than the other groups ($p<0.01$). Specific anti T. cruzi IgG titers, showed to be 10 times higher in group 1 than the control, with an increased relation of IgG2a/IgG1. Tissue inflammation is one of the most important objectives to consider in order to preventing the pathogenesis of Chagas disease. Histopathological analysis showed that animals treated with Ag alone, BNZ or PBS had important inflammatory foci, and even in the PBS group necrotic cells and amastigote nests were observed. However, in group 1 no signs of tissue damage were found and the seric activity of CK and LDH (as markers of muscle injury) were lower than control ($p<0.01$). These results would suggest that treatment with Ag+OMP19 during acute infection is capable of enhancing both the cellular and humoral response by preventing tissue inflammation.

0909 - LEISHMANIA SP-TRYPANOSOMA CRUZI CO-INFECTION IN PATIENTS FROM OF THE NORTH THE SALTA PROVINCE, ARGENTINA.

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UNIVERSIDAD NACIONAL DE SALTA

In several areas of Latin America (including northern Argentina) the geographical distribution of Tegumentary Leishmaniasis (TL) overlaps with transmission areas of American trypanosomiasis (Chagas disease´s). Mixed infections due to Leishmania sp. and Trypanosoma cruzi have been reported in patients showing clinical symptoms of TL, ranging between 12 to 70 %. Prevalence of Leishmania sp. and T. cruzi mixed infection is unknown for northern Salta. In the present study, we examined the prevalence of co-infections by Leishmania sp.-T. cruzi in patients attending the Instituto de Investigaciones de Enfermedades Tropicales (IIET-UNSa)-Oran, from Salta province. During the 2018 period, 253 patients visited the IIET for the diagnosis of LT by microscopy (smear). In addition, conventional serology ELISA Chagastest Rec.v3.0 (Wiener Lab, Rosario) was performed. To identify the possible lineage of T. cruzi, ELISA-TSSAVI was performed in sero-reactive patients by conventional serology. Of the total of 253 patients, 80 % men, the average age was 40 years (1 - 88 years old). Fifty seven % (144/253) were positive by microscopy for TL, while 10.5 % (23/218) were positive for serology for T. cruzi infection. Co-infection Leishmania sp-T. cruzi was 9.7 % (14/144). The reactivity for ELISA-TSSAVI was 72 % (13/18). These results highlight the importance of the diagnosis of Chagas disease in areas where tegumentary leishmaniasis is endemic. Our TSSAVI reactivity results suggest infection with TcII and/or TcV and/or TcVI lineages of T. cruzi in the area.

0931 - SERUM PROFILE OF CYTOKINES AND CHEMOKINES IN PREGNANT WOMEN INFECTED WITH TRYPANOSOMA CRUZI FROM NON-ENDEMIC AREA, ASSOCIATED WITH CONNATAL TRANSMISSION

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INP "DR M FATALA-CHABEN" ANLIS MALBRAN; UNIVERSIDAD ABIERTA INTERAMERICANA (1); INP "DR M FATALA-CHABEN" ANLIS MALBRAN (2); ADMINISTRACIÓN DE LABORATORIOS E INSTITUTOS DE SALUD ¨DR C. MALBRÁN¨, CENTRO NACIONAL DE GENÉTICA (3); CONICET (4); INP "DR M FATALA-CHABEN" ANLIS MALBRAN; UNIVERSIDAD ABIERTA INTERAMERICANA; CONICET (5)

Mother-to-child transmission of T. cruzi represents a challenge in controlling parasite dissemination in endemic and non-endemic regions. Its occurrence will depend on interactions among the parasite, the placenta, the parasitic load and the immune responses of the mother and the fetus. In our laboratory, we have described that chronically T. cruzi-infected pregnant women who gave birth to uninfected children, presented higher serological levels of TNF-alfa; and IL-15. On the other hand, infected women who transmitted the infection to their children showed higher levels of IL-12p70 and six times higher parasitic load than those who gave

birth to uninfected children. The aim of this study was to continue characterizing the immunological profile in pregnant women infected with T. cruzi, regarding the transmission of the infection to their children. The groups of study were pregnant women who gave birth to infected or uninfected children and their control groups: T. cruzi-infected and uninfected non pregnant women, and uninfected pregnant women. Serological levels of RANTES, MIG and MCP-1 chemokines were determined with a cytometric bead array. MIG and MCP-1 levels were increased by T. cruzi infection whereas there were no differences in these chemokines due to pregnancy. However, in women who gave birth to infected babies, MIG and MCP-1 were significantly higher than in those women who had babies without infection. Regarding RANTES, no significant differences were observed among groups. Together with our previous results, these findings indicate that women who transmitted the parasite to their babies presented a pro-inflammatory profile supporting a more active infection and the usefulness of immunological biomarkers to predict the risk of congenital transmission with T. cruzi.

This project was supported by ANLIS C.G. Malbrán, PICT 2016-2693, Subsidio Fundación A.J. Roemmers, and CAECIHS - UAI.

0936 - A DEMOGRAPHIC-URBAN STUDY IN A COHORT OF PATIENTS WITH CHRONIC CHAGAS DISEASE IN 2011-2018 PERIOD IN ARGENTINA. SM LÓPEZ, Y HERNÁNDEZ, N PRADO, A RIARTE. (SAP-2019). INSTITUTO NACIONAL DE PARASITOLOGÍA (INP) DR. M FATALA CHABEN ANLIS MALBRAN BUENOS AIRES. ARGENTINA.

Stella Maris LOPEZ | Yolanda HERNANDEZ VAZQUEZ | Nilda Graciela PRADO | Adelina RIARTE

INP "DR M FATALA-CHABEN" ANLIS MALBRAN

Internal and external migrations contribute to the reduction of inequalities in the world. Migrations seek a balance between access to the labor market and the benefits and costs of migrations. Chronic Chagas disease (CHD) population who received medical care and treatment at the INP comes from Argentinian endemic areas and bordering countries such as Bolivia, and live in urban areas on the outskirts of Buenos Aires. These people are included in the new epidemiological concept of urbanization of CHD. The aim of the present work was to make visible the socio- labor and economic features of a cohort of CHD people over the last decade. An observational study of 376 patients was performed. We worked with secondary sources and patient admission registry (Database Diagnostic Department) in the period 2011-2018. Variables used were origin, occupation, gender, age, number of children and residence. The data were analyzed by SPSS 15.0 software. Twenty-eight % of people came from the NOA* and 28% from the NEA*. Twenty-three % belong to migration from bordering countries, as Bolivia and Paraguay, 66 % to Argentinian migration being foreign men 17 %. These data match with the 2010 Argentine Census (Law No. 25,781 Migrations). Seventy-three % of the active population varies from 40 to 60 years. The majority live in concubinage and 60 % have between 2/4 children. Fifty-eight% have formal or informal paid work, 41 % receive a salary and 18 % work on their own. The job categories are mason/casual work 12 %, employees 22 %, workers 10 %, professionals 0.8 %, other 13 %. Unemployment is greater in men. Housewives are 61 %; 34 % have paid employment, 12 % work in family homes and 22 % in other jobs. Only 2 women work on their own. Adherence to medical controls was higher in women (57 %). CONCLUSIONS. The low labor movement and precariousness, the residences in peripheral areas, the axes as gender, work types, reveal the socio-economic determinants of social inequality and health of CHD in Argentina. The housewives 61 % of our CHD population show mayor adherence to medical controls. The economic impact of housewives represents 22.6 % of the GDP** in Mexico and 24.9% in Peru (Carvajal, 2015°). In Argentina there is no data.

*Endemic regions from Argentina. **Gross Domestic Product. °DOI: http://dx.doi.org/10.24201/edu.v30i3.1497.

0947 - RECOMBINANT MYCOBACTERIUM BOVIS BCG IS A PROMISING PLATFORM TO DEVELOP VACCINES AGAINST TRYPANSOMA CRUZI INFECTIONS

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Chagas disease is caused by human infection with the parasite Trypanosoma cruzi, affecting more than 7 million people worldwide, and there is not yet a vaccine available to control this protozoan infection. BCG vaccine has been extensively used as antigens delivery platform to reach immunity against many infection models, however it has not been described in T. cruzi infection. In the present study, we evaluated recombinant BCG (rBCG) expressing three T. cruzi antigens: two derived from Trans-sialidase (NT-TS, CT-TS) and one from Cruzipain (CZf). Each antigen was cloned into two different vectors able to replicate in Mycobacterium bovis and each construction was transformed in the BCG Pasteur strain. We immunized groups of BALB/c mice with the six rBCGs and controls with BCG Pasteur and PBS. Thirty days after the last immunization animals were challenged with 1,000 T. cruzi. The immunoprotective potential of rBCGs strains against T. cruzi was evaluated and a significant survival rates after challenge were observed only in NT-TS group ($p<0.05$). Histological analysis of NT-TS mice during the chronic phase of the infection revealed a decrease of heart inflammatory infiltrates and fibrosis ($p<0.05$; NT-TS vs control group). Cellular response was evaluated after mice immunizations with NT-TS, BCG Pasteur or PBS by stimulating splenocytes in vitro with recombinant antigens. CD4+ T cells of NT-TS immunized mice increased: proliferative capacity (Ki67+) and cytokine intracellular production (IFN-γ and IL-17) as compared with controls groups ($p<0.05$). Furthermore CD8+/CD107+ T cells exhibited a high IFN-γ; production compared with control groups ($p<0.05$). We conclude that the immunization with NT-TS expressed in rBCG conferred a very significant protection that was correlated with the activation of CD4+ T cells corresponding to both a TH1 and TH17 profile. More studies aimed to increase antigen expression could allow to improve the response obtained with this vaccine.

0952 - TOXOPLASMA GONDII IN MEAT PRODUCTS FOR HUMAN CONSUMPTION

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Toxoplasma gondii is an intracellular protozoan with a worldwide prevalence in human and animal populations. Usually, the risk of infection is associated with contact with the feces of infected cats, although there are other means of infection inherent to the life cycle of this pathogen.T. gondii is capable of infecting a wide range of warm-blooded animals that include birds and mammals. In infected (non-feline) animals, the parasite forms resistance structures called cysts, which may be present in different tissues including muscle. Since each cyst can contain 10 to 1,000 parasites, the consumption of meat without proper cooking or the handling of raw meat itself constitutes an important route of infection. The aim of this study was to conduct a small scale survey in which we assessed the presence and viability of T. gondii in meat samples for human consumption. To determine the prevalence of this infection, 18 pork meat products were acquired in retail stores from 11 localities of Buenos Aires City and surrounding areas. The presence of the parasite was confirmed by hemi-nested PCR for 12 out of 18

samples. To evaluate T. gondii viability, pepsin digested pork material was inoculated in mice (2-3 per sample). Thirty days after inoculation, the animals were euthanized and serum, brain, liver and heart samples were collected. A total of 10 mice were positive for antibodies against T. gondii, measured by indirect ELISA, representing 8 meat samples. On the other hand, molecular detection in DNA extracted from mouse brains showed that the parasite was present and viable in 9 meat samples. Additionally, in order to detect possible variations in T. gondii tropism, we analyzed liver and heart samples by hemi-nested PCR. We confirmed the presence of the parasite in both organs in 7 meat samples. Future experiments will be focused on the characterization of T. gondii strain genotypes to gain insight into the distribution and variability of the parasite in meat products.

0963 - COLONIES INITIALIZATION UNDER LABORATORY CONDITIONS OF NYSSOMYIA NEIVAI AND MIGONEMYIA MIGONEI (PSYCHODIDAE: PHLEBOTOMINAE) IN THE NORTH OF SALTA PROVINCE, ARGENTINA.

Griselda Noemí COPA (1) | Maria Cristina ALMAZAN(1) | Gabriela Del Valle FLORES(2) | Andrés ESCALADA(3) | Lorena Vanesa ARAMAYO(1) | Ruben CIMINO(4) | Julio NASSER(1) | José Fernando GIL(5)

CÁTEDRA DE QUÍMICA BIOLÓGICA. FACULTAD DE CIENCIAS NATURALES. UNIVERSIDAD NACIONAL DE SALTA (1); INSTITUTO DE INVESTIGACIONES TROPICALES- UNSA - SEDE ORÁN (2); INSTITUTO DE INVESTIGACIONES DE ENFERMEDADES TROPICALES (3); INSTITUTO DE INVESTIGACIONES DE ENFERMEDADES TROPICALES - UNSA- SEDE ORÁN (4); INENCO-CONICET (5)

Leishmaniases are vector-borne diseases with sand fly insects (Psychodidae: Phlebotominae) as vectors. In Oran (Salta, Argentina) Nyssomyia neivai is the most prevalent species followed by Migonemyia migonei, they have medical relevance because were found infected with Leishmania sp. in Argentina and Brazil. The establishment and maintenance of sand flies in colonies results key to study their biology, behavior, and relationships with pathogens. The aim of our work was to study the life cycle of Ny. neivai and Mg. migonei under laboratory conditions and to elaborate a horizontal life table for them. For this, sand flies were captured in a peridomiciliary area of Orán city. The blood fed females were captured using manual aspirators both on domestic animals and tree bark. A female with 5 males were maintained in rearing pots at 25 ± 2 ° C and 85-95 % relative humidity. Larval food consisted of a mixture of rabbit feces, fish feed, rabbit feed, while adult sand fly food provided was sugar solution (30 %). A total of 82 females were conditioned for oviposition, the 41.4 % of them survived and oviposited. Thirty-two specimens were Ny. neivai and two Mg. migonei. The average number of eggs laid per female were 40.81 (Ny. neivai) and 59.50 (Mg. migonei). A total of 78 adults of Ny. neivai and 27 of Mg. migonei ($p<0.001$) emerged under laboratory conditions. For Ny. neivai and Mg. migonei, the time range occurred between the egg and adult stages was 37 and 36 days, respectively. The proportions of the original surviving cohorts (lx) in each stage, for both species, were higher in the first stage (L1). The proportion of deaths per stage (dx) for Ny. neivai was higher in eggs and L1, while in Mg. migonei was in L2. Following this protocol, sand fly colonies could be initiated under laboratory conditions, which will allow the development of future projects for incriminating vectors and reservoirs in the north of Argentina.

0967 - VEGETATION COVER AND HUMIDITY INFLUENCE ON THE ABUNDANCE OF SANDFLIES IN COLONIA SANTA ROSA LOCALITY, NORTHWEST OF ARGENTINA.

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Tegumentary Leishmaniasis (TL) is endemic in northern of Argentina, with a zoonotic transmission pattern in northern of Salta and Oran department is the most affected. The vegetation cover and humidity can influence the presence and abundance of sandflies. The aim of this work was to analyze the potential influence of the vegetation cover and the humidity on the sandflies species captured in Colonia Santa Rosa locality (CSR) from the Salta province. To sandflies capture, CDC traps were placed in 14 sites during four nights in January 2016, between 18 pm and 7 am. The sampling sites were distributed in downtown housing yards, periphery and edges of residual vegetation. The species were determined through identification of spermatheca, cibarios and genitalia. The NDVI (normalized difference vegetation index; estimate the vegetation cover) and NDWI (normalized difference water index; estimate the humidity) for the study area were obtained from Google Engine. Then, using the QGIS 3.6 software, circular buffers of 100 meters of radius were generated whose centroids were the sampling sites. The average of the values of the NDVI and NDWI pixels extracted by means of the circular area were used to perform a correlation analysis with the abundance of the different species of sandflies using the non-parametric Spearman method. A total of 435 sandflies were captured. The species found and their abundances expressed by capture effort were: Nyssomyia neivai (133.33 %), Mygonemyia migonei (5.66 %), Cortelezzii Complex (4 %), Evandromyia sallesi (0.33 %) and sp (2 %). The values of NDVI and NDWI versus the total abundance of sandflies ($r= 0.78$; $r= 0.70$; $p<0.05$) and Nyssomyia neivai ($r= 0.76$; $r= 0.68$; $p<0.05$) showed statistically significant correlations. The effect of vegetation cover and humidity on the abundance of sandflies can be used potentially as a tool to generate interventions for control and prevention of LT in northern Salta.

0972 - EVALUATION OF THE IMMUNOGENICITY OF THE RECOMBINANT PROTEIN GSTMU OF FASCIOLA HEPATICA (RFHGSTMU) ADSORBED ON ALUMINUM HYDROXIDE IN SHEEP

Vanesa FERNANDEZ | Javier SOLA | María Celeste MORAN | Silvina GUTIERREZ | Silvia Marcela ESTEIN

CIVETAN CONICET

Fasciola hepatica is a zoonosis which causes significant economic losses in ruminants. The development of a vaccine emerges as an alternative for the control of this disease. The recombinant protein GST Mu from F. hepatica (rFhGSTMu) adsorbed on aluminum hydroxide (rGSTMu + $Al(OH)_3$) conferred 90 % protection against this parasite in the mouse model. The objective of this work was to evaluate the humoral and cellular immune response against this vaccine in the susceptible species. Corriedale female sheeps were immunized twice every 30 days. The humoral immune response was analyzed by an indirect ELISA. Blood was obtained every 15 days from day 0 to 75. The in vivo immune cell response was evaluated on the day 75 by performing the intradermal test with rFhGSTMu. Specific serum IgG antibodies increased after each boost unlike that observed in the control group (without immunization), which maintained baseline levels throughout the trial. The differences were maximum and significative ($p<0.05$) two weeks after the last immunization. The presence of a cellular immune response was not detected in vivo. This preliminary result indicates that rGSTMu + $Al(OH)_3$ is immunogenic in sheep. In the future, we will evaluate the protection conferred against F. hepatica in sheep.

AACyTAL I

Chairs: Marcelo Asprea | Eliana Cicale | Judith Van Luijk

0030 - ENVIRONMENTAL ENRICHMENT IN STANDARD-SIZED CAGES: EFFECTS ON FEMALE SWISS MICE

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IBCN, FACULTAD DE MEDICINA, UNIVERSIDAD DE BUENOS AIRES (1); LABORATORIO DE ANIMALES DE EXPERIMENTACIÓN (LAE), FCV,UNLP (2)

Environmental enrichment (EE) consists on increasing the complexity of the cages, which promotes the species' specific behavior. EE has been used to improve the welfare of laboratory animals as well as a treatment per se in experiments in the field of neuroscience. We aimed to study EE effects in standard-sized cages, on several biological parameters. Female SWISS mice were housed in 3 conditions (20 mice/group): no enrichment (NE), simple enrichment (SE), and complex enrichment (CE). The experiment lasted 8 weeks and afterwards, anxiety-like behavior was assessed using a modified-novelty suppressed feeding (NSF) test. Brains were collected to determine the survival of newborn cells in the hippocampus (HC). Additionally, corticosterone metabolites were measured in fecal samples. Mice in CE had higher levels of corticosterone in feces and behaved less anxiously in the NSF test than mice from the NE group ($p<0.05$). Correspondingly, the number of new cells in the HC was significantly higher in both the enriched groups ($p<0.01$). The higher levels of corticosterone in the CE might have been due to an overall increase in the in-cage activity, therefore reducing boredom; while the CE also reduced a negatively-valenced affective state like anxiety. These findings are consistent with an improvement of animal welfare. Furthermore, both enrichments had an adaptive value to the brain, as they increased the survival of new cells in the HC. SE is commonly used without proper standardization, but it might generate confounded effects at least in neuroscience experiments. Additional studies are needed to assess the effect of EE in standard-sized cages.

0033- HIGHLIGHTS ALONG THE HISTORICAL RECORD OF THE FCEyN, UBA INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) AND 3 Rs IMPLEMENTATION

Fernando A. DYZENCHAUZ (1) | Lirane MACHADO MOUTINHO(2) | Zaira NAGUILA(3)

IBBEA, CONICET-UBA (1); IFIBYNE, CONICET-UBA (2); BIOTERIO FCEN-UBA (3)

There is a growing social awareness on issues related to the protection of animal welfare. In the year 2011 was founded the FCEN/IACUC whose mission is to adopt principles, policies, programs, and regulations aimed at ensuring the ethical use of research animals. To achieve this goal, we worked on two axes: 1) a required course for teachers, researchers, and students of the faculty involved in the use of animals, with a 5 years validity period, and 2) the evaluation of the experimental protocols dealing with animals. Our IACUC undertook the endeavor of the 3Rs implementation. We developed a form for submission of experimental protocols, with evaluation criteria based on the analysis of a wide array of considerations, such as objective and rationale of the project, technical and environmental aspects, experimental and surgical procedures, classification of severity of the procedure, justification for the use of animals with regards to alternative methods, experimental design, anaesthesia, analgesia, and humanitarian end point. Since its founding, 125 protocols were evaluated. It is noteworthy the diversity of the areas of knowledge of the analyzed protocols, while 5% of them include wild animals and 95%, laboratory animals. Disaggregating data by species, the mouse represents 70% of the animals used, rats (11%), fish (9%), frogs (8%), and birds (5%). Strikingly, nearly a third of the protocols submitted for evaluation, have not continued in the evaluation process due to the neglect of those who submitted them. Additionally, there is an evident bias in the gender of animals used, since more than 70% of the protocols submitted by the scientific community of the FCEN-UBA, similarly to the international scientific community, use only males as experimental subjects. Finally, in order to monitor the compliance with the welfare assurance of the animals used in research, the IACUC has implemented an oversight of the protocols wherever the experiments are carried out.

0061 - REFINEMENT OF A FEMUR SURGERY TO TEST DENTAL IMPLANTS IN RATS: A PILOT STUDY

Katya Mariel KLUG GOMEZ | Agustina RESASCO | **María Clara VERCELLINI** | José Luis BELTRANO | Martín CARRIQUIRIBORDE | Ana Cristina CARRANZA MARTÍN | Juan Martín LABORDE | Fabricio Alejandro MASCHI | María Del Pilar CAGLIADA | Cecilia CARBONE | Miguel AYALA

LABORATORIO DE ANIMALES DE EXPERIMENTACIÓN (LAE) FACULTAD DE CIENCIAS VETERINARIAS, UNLP

The refinement of the different techniques is an important part of Laboratory Animal Science. In our lab we have stablish a model to bone regeneration. In order to refine the technique, we aimed to study how two types of bone perforation (one long defect vs two smaller ones) would affect animal welfare. For this purpose, 8 WKAH/HokLAE rats were employed, housed in SPF conditions. After the surgical procedure, pain management and antibiotic therapy were given to the rats. Their weight, food consumption and performance in the burrowing test were measured before and after the surgery, and compared using paired t-tests. No significant differences in food consumption were detected between groups after a week ($p\geq0.05$). Nevertheless, a trend in body weight reduction was observed after that period for the group with the long defect ($p\geq 0.05 \leq 0.1$), whereas significant differences were observed for the rats with the double defect ($p<0.05$). Regarding the burrowing test, significant differences were observed 72 hs after the surgery only in the rats with the long defect ($p<0.05$). The original values for the burrowing test were restored when it was repeated a week after the procedure ($p\geq0.05$). As deviations from the original values were observed, further research into these two models will be performed in order to improve the refinement of this technique.

0070 - A RETROSPECTIVE ANALYSIS OF PROTOCOL REVIEW: THREE YEARS OF IBYME-IACUC

Natalia Alejandra VASTA | Rosa María MORALES | Amalia BOTO | Graciela SANITÁ | **Ernesto GULIN**

INSTITUTO DE BIOLOGÍA Y MEDICINA EXPERIMENTAL (IBYME-CONICET)

The Instituto de Biología y Medicina Experimental Institutional Animal Care and Use Committee (IBYME-IACUC) oversees laboratory animal welfare in research and reviews the protocols involving animals used at the institution. The lack of a National Law aiming at regulating and protecting the use of animals for educational and research purposes undermines a strict control on the procedures. Moreover, not only national and international funding agencies but also most of the peer-reviewed scientific journals require an IACUC approval. In 2016, the Committee Board at IBYME appointed an internal IACUC, composed of a veterinarian, laboratory animal technologists, research scientists from different disciplines and community representatives. This work aims to report the key issues dealt with while reviewing and approving the protocols submitted to the IBYME-IACUC during 2016-2018. Along this 3-year period, 150 protocols were submitted and 144 were finally approved (2016= 57; 2017= 57; 2018= 30). The main areas were represented as follows: cancer constitutes 31%, reproduction 27 % while immunology, neurosciences and endocrinology include

13 % of the projects submitted, allocated jointly to these three areas, and 3 % for other categories. The most used animal species were mice (Mus musculus) in 82 % of the protocols, followed by rats (Rattus norvegicus) (13 %) and 5 % of other species. Regarding mice strains, most protocols employed C57BL/6 or BALB/c. The severity classification of the procedures was based on EU Directive (2010/63). Thus, procedures classified as mild or moderate were 42 %, while 10 % were severe, 3 % with no recovery or mild and 3 % had no recovery. Considering the lack of local database on the use of laboratory animals, this work provides updated information of the local scenario of animal use for research and may contribute to developing long term scientific policies that ensure the welfare of animals used in research to help fulfill IACUC's responsibilities in Argentina.

0161 - OPTIMAL NUTRITION MURINE MODEL FOR THE STUDY OF NON-TRANSMISSIBLE CHRONIC DISEASES: A PRELIMINARY STUDY

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The development of animal models of optimal nutrition, understood as one that acts as a protective factor against chronic non communicable diseases (NCDs) is an area of interest in medical sciences. The general objective of the work was to evaluate the nutritional adequacy of experimental diets based on natural ingredients rich in linolenic acid w3 (ALA; C18:3) in experimental mice through multiparametric determinations. Thirty male mice of the Balb-c strain (28 days) from the Bioterium of the Faculty of Medicine-UNNE (CICUAL MED-UNNE Res protocol No. 02/17) were used. Mice were distributed randomly in 3 lots (10 animals each) provided with water and food ad libitum and were fed for 70 days: LOT 1 CONTROL= commercial balanced diet, LOT 2= diet B with crushed chia seeds (Hispanic sage) and LOT 3= diet C with crushed flax seeds (Linnum usitatissimum L). Growth indicators, biochemical parameters, histological and endogenous bioconversion of ALA were determined. The data were processed with the Prism 6.0 software. Weights did not change significantly among lots. The biochemical levels of glucose, cholesterol and triglycerides were lower in the experimental lots compared to control. Histological studies with H / E and PAS stains did not reveal apparent damage to the tissues in both experimental groups. Diet B fed mice showed bioconversion of ALA in 6.4 ± 0.32 % of eicosapentanoic acid (EPA, C 22: 5) and 22.7 ± 1.13 % of docosahexanoic acid (DHA, C 22: 6) in brains. Mice fed with diet C showed no bioconversion in EPA and 114.30 ± 5.7 % in DHA. In conclusion, diets B and C demonstrated lipid lowering and hypoglycemic properties, induced optimal nutrition without causing histopathological alterations. Diet C formulated with crushed flax seed caused the greatest bioconversion in brains of ALA in DHA. This study might contribute to obtain murine experimental models based in dietary conditioning for the study of NCDs.

0325 - PRIMED B LYMPHOCYTE DEPLETION BY POPLITEAL LYMPH NODE RESECTION SURGERY IN A MURINE MODEL OF DENGUE VIRUS INFECTION

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INSTITUTO DE QUÍMICA BIOLÓGICA DE LA FACULTAD DE CIENCIAS EXACTAS Y NATURALES (IQUIBICEN) (1); BIOTERIO FCEN-UBA (2); FUNDACIÓN INFANT (3)

Strategies designed to study B cell responses to viral infections in mice comprise passive transfer of antigen-specific antibodies, adoptive transfer of primed B lymphocytes or use of B-cell-deficient mice, among others. The objective of the present work is to describe ipsilateral popliteal lymph node resection surgery as a strategy to abrogate B cell responses in a mouse model of dengue virus (DENV) infection. C57BL/6 mice were inoculated with UV-inactivated DENV-1 or DENV-2 (equivalent of $5x10^5$ PFU) via footpad (f.p.). On day four, the ipsilateral popliteal lymph nodes in the hind legs corresponding to the inoculation site were removed. Control mice were inoculated with UV-inactivated DENV-1 via f.p. and underwent mock surgery or were inoculated with C6/36 cell supernatant (placebo) f.p. Briefly, C57BL/6 mice were anesthetized using an intraperitoneal injection of a mixture of ketamine and xylazine (100 mg/kg and 10 mg/kg of body weight, respectively, via i.p. injection). After depilation and skin antisepsis, the ipsilateral popliteal lymph nodes were removed. After surgery enrofloxacin (0.1 mg/ml, diluted in drinking water) was administered for 3 days as prophylaxis and tramadol (200 µl of a solution 100 mg/ml, diluted in 1 liter of drinking water) was administered for 4 days as an analgesic. Post-surgical wound healing and behavior was monitored. All the animals being operated survived the surgery and did not show mobility impairments in the operated member or disturbances in normal behavior. The efficacy of B cell depletion was tested at 40 days post-inoculation by DENV-specific IgG immunoassay. DENV-1 and DENV-2 IgG endpoint titers were significantly reduced 13- and 36- times, respectively, in lymph node resected mice, compared to animals that received mock surgery. The results demonstrate that popliteal lymph node resection was a successful strategy to deplete B cell response to DENV inoculation in mice

0497 - USE OF INTRA-RECTAL VIA IN CF1 MICE FOR THE ADMINISTRATION OF A COMBINATION OF ACEPROMAZIN AND MIDAZOLAM AS ANESTHETIC PREMEDICATION

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LAB. DE CS. VETERINARIAS, INSTITUTO NACIONAL DE MEDICAMENTOS, ADMINISTRACIÓN NACIONAL DE MEDICAMENTOS, ALIMENTOS Y TECNOLOGÍA MÉDICA (ANMAT)

Intraperitoneal is the route of administration for anesthetic drugs generally used in mice. It requires a particular physical restraint that generates stress. The aim of the present work is to evaluate the intra-rectal (IR) route to administer an alternative drug combination that would allow, with low doses of drug, a comfortable handling of the animal and also to evaluate the impact produced by the repeated administration of acepromazine/midazolam IR in CF1 mice. At the initial stage, 12 CF1 mice were used. Each animal was administered acepromazine maleate and midazolam hydrochloride by IR using an intravenous Teflon catheter, previous manual emptying of the rectal ampoule, registering latency and duration of the effect in each case. In all the animals, the latency time recorded was 3 to 5 minutes and the duration of the effect was between 30 and 60 minutes. At the second stage, 10 female CF1 mice were used. The treated group was composed of 7 animals to which were administered the same dose as the previous group and by the same route, using the same materials and the same technique. The control group consisted of 3 animals that were administered the same volume of physiological solution. The maneuver was repeated daily for 12 consecutive days. At the end of the trial, the animals were euthanized. Necropsy examinations were made, taking samples of the rectum and colon that were processed according to routine techniques. There were no lesions at the epithelial level or inflammatory reaction, nor congestion and vascular edema in the treated animals. No significant statistical differences were observed between the treated animals and controls. The IR administration is a simple and fast option for the operator and it is safe for the animal. Additionally, the IR route for repeated administration of this pre-

anesthetic combination has not presented side effects that should be considered at the time of use. It constitutes an alternative that offers an excellent margin of safety and contributes to animal welfare, as it is less invasive.

0609 - SUCCESSFUL RECOVERY OF A MOUSE LINE FROM SPERMATOZOA: FIRST CASE REPORT FROM THE IBYME-CONICET CRYOPRESERVATION SERVICE

Vanina Gabriela DA ROS | Diego GELMAN | Patricia CUASNICÚ | Debora Juana COHEN

IBYME-CONICET

The transfer of genetically modified mice from one Animal Facility to another is not allowed if the animals to be transferred have pathogens different from those present in the recipient facility. Recently, a research group requested our service to introduce a BALB/C mouse line carrying the desired gene modification in homozygosis into a new facility. For this purpose, the method chosen was to first perform in vitro fertilization using spermatozoa from the genetically modified males followed by uterine transfer of the in vitro generated blastocysts into pseudopregnant wild type recipients. For this purpose, epididymal sperm were recovered from a donor male of proven fertility and subjected to in vitro capacitation in fertilization medium (HTF). On the other hand, 5 prepuberal wild type BALB/C females were subjected to ovarian superstimulation with injections of eCG and hCG separated by 48 h. Twelve hours after hCG administration, the cumulus-oocytes complexes were collected from the females and inseminated. Gametes were co-incubated in HTF until eggs reached the 2-cell stage. The resulting 71 2-cell embryos (64 % fertilization rate) were incubated in an appropriate medium (KSOM) supplemented with amino acids for embryonic development. The 14 blastocysts (20 % embryo development rate) recovered after 4 days of culture were transferred into a pseudopregnant female (previously mated with a vasectomized male) using a commercial device (NSETTM) without the need of surgery or anesthesia. Three weeks later, 3 BALB/C pups were born in the new facility (21 % live born rate). PCR genotyping showed that all pups carried the desired transgene in heterozygosis. To our knowledge, these results showing the successful recovery of a mouse line from sperm of a male housed in an external Facility represent the first case reported so far in our country.

0759 - TEACH / LEARN WITH 3D TECHNOLOGY. AN ALTERNATIVE TO THE USE OF ANIMALS

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FACULTAD DE CS. VETERINARIAS - UBA

Students Students in most countries learn procedures in healthy animals. At some professional careers such as Veterinary Medicine and the Tecnicatura en Gestión Integral de Bioterios, because of ethical or humanitarian reasons, they are opposed to use animals during training activities. This paper presents an alternative method for students to develop manual skills for their professional work, using inanimate 3D printed models. This proposal is developed during the subjects "Técnicas de Bioterio" I to V (from first to third year from this career) to help students to learn different techniques and acquire skill and confidence. The proposal is based on the so-called rule of the three R's; specifically, the Replacement for inanimate animal models in teaching and training, complementing it with audio-visual systems, computer simulators and virtual reality. Students find an attractive and innovative alternative in this way in which they are being trained, and besides that, they find it simple and economical, and this covers their expectations. An Integral Project of Digital Technologies Inclusion is carried out by FCV UBA, because of this, the Faculty acquired a 3D printer "Replikat XY" (Argentine Industry) that works with different types of materials and consolidated a design team in order to support professors and different technical-pedagogical projects. Thanks to its unconditional contribution, an excellent quality 3D rat bio model could be obtained so students can practice different techniques by using them to improve their skills (clamping techniques, inoculation, even surgical manoeuvres). The inanimate animal bio models developed are made of synthetic material FLEX of 1.75 mm in diameter, white, PRINT A LOT®, printed in 3D with external morphological characteristics similar to those of live rodents. Taking into account that being trained on subjection and inoculation techniques are specific professional skills that should be acquired by all students from "Tecnicatura Universitaria en Gestión Integral de Bioterios" and Veterinary, the use of 3D printed animal models are considered a safe alternative method that allows students to achieve more practice and acquire these professional skills that complement practices with live animals.

0880 - ANALYSIS AND IMPORTANCE OF PATHOLOGICAL BACKGROUND LESIONS OF RAT AND MOUSE HEART: PRELIMINARY RESULT

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Background (incidental) lesions have been described as findings that are usually thought of as a change in tissue morphology outside of the range of normal variation for a particular species or strain. The myocardium is susceptible to a variety of morphologic changes in routine toxicity studies and background lesions need to be differentiated from real lesions related to drug treatment. The aim of this study was to do a retrospective analysis of background lesions in the heart in Wistar/Cmedc rats and Balb/c Cmedc mice. They were healthy animal from production area and control groups of preclinical and toxicological studies reported at Centro de Medicina Comparada (ICiVet-Litoral) from 2016 to 2019. Animals were necropsied and hearts were fixed in 10 % buffered formalin, embedded in paraffin, sectioned at 4 µm, and stained routinely with hematoxylin and eosin. Heart lesions from 29 rats (22 male and 7 female) and 34 mice (14 male and 20 female) were reviewed. Background lesions in the heart were observed in 22 of rats (76 %) and 17 of mice (50 %). The following lesion were describer: cardiomyocyte vacuolation in 9 rats (31 %) and 2 mice (6 %), inflammatory cell infiltration in 5 rats (17 %) and 6 mice (18 %), valvular myxomatous degeneration in 7 rats (24 %), aortic cartilaginous metaplasia in 6 rats (21 %), hemorrhage in 1 rat (3 %), thrombi in the atrium in 3 mice (9 %) and valvular endocarditis, cardiomyocyte atrophy, mineralization and fibroplasia in 1 mouse (3 %). Pathologists need to recognize background lesions in acute and chronic toxicity studies because many of the lesions can be mistaken or increased by treatment-related findings in preclinical studies. The importance of background lesions in toxicopathology studies is variable and related to the change type, severity and size.

0910 - EVALUATION OF HEMODYNAMIC PARAMETERS IN BLOOD VESSELS OF THE POSTERIOR SEGMENT OF THE RABBIT EYE USING COLOR DOPPLER ULTRASOUND.

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Doppler ultrasound (US) evaluation is a complementary technique to B-mode scanning and allows a non-invasive study of local vascularization, identifying alterations of blood vessels diameter and their path, and determining quantitative parameters of blood flow and vascular resistance. Evaluation of the effects of

pharmacological and surgical interventions in the ocular circulation is important in many retinal diseases such as diabetic retinopathy, age-related macular degeneration, glaucomatous optic neuropathy and retinal vascular occlusion. The aim of this work is to report the development of the Doppler US technique for evaluation of hemodynamic parameters in rabbit eyes. Twenty New Zealand adult male rabbits from CMC (ICIVET Litoral) were used. After systemic and ocular local anesthesia, the animals were subjected to US by a 6-14 MHz linear probe (L14-6P, Mindray) added to a color Doppler equipment (Z6 Vet, Mindray, China), carefully supporting the transducer on the eyeball. Color and spectral Doppler settings were optimized for evaluation of blood velocity (peak systolic velocity, end-diastolic velocity, and mean velocity), the resistance index (RI), and the pulsatility index (PI) of the central retinal artery and the central retinal vein. Blood velocity, RI and PI were obtained for each eye of every rabbit on the study. Doppler evaluation allowing us to determine and characterize hemodynamic parameters of the vessels of the posterior segment of rabbit eyes. There have been few previous reports of using Doppler ultrasound to measure blood velocity in the retinal vessels in laboratory animals due to the small diameters and low flow rates of these vessels. This technique provides a different and non-invasive tool for the evaluation of hemodynamic parameters in ophthalmological preclinical studies.

0913 - IMPACT ON REFINEMENT ON EXPERIMENTS FROM TWO YEARS OF IACUC ANALYSIS

Ramiro REARTE | Martin BONAMI | Carla MITACEK | Paula BLANCO | Cecilia STORNELLI | Julieta DE IRAOLA | Miguel AYALA | Andrea DELLARUPE | Gaston MORE | **Fabricio MASCHI**

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Research studies that involve experimental animals should be performed considering the relevance of species used, experimental design and the number of experimental individuals included in every study according to fulfill the three R's (replacement, reduction and refinement) postulates. The objective of current work wasto describe the research studies reviewed by FCV-UNLP IACUC respect the animal species involve and the studysample size depending on the level of distress produced by the experimental procedure. Every research study presented to FCV-UNLP'sIACUC during a two-year period (2017-2018) was included in this study (n= 69) and were classified in three groupsaccording to the severity of interventionson animals [A (n= 31); B (n= 21); C (n= 9)]. The distributions of species by severity aggressionclasses was describedgraphically with a stacked column graph. The most of research studies that involve low level of aggressions were carry out in live stock species, whereas those studies that imply a high level of aggressions are mostly performed in rodents. Furthermore, the number of experimental units by experimental procedure is higher in number and variation in Category A (mean= 197; range 3-900) than B and C (mean= 84, 20; range 6-582, 6-542; respectively). Thoseresults combined with commission observation suggest that low injuries experimental procedures tend to use an excess of experimental units, conversely high categories experiments show an apparent excessive reduction of experimental units. Determination of number of animals included in experimental procedures should be supported by power analysis based on size effect to prove.

0950 - THE STRESS RESPONSE EVALUATION IN VICUÑAS SUBJECTED TO DIFFERENT TYPES OF SHEARING

Nadia RAMOS(1) | **Silvina Soledad MAIDANA** (2) | Mercedes ODEÓN(3) | Samuel MIÑO(2) | Sandra ROMERO(4) | Marcelo ECHENIQUE(1) | Sonia Alejandra ROMERA(2)

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The South American camelids (SACs) "llama" (Lama glama) and "vicuña" (Vicugna vicugna) possess natural fibers of exceptional fineness. Currently, vicuña´s fiber is obtained by the practice of capture, shearing and releasing back to nature. This practice is performed in two management models that, due to their status as wildlife, respond to conservation and sustainable use policies: captivity (INTA Abra Pampa) and Silvestre (chaku), carried out by native people communities. Then, the objective of this study is to assess the level of stress in both modalities. The levels of glucose and protein were quantified after manual and mechanical shearing of vicuñas and defined as stress indicators. These stress indicators were assessed in trapping and shearing of wild vicuñas comparatively with the shearing of vicuñas in captivity. The sample was taken in both cases immediately post shearing. The glucose concentration was measured with test strips (values expressed in mg/dl). The plasma was recovered to quantified plasma protein measurements by Lowry's method. In addition, for standarization of interleukinas qPCR, primers of different interleukins were designed based on the genome of Camelus dromedarius, to assess stress at the level of gene expression. To obtain the positive controls, DNA was extracted from vicuña tissue with a commercial kit. We evaluate the specificity of primers by end-point PCR since they are designed based on another species of camelide deposited in the database. The results would indicate that the vicuñas mechanically sheared had lower concentrations of glucose (164.8 vs. 232.6 mg/dl) and total proteins (2891 vs. 6287 µg/ul) that manual shearing in silvestry condition. In captivity the levels of glucose were (100 vs. 133 mg/dl) and total proteins (962 vs. 1131 µg/ul) mechanically and manual shearing respectively. Due to the results obtained mechanical shearing generates less stress response in both silvestry and captivity. Develop of methodologies adapted to local needs evaluating physiological, biochemical and behavioral parameters as indicators of well-being will allow increasing the productivity of camelids, reducing stress in the different managements an added value for commercialization of fibre, responding to the demands of an international market ethically sensitive to animal abuse and regulatory requirements for wildlife conservation and management.

0990 - European Quality In Preclinical Data (EQIPD) educational program on preclinical research and data integrity

KE Wever | L. Monk | M. Ritskes-Hoitinga | T. Steckler | MR Macleod

EVIDENCE-BASED LABORATORY ANIMAL SCIENCE AND SYSTEMATIC REVIEW CENTRE FOR LABORATORY ANIMAL EXPERIMENTATION (SYRCLE), DEPARTMENT FOR HEALTH EVIDENCE, RADBOUDUNIVERSITY MEDICAL CENTER, NIJMEGEN, THE NETHERLANDS

Robust data from animal experiments are key drivers for decision making in the pharmaceutical industry and in basic research. Recent publications report shortcomings in the robustness and validity of data from animal studies, which often impact the transition from preclinical to clinical testing. European Quality in Preclinical data (EQIPD) is an Innovative Medicines Initiative (IMI) funded project that aims to make a lasting change in the rigour and robustness of pre-clinical animal research. Researchers should be more aware of the criteria and principles which increase preclinical research robustness and quality, to make a sustainable change in the way animal experiments are conducted and reported. Developing an educational program ensuring research-community wide expansion of knowledge on these topics is therefore part of our approach. Our main objective is to provide an online training program aimed at early career scientists and quality professionals,

through which essential training on quality principles can be achieved. The scope of our training program covers i.a. general scientific integrity, experimental design, validity, data handling and statistics, transparent reporting, systematic review of animal studies, the set-up of academia and industry collaborations and the implementation of quality management systems in discovery research environments. For each of these, existing open access online training materials are being identified and evaluated with a view to being collated into a complete training program. In addition, a yearly EQIPD summer school provides the opportunity for face-to-face training and networking, to achieve cross-fertilization and sharing of expertise. For more information, please visit https://quality-preclinical-data.eu/.

Oncología/ Oncology VI

Chairs: Denise Belgorosky | Fátima Ladelfa

0508 - EXPRESSION OF ESTROGEN RECEPTOR ALPHA VARIANTS (ERALPHA46, ERALPHA66) AND C-FOS IN RAT MAMMARY GLAND AND MAMMARY TUMORS

Alicia GUTIÉRREZ (1) | Lorena SAMBUCO(2) | Laura ÁLVAREZ(1) | Mariel NÚÑEZ(2) | Rosa BERGOC(2) | Elsa ZOTTA(3) | Gabriela MARTÍN(2) | Andrea Silvana RANDI(1)

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Breast cancer is currently the leading cause of cancer death among women worldwide. AP-1 (c-Fos/c-Jun) is associated with proliferation, while cytoplasmic c-Fos activates phospholipid synthesis in cells induced to differentiate or grow. Estrogen receptor alpha 46 (ERalpha46) is a splice variant of full-length ERalpha66 and it is known that it has an inhibitory role in cancer cell growth. Our aim was to investigate c-Fos localization, its relationship to AP-1, and to ERalpha46 and ERalpha66 isoforms in rat mammary gland development and carcinogenic transformation, and tumors. Female rats were injected: a) saline solution (Control, CMG) or b) N-Nitroso-N-methyl urea (NMU, 50 mg/kg), and samples were taken at 60, 90, 120 and 150 days of life. In addition, we analyzed hormone-dependent (HD) and independent (HI) tumors in ovariectomized rats, and intact tumors (IT) in non-ovariectomized ones. Our results show that, in CMG, nuclear c-Fos and proliferation (PCNA)(IHC) decreased with age ($p<0.05$ at 150 vs. 60 days), AP-1 content (p-c-Jun immunoprecipitation and c-Fos WB) was low ($p<0.01$), and nuclear ERalpha46/ERalpha66 ratio (WB) was enhancing with age, being greater than 1. In NMU, nuclear c-Fos and proliferation increased with carcinogenic transformation ($p<0.01$ at 120-150 vs. 60 days), AP-1 content was high ($p<0.001$ at 120 vs. 60 days), and nuclear ERalpha46/ERalpha66 was decreasing with age, being below 1. As tumor grade increased, proliferation ($p<0.01$), nuclear c-Fos ($p<0.05$) and AP-1 ($p<0.001$) expression were negatively associated to ERalpha46/ERalpha66 ($p<0.01$) in IT. In HD, nuclear ERalpha46/ERalpha66, nuclear c-Fos, AP-1 levels, and proliferation were lower than in HI ($p<0.05$), whose growth is estrogen-independent. Collectively, our findings support the notion that variant detection and ERalpha46/ERalpha66 ratio could shed light on the role of ERalpha isoforms in mammary gland transformation and help better predict breast cancer response to anti-hormonal therapy.

0510 - STUDY OF TRASTUZUMAB AND TRASTUZUMAB-EMTANSINE EFFECTS ON HER2+ HUMAN BREAST CANCER 3D CELL CULTURES

Marianela ABRIGO | Ivana J. TAPIA | Virginia Judith WOLOS | Romina A. KARAS | Miriam Judith DIAMENT | Elisa D. BAL DE KIER JOFFÉ | Gabriel FISZMAN

UNIVERSIDAD DE BUENOS AIRES, INSTITUTO DE ONCOLOGÍA "ÁNGEL H. ROFFO"

Trastuzumab (Tz) and Trastuzumab-emtansine (T-DM1) are therapeutic monoclonal antibodies that bind HER2 and show substantial antitumor efficacy in HER2+ breast cancer. However, resistance to trastuzumab emerges in most treated patients. Multicellular tumor spheroids represent a 3D in vitro model useful to study the mechanisms of action and resistance development in HER2+ treatment. Our aim was to assess the effect of Tz and T-DM1, either isolated or sequentially combined, on cell cycle distribution and apoptosis of 3D breast cancer BT-474 spheroids. We found that a long Tz 50 µg/ml treatment (12 days) modulates the cell cycle inducing a significant increase in G0/G1 cell population compared to a short treatment of 5 days or to IgG as control ($p<0.01$). T-DM1 (1 and 10 µg/ml) compared with Tz exerted an earlier and opposite effect, reducing G0/G1 population since 5 days treatment ($p<0.01$). This inhibition of G0/G1 was associated with a significant increase in G2/M population by T-DM1 (10 µg/ml). Interestingly, treatments combined in a sequential form did not reduce the G0/G1 cell cycle phase as much as the isolated treatment did. We also analyzed the effect of these drugs on cell death at 12 days treatment to evaluate apoptotic cells. We found that both drugs induced an increase in the percentage of apoptotic cells (Tz, 39.2 ± 4.4%; T-DM1 1 and 10 µg/ml, 62.6 ± 3.6 and 87.0 ± 0.9% respectively vs. IgG, 27.0 ± 5.2 %). Then we wanted to evaluate whether T-DM1, as previously shown by Tz, induces resistance after spheroid treatment. We found that monolayers derived from T-DM1 10 ug/ml 9 days treated spheroids acquired an evident resistance to the same concentration ($p<0,01$). In conclusion, Tz and T-DM1 were cytotoxic to BT474 cells in a different way; Tz arrests cells in G0/G1 phase maybe driving them to a dormancy state and T-DM1 was more cytotoxic inducing cell death after G2/M phase cell cycle arrest. In both cases, after treatment in 3D, the cells acquire resistance.

0511 - HISTAMINE H2 RECEPTOR AND MRP4/ABCC4 AS MOLECULAR TARGETS FOR ACUTE MYELOID LEUKEMIA

Angela Paola RODRÍGUEZ GONZÁLEZ (1) | Ana SAHORES(2) | María MAY(2) | Nicolas DI SIERVI(2) | Carlos DAVIO(2) | Carina Claudia SHAYO(1)

LABORATORIO DE PATOLOGÍA Y FARMACOLOGÍA MOLECULAR, IBYME-CONICET (1); LABORATORIO DE FARMACOLOGÍA Y ONCOLOGÍA MOLECULAR, ININFA-UBA-CONICET (2)

Acute myeloid leukemia (AML) is a heterogeneous clonal disorder in which early hematopoietic cells fail to differentiate and do not undergo programmed cell death nor apoptosis. We previously showed that increments in intracellular cAMP levels play an important role in leukemic cell proliferation and differentiation. Histamine, through H2 receptor (H2R), stimulates cAMP formation and up-regulates MRP4 expression, responsible for cAMP efflux. The aim of this study was to evaluate the effect of combining treatment with H2R agonists, histamine (HDC) or amthamine (A), together with a MRP4 inhibitor (ceefourin1) or non-specific MRP4 inhibitors (probenecid and MK571) upon proliferation and differentiation of AML cell models. U937, HL60 and KG1a cell proliferation after 72 h treatment with 100µM HDC or 10 µM A and different concentrations of MRP4 inhibitors was assessed by cell count. H2R stimulation enhanced the concentration-dependent anti-proliferative effect of MRP4 inhibitors. Next, CD88 expression was evaluated by Western blot, as an AML terminal differentiation marker. HDC and A in combination with MK571 or probenecid augmented CD88 expression compared to single treatments. In accordance, c-Myc expression was down-regulated to a greater extent in the combined treatment. However, neither ceefourin1 nor its combined treatments induced CD88 expression, but induced

apoptotic markers (caspase-3 and PARP activation). To evaluate the effect of ceefourin1 on leukemia cell proliferation in vivo, Swiss nu/nu mice were subcutaneously injected with U937 cells. Mice were treated with ceefourin1 for 3 weeks (i.p., 10 mg/kg daily), xenografts were measured periodically and their morphology was assessed by H&E staining. Although tumor growth was not affected, a significant increase in the apoptotic index was observed in mice treated with ceefourin1. Taken together, our results contribute to the rational basis of a polypharmacological approach in AML using H2R ligands and MRP4 inhibitors.

0513 - CD4, CD8 AND TREG LYMPHOCYTES IN THE TUMOR MICROENVIRONMENT OF M-406 MURINE TRIPLE NEGATIVE MAMMARY TUMOR GROWING IN HOSTS WITH DIFFERENT SUSCEPTIBILITIES

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The tumor microenvironment contributes to several aspects of carcinogenesis and, therefore, offers promising targets for cancer therapy. CBi- mice was artificially selected for body conformation from CBi mice. The M-406 mammary adenocarcinoma appeared spontaneously in an inbred CBi female mouse and it is maintained in vivo in mice of the same line. Previously, we observed that when inbred CBi- mice were s.c. challenged with M-406 they showed 100 % takes and 100 % regressions (resistance). In contrast, the tumor grew exponentially (susceptibility) in CBi mice and in F1 hybrids (CBi x CBi- and CBi- x CBi). Our aim was to determine the participation of CD4, CD8 and Treg lymphocytes in tumors growing in hosts with different susceptibilities. Mice of the three genotypes were s.c. challenged with M-406 and when growing tumors reached the maximum size ethically allowed, they were sacrificed, tumor samples were obtained, fixed and included in paraffin. Also, tumors being rejected (CBi-) were excised at 12-17 days. CD4, CD8 and Treg cells were quantified by immunohistochemistry with antibodies for CD4, CD8 and Foxp3 in 30 fields of 1,000X. The number of CD4+ and CD8+ cells were higher and the number of Treg lower in CBi- than in CBi and F1, being CBi- > F1 for CD4 ($p<0.05$) and CD8 ($p<0.01$), and CBi- < F1 for Tregs ($p<0.001$). No significant differences were found for CBi vs. CBi- or F1 for the three types of cells tested. Also, the CD8/Treg ratio was higher in CBi- compared to CBi ($p<0.05$) and F1 ($p<0.001$). CD4/Treg ratio was higher in CBi- than in F1 ($p<0.001$). These results allow us to conclude that: 1) The decrease of Tregs and increase of CD8 and CD4 in CBi- tumors would allow the antitumor immune response to proceed and explain, at least partially, the rejection of M-406; 2) Higher number of intratumor Tregs would play a role in the susceptibility to M-406 shown by CBi and F1 mice; 3) The higher ratio CD8/Treg increased the differences among susceptible and resistant mice.

0522 - ACTIVITY OF INTESTINAL EFFLUX PUMPS IN M-406 MAMMARY ADENOCARCINOMA BEARING MICE WITH METABOLIC SYNDROME (MS) TREATED WITH CYCLOPHOSPHAMIDE (CY) METRONOMIC CHEMOTHERAPY (MC)

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High fat diet (HFD) cause obesity and MS, which are associated with breast cancer progression. MC (chronic, low dose, with no extended rest periods, drug administration) is a promising treatment strategy with low toxicity. Some chemotherapeutic drugs are substrates of multidrug resistance-associated protein 2 (Mrp2) and P-glycoprotein (P-gp), efflux pumps that limit the intestinal absorption and are modified by alterations produced in MS. We previously demonstrated the induction of MS in CBi male mice. The aim was to assess the alteration produced by MS in Mrp2 and P-gp activity as intestinal biochemistry barrier and its influence on the therapeutic effect of Cy MC. CBi male mice (5 weeks n= 20/group), were fed with a chow diet (C) and a diet with 40 % calories of fat (HFD) throughout the experiment. At 16 weeks, the development of MS was confirmed by biochemical and morphological parameters. The activity of Mrp2 and P-gp was evaluated using the in vitro model of everted intestinal sacs. Once the MS features were settled, mice were challenged s.c. with M-406 (day 0); when the tumor was palpable, mice were distributed into 4 groups (n= 8/group): GI: C no treatment, GII:C+Cy (30 mg/kg/day in drinking water), GIII: HFD no treatment and GIV: HFD+Cy. Efflux of Mrp2 substrate DNP-SG decreased 64% in HFD respect to C ($p<0.05$); transport rate of rhodamine 123 by P-gp decreased 55 % in HFD vs. C ($p<0.05$). At the end of the experiment (day 22), the tumor volume was lower in GII vs GI and in GIV vs GIII ($p<0.0001$).The % inhibition of tumor growth in GII was greater than that of GIV ($p= 0.0524$). It was observed a 30% decrease in body weight in GIV, indicating toxicity. We conclude that the induction of MS impairs the intestinal activity of Mrp2 and P-gp and these modifications may produce a change in Cy absorption, leading to toxicity along with therapeutic effect; also, MC has lower antitumor effectiveness in animals with MS so that the drug schedule should be re-designed.

0536 - ANTITUMOR EFFECT OF RAC1 INHIBITION IN 2D, 3D AND IN VIVO GLIOBLASTOMA MODELS

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Glioblastoma Multiforme is the most common central nervous system cancer among adult patients with one of the worst prognoses. Despite current therapeutic efforts, cancer recurrence is detected close to the origin site in nearly all cases, clearly showing the necessity of developing novel therapies for the treatment of this disease. Rac1, a key member of the family of the Rho-GTPases, presents itself as an interesting molecular target for the development of novel therapies since it regulates relevant cellular functions, including cell migration and proliferation. Aberrant Rac1 overactivation has been linked to tumor progression and invasion in several cancers, including glioblastoma. We hypothesize that the inhibition of Rac1 could present an antineoplastic effect on glioblastomas, capable of negatively modulating tumoral aggression. The aim of this work is to evaluate 1A116, a Rac1 inhibitor developed by our group, in different experimental settings using two human Glioblastoma cell lines: LN229 and U87-MG. We first analyzed the effect of different 1A116 concentrations on glioblastoma cells growing in a 2D monolayer (IC50 (µM) of 35 and 19 respectively) as well as in a 3D spheroid model, showing an IC50 of 41 µM in LN229 and no effect in U87. We further analyzed the effect of 20 mg/kg.day 1A116 treatment on LN229 and U87 in vivo s.c. growth. Treatment with 1A116 showed a 47 % ($p<0.05$) decrease in tumor growth rate when compared to control in LN229, with no effect on U87 in vivo growth, as was observed in the spheroid models. Finally, we demonstrate that 1A116 antitumor effect in 3D and in vivo settings correlate with the degree of Rac1

pathway activation. Our experiments show a selective effect of the inhibitor 1A116 among different human glioblastoma cell lines. This highlights 1A116 as a promising candidate for the development of a novel therapeutic strategy in a pathology with great unsatisfied medical needs.

0556 - NORCANTHARIDIN, PRECLINICAL SETTINGS OF A NEW PLAYER FOR TRIPLE NEGATIVE BREAST CANCER TREATMENT

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Triple negative breast cancer (TNBC) is a subgroup of mammary cancer that do not express estrogen or progesterone receptors and neither overexpress the HER2 receptor. These tumors are very aggressive, and patients present high recurrence and metastasis rates. It has been described that Norcantharidin (NCTD) can inhibit the progression of several types of cancer however, the effect on breast cancer has not been studied yet. For this reason, we have evaluated the effect of NCTD on human (HS578T) and murine (4T1) TNBC cell lines. NCTD exhibited an important antiproliferative effect, with an IC50 of 56 µM for HS578T and 35 µM for 4T1 cell lines, obtained by MTS assay. This antiproliferative effect was consequence of a time-sustained reduction in ERK activated levels (p-ERK) as well as an increase in the Sub-G0 cell cycle fraction, compatible with the presence of apoptotic cells. In both cell lines, NCTD significantly reduced adhesive and migratory capacities ($p<0.05$, ANOVA test) also displaying animportant reduction in MMP-9 secreted activity. Although these parameters could have a direct implication in the malignant progression, clonogenic and in vivo assays showed an inverse behavior. In this regard, the pretreatment of 4T1 cells with NCTD induced an increase in the number of in vitro colonies and no effect could be detected in the amount of experimental lung metastatic nodes. Even though some results obtained are encouraging, we must seek the appropriated therapeutic strategy, probably combining with another drug, in order to allow and effective use of NCTD for the treatment of triple negative breast cancer.

0557 - ANTICANCER ACTIVITY OF NOVEL COPPER(II) COMPOUND WITH A SCHIFF BASE AGAINST 2D AND 3D HUMAN BREAST CANCER MODELS.

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The development of copper complex has shown to be very potential as antitumor agents. Several findings provide evidence that copper ions are capable of interacting directly with proteins and DNA, causing site-specific damage. We attempt to evaluate the biological activity of a novel copper complex against cancer cell lines: HT29 (colon), MG-63 (osteosarcoma), A549 (lung), MDA-MB-231 (breast) and MCF7 (breast) and one normal cell line L929 (mouse fribroblast), using MTT assay. The complex significantly reduced the cell viability in all cell lines tested (HT29 IC50: 9.71 µM; MG-63 IC50: 6.63 µM; A549 IC50: 2.82 µM; MDA-MB-231 IC50: 1.56 µM; MCF7 IC50: 1.70 µM; L929 IC50: 4.23 µM) ($p<0.001$). Further analysis in more sensitive cell lines (MCF7 and MDA.MB.231) showed a great genotoxicity, determined by comet assay, and inhibition of the cell proteosomal activity. On the other hand, MCF7 spheroids were cultured by the hanging drop technique and the effect of the compound on cell viability was evaluated by resazurin reduction assay. The compound diminished the cell viability on spheroids affecting the spherical shape and the cell migration. Finally, we analyse the effect of the complex on cancer stem cells (CSC). Flow cytometry analysis showed that the compound generates a decrease of CSC-characteristically CD44high/CD24low cell populations. Moreover, pre-treatment of cells with the compound lead to a decrease in the development of mammospheres. In summary, this copper complex displays anticancer activity, increasing the genotoxicity and cytotoxicity on 2D and 3D.

0566 - IN VITRO STUDIES OF THE TGFBETA/SMAD PATHWAY INDUCED BY BORON NEUTRON CAPTURE THERAPY (BNCT)

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Introduction: BNCT is based on the nuclear reaction 10B (n, alpha) ^{7}Li and the radiation field produced is a mixture of high and low LET components which activates the DNA damage response (DDR). It is well known the role of TGF beta1 in homoeostatic growth control; however it has been less studied its complex role in regulating responses to genotoxic stress (through transcription factors Smads). The aim of these studies was to analyze the TGF beta/Smad signaling pathway as part of the DDR arising from BNCT. HT29 human colon cancer cells were seeded in bottles of 25cm2 and distributed in 4 groups: 1) Control; 2) BNCT (BPA + neutrons); 3) NCT (neutrons alone) and 4) Bystander effect from BNCT. The irradiation was carried out in RA3 reactor (Neutron flux of 1.1010 n/cm^{2}sec). After 2 h of incubation at 37 °C, the total RNA was extracted with Trizol and real time PCR was performed for each gene: TGF beta1, Smad2 Smad7, ATM and ATR. The expression of TGF beta1 and Smad7 increased in all the BNCT and NCT groups respect to the Control ($p<0.05$). The indirectly irradiated group (Bystander) showed an increase only for TGF beta 1. The expression of Smad2 decreased respect to the Control group in all the irradiated groups. ATM showed a significative decrease, while ATR showed an increase ($p<0.05$). The increase in the expression of TGF beta and Smad7 on the one hand, and the decrease of Smad2 on the other, for all the groups irradiated would be consistent with the decrease in cell survival previously observed by our group. The increased expression of Smad7 would act by inhibiting this pathway through the blockade of Smad2 and therefore increasing the genetic instability. ATR instead of ATM would mediate the DDR. The description of the mechanisms will allow studying strategies of manipulation of the cellular response with potential translation to the clinic.

0569 - STARVATION-INDUCED AUTOPHAGY MODIFIES THE EXOSOMES COMPOSITION FROM PANCREATIC CANCER CELLS

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Pancreatic adenocarcinoma (PDAC) is a highly deathly cancer with neither a prognostic marker nor effective treatment. Exosomes are 50-130 nm extracellular vesicles that are released from most tissues, although their composition is in close relation with the origin cell. These vesicles are important mediators of intercellular communications and play pivotal roles in cancer progression, metastasis and chemoresistance. Tetraspanins are widely accepted exosomal markers (CD63, CD9, CD81). Recent publications suggest a close relationship between exosomes release and the autophagy pathway. The aim of this work was to evaluate tetraspanins composition in exosomes from PDAC cells in response to starvation as a physiological autophagy inductor. Total exosomal fractions were purified from MiaPaca2 and Panc1 PDAC cell lines by

ultracentrifugation. Then, specific exosomes populations were selected by different antibodies against tetraspanins attached to magnetic beads. Eventually, bead-bound exosomes are probed for specific markers and analyzed by flow cytometry. We observed a significant increase of the exosomal marker CD63 upon 1h starvation versus basal condition in CD9+ vesicles (MFI, MiaPaca2: 183.2 vs. 97.8, $p<0.01$ and Panc1: 165.8 vs. 127.4, $p<0.05$). In the same way, increase in CD9 signal was detected in exosomes selected by CD63 (MFI, MiaPaca2: 76.0 vs. 48.7, $p<0.05$ and Panc1: 81.9 vs. 58.2, $p<0.01$). Similar results were acquired for CD63 in CD81-selected exosomes (MFI, MiaPaca2: 166.8 vs. 111.3, $p<0.05$ and Panc1: 161.3 vs.109.0, $p<0.01$). Additionally, in other series of results, no significant difference was observed between both cell lines in exosomes after 48h of culture in basal condition. In conclusion, autophagy status quickly modulates the exosomal fraction tetraspanins profile of PDAC cells.

0587 - IN VITRO STUDY OF THE ROLE OF VAV PROTEINS IN MELANOMA

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Melanoma is the most dangerous form of skin cancer, accounting for the third highest number of lives lost across all cancers. Since the incidence of melanoma is steadily increasing in the population, finding prognostic and therapeutic targets appears a crucial task. Vav proteins are guanosine nucleotide exchange factors (GEFs) of the Rho GTPase family. As GEFs, they modulate processes highly associated to the development of cancer and metastasis, mostly through their GTPase regulatory function, but their involvement in melanoma is yet to be elucidated. Previous results of our group demonstrated by in vitro studies a role for Vav2 in melanoma cells proliferation and migration. To further explore the role of this protein in melanoma development, we performed in vivo experiments. Using shRNA techniques and transfection with expression vectors, we modulated Vav2 expression or activity in B16-F0 melanoma cell lines. These cells ($2x10^5$ cells/100 µL) were subcutaneously injected in the right flank of 8 weeks-old C57Bl6 female mice (n= 7-9/group). Tumor volume was measured with a calliper biweekly during 3 weeks. At the end of the experiment mice were sacrificed to collect tumor samples and lungs. Growth kinetics indicated that tumors with reduced expression of Vav2 grow slowly than the control (doubling time 1.71 ± 0.08 versus 1.53 ± 0.11; $p<0.05$), whereas tumors expressing a catalytically active version of Vav2 grew faster (doubling time 1.07 ± 0.42; $p<0.05$). Exploring histological sections of lungs derived from these mice stained with haematoxylin/eosin, we observed that cells expressing the catalytically active version of Vav2 were able to colonize lungs, generating metastasis and micrometastasis in some animals ($p= 0.0293$; Chi-squared test) whereas no metastasis was observed in lungs from the other groups. Altogether, our data indicate a putative role for Vav2 in the control of several processes associated to melanoma growth and the development of metastasis.

0590 - DIETARY INTERVENTION WITH YERBA MATE EXTRACT AS A PROMISING COMPLEMENTARY TREATMENT FOR COLORECTAL CANCER

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Yerba Mate (Ilex paraguariensis) is a native plant from South America. It has a large amount of bioactive compounds and has been reported to have biological activities. Colorectal cancer (CRC) is the second leading cause of cancer death worldwide so efficient strategies for the treatment are being explored, including dietary intervention. Hence, this study investigated the antitumor activity of Yerba Mate extract (YMe) using in vitro and in vivo CRC models. YMe was generated by aqueous extraction and bioactive compounds were identified and quantified by RP-HPLC. The most abundant polyphenol was chlorogenic acid (CA). We evaluated the YMe effect on cell proliferation using CT26 and COLO 205 colon cancer cell lines. The treatment with YMe inhibited cell proliferation of CT26 and COLO 205 with IC50 values of 0.25 mg/mL and 0.46 mg/mL, respectively. Using TUNEL assay we demonstrated that YMe induce apoptosis (30 %). On the other hand, we explore whether CA is the molecule responsible of biological effects of the YMe. Both YMe and commercial CA inhibited cell proliferation, adhesion, migration and the invasive capacity of tumor cells. However, YMe was the most potent inhibitor at a concentration lower than the concentrations used of CA. The effect of YMe on tumor progression was also studied in vivo using a syngeneic tumor model (CT26 cells). Animals received YMe by oral administration in a dose of 1.6 g/kg/day before and after cell inoculation. The results showed that YMe reduce new vessel formation around the tumor, delayed tumor onset and showed a reduction of tumor volume. The effects of the combination of YMe with 5-fluorouracil (5-FU) was also evaluated in mice. The results suggest that this combination increased susceptibility of the colon cancer cells to the cytotoxicity of 5-FU. In conclusion, our study suggests that YMe can be a promising candidate as healthy food sources in human nutrition, and also be considered a natural source of potential antitumor agents.

0598 - ASSESSMENT OF THE ANTICANCER ACTIVITY OF PROTEINS EXTRACTED FROM THE BROWN MACROALGAE UNDARIA PINNATIFIDA, AN INVASIVE KELP IN ARGENTINA

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Cancer has become the leading cause of death in developed countries and the second in developing countries. Therefore, finding safe and effective immunotherapeutic agents for the treatment of the disease has become a key topic. In recent years, one of the main approaches is based on the search for compounds in natural resources. For example, the chemical components from algae. Macroalgal proteins currently represent good candidate raw materials for the extraction of novel biofunctional peptides. Undaria pinnatifida is an edible brown alga, native from Asian countries where it has an annual winter growth cycle. In some introduced sites it can persist throughout the year, potentially exacerbating its impacts, as occurs in the southern coasts of Argentina. For this reason, the aim of the present work is to characterize and evaluate the cytotoxic capacity on cancer cells of the extracts from U. pinnatifida. The protein extract (PE) was obtained by the pH-shift method and ultrasound, using alkaline protein solubilization followed by isoelectric precipitation. Proteins present in the PE were analyzed by size-exclusion liquid chromatography (SEC-FPLC). Hemolytic activity, cellular activation and cytotoxic capacity of the PE were evaluated on red blood cell, PBMCs cells and MCF-7 cells, respectively. In addition, the cytokines and growth factors levels were evaluated by ELISA (IL-6, IL-10, IFN-g and TGF-ß). The protein content of the PE was calculated as 6.3 ± 0.5 mg g^{-1} biomass (dry weight basis). PE presents antitumor activity with cytotoxicity against MCF-7 cells (60-82 ± 5 %). We obtained that the PE produces a greater metabolic relative viability on PBMCs (214.7-270.0 ± 0.5 %). PE affects the production of cytokines. Moreover, the PE did not affect human red blood cells

(6.0-9.3 ± 0.4 % hemolysis). These preliminary results are promising for the development of new therapeutic agents or as immunotherapeutic treatments against cancer.

0599 - HYPOXIC FIBROBLAST-BEARING TUMOR IMPAIRS ESSENTIAL EVENT TO PHOTODYNAMIC THERAPY.

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Tumor microenvironment (TME) is a dynamic evolutionary ecosystem where fibroblasts are recruited in order to provide the niche to support growth. Owing to the rapid tumor proliferation, hypoxic regions appearedwherein HIF-1a; acts as the main molecular mediator of adaptability. In this sense, we have previously reported that this transcription factor confers therapeutic resistance to photodynamic therapy (PDT). PDT is based on interactions between light, oxygen and a photosensitizer (PS), leading to phototoxic reactions that culminate in cell death. However, the role of fibroblasts in promoting or impaired PDT outcome have not yet been fully addressed. In this study, we demonstrated the consequences of a hypoxic stromal phenotype on tumor mass for exploring PDT response. We mimic TME complexity implementing colon cancer cells and fibroblasts 3D cultures called spheroids. The homotypic spheroids diameter was homogeneous and proportional to the cell seeding concentration. Using hypoxia reporting lines, we verified that homotypic spheroids HIF-1 activity exhibited a size-dependent radial enhancement from the periphery towards the center. When co-cultured, fibroblasts localized on the hypoxic core, which manifested the influence of hypoxia on the stromal cells pattern distribution. The target selectivity of the PS displayed a PpIX accumulation in a positive relationship with Me-ALA concentration. In stromal spheroids, the PS distribution was homogeneous while decreased in hypoxic areas of tumor 3D. Low-concentration of Me-ALA showed a stromal-preferential PpIX generation. Interestingly, in heterotypic ones, the cross-talk between cancer cells and fibroblasts attenuated PpIX accumulation. Overall, our data suggest that stroma and tumor act in an integrated, reciprocal fashion which could ultimately influence on therapeutic resistance. Therefore, only simultaneous targeting of both population with combination therapies may be a successful approach for treating cancer.

0603 - EFFECT OF AZT TREATMENT ON PROTEINS VINCULATED TO TUMORAL DEVELOPMENT AND TERT EXTRA TELOMERIC ACTIVITIES IN THE HUMAN BREAST ADENOCARCINOMA CELL LINE MDA MB 231.

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One of the main features of cancer cells is the replicative immortality. In most human tumors, this is possible due to the activity of a holoenzyme called telomerase, which specifically elongates telomeres. Recent works have demonstrated that TERT, the catalytic subunit of telomerase, acts as a transcriptional modulator in several oncogenic signaling pathways. Additionally, our group reported that azidothymidine (AZT) not only acts as a telomerase inhibitor, but also downregulates the expression of TERT and has a potential modulating effect on extratelomeric activities related to tumor progression in a murine model. Our hypothesis proposes that there are variations on the expression level of proteins involved in key processes of tumor development, which are induced by the alteration of TERT. Consequently, the objective is to identify those variations in the human mammary adenocarcinoma cell line MDA MB 231, after the treatment with AZT. In order to achieve this objective, we determined the IC50 of AZT in MDA MB 231 cells, through a cell proliferation assay (57.03 ± 17.10 µM). Subsequently, we treated cells with AZT at a final concentration of 10 µM, during 24 and 96 hours. We obtained the sub proteomes (Cytoplasm, Organelles and Nucleus) from the samples, and their analysis was performed by using the LFQ proteomics method (HPLC - mass spectrometry). The obtained data was analyzed by Perseus Software. Based on these results, we observed significant differences in the expression of cancer related proteins (ANOVA, $p<0.05$) leading us to postulate AZT as a modulator of both telomeric and extra telomeric activities for human cancer treatment.

0621 - IMMUNOTHERAPY AGAINST MELANOMA: NOVEL PROTOCOL FOR IMMUNOGENIC MATURATION OF DENDRITIC CELLS

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Dendritic cells (DCs) have been used as immunotherapeutic in multiple clinical trials with varying success, but strategies for activating are not yet fully elucidated. Recently, have been demonstrated that immunogenic cell death (ICD) enhances DC maturation and DC ability to stimulate immune effector cells, thus ICD could represent a novel tool for improving DCs-based immunotherapy. Photodynamic Therapy (PDT), an antitumor therapeutic that combines photosensitizing agents, O2 and light to create a harmful photochemical reaction, is a well described inducer of ICD. Based on these findings, we propose to optimize DCs maturation protocol by loading them with PDT-treated B16-OVA melanoma cells (Mel-PDT). Firstly, we induced bone marrow-derived DCs differentiation by using 10 or 30 % J558-conditioned medium (CM) containing GM-CSF. Floating and attached cells were separately examined for their CD11c differentiation marker expression by flow cytometry. Attached cells showed to be the optimal condition DC for differentiation process (75.2 % CD11c+). Next, the ability of tumor photosensitization to promote DCs maturation was compared to activation triggered by known DC activating LPS (0.5 µg/ml). In the former protocol, DCs were loaded with Mel-PDT in 1:1, 1:2 and 1:3 ratios (DCs:TCs ratio) and the maturation marker was assessed by flow cytometry. The best maturation regimen were DCs co-cultured with Mel-PDT in 1:1 ratio (53.3 % CD86+), even higher than LPS-induced maturation (45.6 % CD86+). To assess the PDT implication on melanoma DCs phagocytosis, bright-field photographs were taken after 24 h of cellular co-incubation. Interestingly, it was observed that DCs preferentially phagocytes Mel-PDT. Overall, our experiments focused on a novel method to obtain tumor lysates from cells putatively undergoing ICD to be used for DC pulsing and to test the maturation of the generated DCs for their future application on antitumor vaccine development.

0631 - ROLE OF G PROTEIN COUPLED RECEPTOR KINASE 2 IN PROLIFERATION OF PANCREATIC CELLS.

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G protein-coupled receptor (GPCR) kinase 2 (GRK2) is known for its role in phosphorylation and desensitization of GPCRs. However, GRK2 has been shown to interact also with non-receptor targets and has been associated to cell migration and proliferation. Since GRK2 is upregulated in pancreatic ductal adenocarcinoma we aimed to evaluate the role of GRK2 in the regulation of proliferation

of pancreatic BxPC3 cell line and to discriminate between phosphorylation dependent and independent mechanisms in this process. In MTS proliferation assays we found that transfection of BxPC3 with wild type GRK2 or kinase death mutant did not modify cell proliferation. However, transfection with a dominant negative mutant of the regulator of G protein signaling (RH) domain of GRK2 significantly decreased proliferation rate (100 ± 15 mock vs. 50 ± 17 % RHmut, $p<0.05$). When we analyzed by Western blot the regulation of survival and proliferative pathways in these conditions, we found an increase in pERK levels in presence of dominant negative mutants of RH domain. On the contrary, inactivation of the RH domain of GRK2 led to a significant decrease in the levels of pAKT (25 ± 5 %, $p<0.05$). Transfection with wild type GRK2 did not modify phosphorylation of ERK nor AKT. These results suggest that GRK2 may be involved in regulation of proliferation of pancreatic BxPc3 by means of its RH domain and diminished AKT activity.

0637 - NOVEL ROLE OF HISTAMINE H4 RECEPTOR IN ANTITUMOR IMMUNITY

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The histamine H4 receptor (H4R) is the last discovered histamine receptor subtype and is mainly expressed in cells of the immune system, showing numerous immunomodulatory roles. Its functional expression is further described in different types of tumors. The use of H4R knockout mice (H4R-KO) could contribute to better understand the pathophysiological relevance of H4R in cancer and other immunological disorders. This study aimed to explore some immune-related responses in H4R-KO in comparison to wild-type (WT) mice and to evaluate the participation of H4R in antitumor immunity in a model of breast cancer. We first evaluated the composition of immune cell subsets in spleens and lymph nodes from H4R-KO and WT Balb/c mice by flow cytometry. We further assessed growth parameters, histological characteristics and the composition of tumor, tumor draining lymph node (TDLN) and spleen immune subsets in a syngeneic model of breast cancer developed orthotopically with LM3 cells. Mice lacking H4R showed reduced CD4+/CD8+ ratio and increased percentage of NK cells in lymph nodes compared to WT mice ($p<0.05$, $n= 5$). To evaluate the role of H4R in antitumor immunity we analyzed the distribution of the cell subsets at 21 and 28 days post-tumor inoculation (p.i.). At day 21 no significant changes were observed neither in tumor weight nor in tumor infiltrating lymphocytes (TILs). TDLN from H4R-KO mice showed decreased percentages of CD4+ T cells and T regulatory cells (CD4+CD25+FoxP3+) and increased CD8+ cells. This was accompanied by increased percentage of NK cells and decreased myeloid derived suppressor cells in spleens from H4R-KO compared to WT mice evident only at day 28 p.i. of LM3 cells ($p<0.05$, $n= 6$). Importantly, tumor-bearing KO mice showed a better survival compared to WT mice at 38 days p.i. We conclude that H4R is a key mediator of immune cell responses, participating in breast cancer antitumor immunity.

0644 - NEUTROPHIL EXTRACELLULAR TRAPS (NETS) DIFFERENTIALLY MODULATE THE GROWTH OF BREAST CANCER CELLS BT474 IN 2D AND 3D CULTURES

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After certain stimuli, polymorphonuclear neutrophils (PMNs) can release neutrophil extracellular traps (NETs) that trap tumoral cells (TC) modulating their growth. The aim of this project was to study whether BT474, a HER2+ human breast cancer cell line, induces PMNs to release NETs, and if these networks modulate TC growth cultured in monolayer (2D) or as spheroids (3D). First, we studied whether BT474 monolayers and spheroids stimulated PMNs obtained from healthy donors. We observed by neutrophil elastase, histone specific antibodies and DAPI staining through fluorescence microscopy, that in both culture conditions the TC promoted the formation and release of NETs, and that these networks grouped together and covered the TC. NETs generated by the stimulation of PMNs with phorbol myristate acetate (PMA), as a positive control, were tested for cytotoxic activity on TC in 2D and 3D cocultures. After 72 hours, we observed a 47 % decrease in the viability ($p<0.001$) of BT4T4 monolayers while we observed a 20% increased cell viability in 3D by MTS:PMS assay ($p<0.01$). In addition, NETs (PMNs-PMA), enhanced spheroids size compared to control ($p<0.001$). Surprisingly, NETs (PMNs-PMA) affected the generation of spheroids employing a hanging drop by co-culture with TC. After 72 hours, the presence of these networks affected their spherical structure which became irregular and disaggregated, and sometimes, gave place to multiple smaller spheroids. We can conclude that BT474 cells can modulate the PMNs activation, promoting the release of NETs. In 2D culture, the NETs were cytotoxic to TC; while, in 3D cultures they promoted spheroids growth. BT474 spheroids may provide an accurate model to study the relationship between a subpopulation of the innate immune response in a 3D structure that mimics the organization of in vivo small avascular tumors.

0648 - NOVEL NUCLEAR LOCALIZATION OF MULTIDRUG RESISTANCE ASSOCIATED PROTEIN 4 (MRP4/ABCC4) IN HUMAN CANCER CELL LINES

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ININFA, UBA-CONICET

The member of ATP Binding Cassette (ABC) family, MRP4 is responsible for substance extrusion, including cAMP. It is typically located in the plasma membrane. MRP4 is overexpressed in pancreatic and hepatocellular cancers, high levels of MRP4 tend to correlate with poor overall survival in patients with pancreatic ductal adenocarcinoma (PDAC). Preliminary results have shown a strong nuclear MRP4 staining in PDACs tumors. Thus, the aim of this study was to determine the novel presence of MRP4 in the nuclei of human PDAC and hepatocellular cancer cell lines. Therefore, MRP4 western blot (WB) assays were performed using enriched nuclei, total membrane and cytosolic isolated fractions (50 µg/lane) obtained by ultracentrifugation of pancreatic BxPC-3 and hepatocellular HepG2 cancer cells. The enrichment was evaluated by histone, actin and GAPDH detection, respectively. Also, immunofluorescent colocalization of MRP4, GAPDH and DAPI was assessed in individual cells and isolated nuclear fraction by confocal microscopy. Our results show that MRP4 localizes in the nuclear fraction of both cancer cell lines, the nuclear/total ratio was 1.3 in BxPC-3 cells and 0.3 in HepG2 cells. Total MRP4 expression in BxPC-3 cells doubles that of HepG2 cells ($p<0.05$) while nuclear and membrane expression were 2.5 ($p<0.01$) and 5-fold ($p<0.001$), respectively compared to HepG2. Mrp4 and DAPI colocalization was confirmed by confocal microscopy in both cell lines and in the isolated nuclear fractions. Besides its typical plasma membrane localization, our findings demonstrate for first time the presence of this transporter in cells nuclei, specifically in pancreatic and hepatocellular cancer cells. Despite the nuclear role of MRP4 is still unknown, we speculate it could modulate the transport of molecules across the nuclear membrane such as cAMP, which is produced by the nuclear soluble form of the adenylate cyclase as a locally restricted signaling involved in CREB modulation.

0649 - HEMEOXYGENASE-1 EXPRESSION AND ROLE IN THYROID CANCER

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Thyroid cancer (TC) is the most common worldwide endocrine tumor. Hemeoxygenase-1 (HO-1) is a microsomal enzyme that catalyzes the degradation of the heme group. Our laboratory, among others, has shown that HO-1 is related to tumor progression. The aim of this work was to study the expression and role of HO-1 in TC. In silico studies showed overexpression of HO-1 in papillary ($p<0.001$) and anaplastic ($p<0.001$) TC compared to non-tumoral thyroid tissue. In TC biopsies, overexpression of HO-1 was found in the tumor respect to non-malignant areas to the tumor (NMT) (Mann Whitney test, $p= 0.0066$; $n= 45$) by IHC. In tumor tissue, HO-1 was expressed in the cytoplasm while, in NMT, HO-1 nuclear expression was found. In addition, a positive correlation between HO-1 expression and tumor size (Chi-square, $p= 0.025$, $n= 33$) was observed. The IHC of fine needle aspiration biopsies samples confirmed HO-1 cytoplasmic expression. Tumor and normal primary cultures (PC) from a patient were established and the expression of HO-1 was evaluated by IF and WB. Hemin treatment of these primary cultures induced cytoplasmic expression of HO-1 in tumor (TPC) and nuclear expression in normal cell cultures (NPC) compared to vehicle-treated cells. Furthermore, induction of HO-1 with hemin (40-80 µM) induced an increase in cell viability in TPC ($p<0.0001$) and in NPC ($p<0.05$) at 96 h. In the TCP-1 thyroid tumor cell line, hemin 40 µM induced an increase in cell viability at 48h ($p<0.05$); but in 8505C thyroid tumor cell line, hemin treatment did not show differences in cell viability compared to its vehicle. Moreover, in both thyroid tumor cell lines, 40 µM hemin induced an increase in cell migration ($p<0.05$). This preliminary results show that HO-1 is strongly overexpressed in TC and that it could be implicated in tumor progression.

0650 - ANTITUMOR EFFICACY OF RACOTUMOMAB IN COMBINATION WITH PD-1 CHECKPOINT BLOCKADE IN A PRECLINICAL MODEL OF NON-SMALL CELL LUNG CANCER

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N-glycolylneuraminic acid (NeuGc) is a sialic acid molecule present in mammalian cells as terminal constituents of membrane glycoconjugates such as gangliosides and glycoproteins. In humans, NeuGc-containing gangliosides are widely expressed in certain aggressive neoplasms, including non-small cell lung cancer (NSCLC), and associated with an adverse prognosis. Racotumomab is an anti-NeuGc anti-idiotype monoclonal antibody that has been approved in Latin American countries as maintenance immunotherapy for advanced NSCLC. Combinatorial approaches involving long-term immunization against tumor-specific antigens together with the anti-PD1 immune-checkpoint inhibitor pembrolizumab is an attractive strategy. In this work, we have examined the antitumor activity of racotumomab in combination with anti-PD1 therapy, using the 3LL Lewis lung carcinoma as a preclinical model of NSCLC in C57BL/6 mice. Immunization with either weekly or biweekly s.c. doses of racotumomab at 50-200 µg/dose formulated in aluminum hydroxide (racotumomab-alum) demonstrated a significant antitumor effect against the progression of lung tumor nodules ($p<0.05$, ANOVA followed by Tukey). Similarly, checkpoint blockade with an anti-mouse PD1 monoclonal antibody injected i.p. at 200 µg/dose exerted a comparable antitumor effect in this 3LL lung model ($p<0.01$, ANOVA followed by Tukey). Interestingly, sequential administration of anti-PD1 therapy followed by repeated immunizations with racotumomab-alum was highly effective against lung nodules and well tolerated, showing a reduction in nodules formation of 62 and 45 % compared to anti-PD1 or racotumomab-vaccinated groups, respectively ($p<0.01$, ANOVA followed by Tukey). Our preclinical data provide support for the combination of anti-PD1 checkpoint blockade with the anti-idiotype monoclonal antibody racotumomab in advanced NSCLC, since combination treatment has a significant additive antitumor effect compared to each individual treatment.

0651 - CISPLATIN CHEMORESISTANCE INCREASES STEMNESS PROPRIETIES OF HUMAN NON-SMALL CELL LUNG CANCER

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Non-small cell lung cancer (NSCLC) is the most frequent lung malignancy in the world. Currently, cisplatin is the standard therapy for this disease, although there is a frequent acquisition of resistance. The presence of cancer stem cells (CSC) is associated with the above-mentioned resistance, and the retinoic acid system, among other systems, has been implicated in the maintenance and expansion of CSC. In order to evaluate the involvement of retinoic acid (ATRA) in growth modulation in a chemoresistance context, we have developed a cisplatin-resistant variant from NSCLC A549 cell line (A549cpr). Monolayers of parental and cisplatin-resistant A549 cells were treated with ATRA (0.3 - 70 µM) and/or Cisplatin for 72 h. While the Cisplatin treatment IC50 (6.87 ± 1.04 and 18.08 ± 1.03 µM for A549 and A549cpr, respectively) induce growth inhibition, ATRA addition did not modify proliferative capacity. Although both cell lines express all nuclear retinoic acid receptors, A549cpr cells showed lower levels of the RARβ isotype, involved in differentiation (determined by RT-qPCR). Moreover, ATRA treatment (1 µM) increased RARβ levels in both A549 variants, indicating that retinoic system is active. Finally, we studied the CSC component of A549 and A549cpr cells through an oncosphere culture. Although no differences were observed in oncosphere formation capacity, A549cpr cells present larger oncosphere colonies. This phenomenon was accompanied with higher Nanog mRNA expression levels, involved in cell pluripotentiality. In all cases, Nanog expression was higher in oncospheres than in the respective monolayers. Our results reinforce the hypothesis that cisplatin resistance may be mediated by an increase in CSC renewal. These findings lead us to propose different combination therapies for targeting CSC.

0666 - CFTR IS INVOLVED IN COLORECTAL CANCER STEM CELL PHENOTYPE

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CFTR is a chloride channel expressed in many epithelial cells and there is a relationship between this channel and cancer. The cancer stem cells (CSC) are responsible of tumorigenesis, secondary focus

formation in metastasis and chemoresistance. We previously found that CFTR is expressed in colorectal cancer cells HCT116 and is associated with cancer stem cells (CSC). To further investigate this, we inhibited CFTR activity with CFTR inhibitor-172 or downregulated CFTR expression with two different shRNA (shCFTR1 or shCFTR2) in HCT116 cells. When these cells were transfected with the shCFTRs, we observed a downregulation of both CFTR protein and mRNA by Western blot, immunofluorescence and RT-PCR. Then we determined the clonogenic growth, a property of CSC, of control HCT116 cells and when CFTR is inhibited after seven days of platting at low density. The number of colonies was diminished in cells where CFTR was inhibited ($p<0.05$). Next we analyzed the expression of CSC markers in these cells. A lower amount of the colon cancer stem cell marker CD133, Nanog, c-Myc and Oct-4 were detected by immunofluorescence staining of HCT116 cells when CFTR is inhibited. From these and our previous results we confirmed that the channel CFTR expression and activity are associated with CSC, indicating that CFTR could have an influence on the CSC properties at least in human colorectal cancer.

0682 - RUNX1 PARTICIPATION IN CHEMOTHERAPY RESISTANCE ON TNBC

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Triple negative breast cancer (TNBC) is associated with early recurrence and low survival rates. Treatment options are limited due to the lack of specific therapeutic targets and are consequently managed with chemotherapy. This highlights the urgent need for new specific therapeutic targets for this group of patients. TNBC is associated with epithelial-mesenchymal transition (EMT) and enrichment in breast cancer stem cell population. Growing evidences strongly suggest that EMT might be involved in tumor chemoresistance. Our group has shown that RUNX1 could be involved in the aggressiveness of ER-/PR- breast tumor. We reported that RUNX1 is able to promote cell migration and regulate tumor related-gene expression, like RSPO3 and GJA1, in a FOXP3-dependent manner. ChIP assays done in our lab on MDA-MB-231 cell line revealed that RUNX1 has the potential to regulate other transcription factors, such as SOX4, involved in EMT process. Besides, we observed a significant up regulation of RUNX1 gene expression in murine tumor cell lines treated with TGFß. Moreover, RUNX1 has been reported to correlate with poor patient prognosis in human samples of TNBC. Our hypothesis is that RUNX1 promotes EMT in TNBC cells, which make them become chemoresistant while leading metastasis to distant organs. The aim of this study was to investigate RUNX1 participation in the generation of chemotherapy resistance in TNBC. To do this, we used TNBC cell lines, doxorubicin (a clinically used drug) and loss of RUNX1 function assays. Here we show that RUNX1 ($p= 0.0067$) and GJA1 ($p<0.0001$) gene expression are significantly up regulated in doxorubicin-treated MDA-MB-231. Interestingly, we observed that loss of RUNX1 transcriptional activity strongly enhance doxorubicin toxicity on MDA-MB-231 ($p<0.05$), showing an improvement in drug's sensitivity. Therefore, RUNX1 may be involved in TNBC chemotherapy resistance, pointing out this transcription factor as a possible new therapeutic target in TNBC.

0729 - A COMBINATION OF IN SILICO AND WET-LAB STRATEGIES TOWARDS THE IDENTIFICATION OF BIOMARKERS OF ENDOMETRIAL CANCER PROGRESSION AND AGGRESSIVENESS

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Endometrial cancer (EC) is the 2nd most frequent gynecological neoplasm, expecting a > 50% increase in the next 20 years. Current diagnosis is based on abnormal uterine bleeding, transvaginal ultrasound or uterine biopsy or curettage with or without hysteroscopy. Final diagnosis and tumor classification is done during hysterectomy. Although highly relevant, there are no established EC molecular biomarkers. Objective: Combine bioinformatics tools to identify potential biomarkers of EC progression and aggressiveness and test their expression in EC cell models. An in silico and wet-lab basic research study was conducted. Bioinformatics: DisGeNET (text mining), GEO, TCGA, HPA databases (data mining), ToppGene (gene priorization), and Statistics: Kaplan-Meier Method, Odds Ratio and Cox Proportional Risk Model (Statistical models) approaches. Cell culture, end-point/Q-RT PCR. Data base analysis of a transcriptomic study (GSE17025; GEO platform) from EC samples identified 39 differentially-expressed genes, based on comparing tumor versus non-tumor; ECC versus NEEC, Grade 1,2 versus 3. Text database (DisGeNET) analysis identified 962 EC-associated genes. Gene prioritization analysis (ToppGene) selected 33 genes. Genes were subsequently assessed in an EC TCGA dataset and evaluated using clinical parameters statistical models ($p<0.05$), selecting 6 genes. Four genes were finally selected based on ToppGene prioritization and HPA data: PLEKHH1, PTCH1, TMPRSS2 and TPX2. Their differential expression was confirmed in EC cell models of aggressiveness (Hec1a-ETV5 and Ishikawa-ETV5) and controls (Hec1a and Ishikawa) (Colas et al, Oncogene 2012). Strategies combining bioinformatics and cell culture/molecular biology approaches identified potential biomarkers of EC progression and aggressiveness. Current studies are assessing their expression in EC patient samples to validate their clinical use.
Note: EC cells provided by Dr. Colas et al. (Vall d'Hebron Research Institute; Barcelona, Spain. European Comm - 7th Framework Project-IRSES).

0730 - TRIPLE NEGATIVE BREAST CANCER MOLECULAR MARKERS IN MCF7 CELLS OVEREXPRESSING A HUMAN EPITHELIAL CADHERIN SPLICE VARIANT

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Breast cancer (BC) is the most common cancer in women worldwide and in Argentina. Alterations in Epithelial cadherin (Ecad) expression/functions are associated with BC progression. We identified a novel human Ecad alternative splice variant (Ecadvar) (delta34DEL-Exon14). MCF7-Ecadvar stable transfectants showed epithelial-to-mesenchymal transition (EMT) changes, and a triple negative (TNBC) profile. Objective: Characterize transcript expression of MCF7-Ecadvar cells and determine similarities with TNBC molecular subtypes. Prospective basic research study using cell culture, molecular biology and bioinformatics approaches. MCF7-Ecadvar and control (MCF7-pcDNA3) cell culture; RNA extraction; end-point and Q-RT PCR. DisGeNET (text mining), STRING (network analysis), GEO database (data mining). MCF7-Ecadvar cells mRNA analysis confirmed an EMT molecular phenotype, with lower ($p<0.05$) levels of wild type E-cadherin, and higher ($p<0.05$) levels of Twist, Slug, Snail, Zeb1 transcriptional repressors, Vimentin and N-cadherin mesenchymal markers than control. MCF7-Ecadvar cells also had lower ($p<0.05$) levels of ER(alpha), PR and HER-2 receptor mRNA, but high ER(beta) levels. Moreover, they showed increased ($p<0.05$) levels of FXYD5/Dys, ACSL4, LDHB, MCT1, MCT4 and Anx2 mRNA TNBC markers, but lack mRNA of MUC1 and Kaiso TNBC-basal like subtype markers. Also, they displayed lower ($p<0.05$) levels of Claudin-3, -4 and -7 TNBC-Claudin-Low markers. All 22 evaluated genes were listed in DisGeNET (term "breast cancer"; CUI=C0006142 & C067822) and 12 in TNBC. STRING identified genes involved in BC, adherent/tight junctions, cell-cell adhesion. 12/22 genes depicted same expression

changes found in Claudin-low TNBC samples (GSE18229 database; Prat et al, 2010). Conclusions: MCF7-Ecadvar cells express markers of Claudin-Low TNBC. We propose the use of this cell model to identify TNBC novel biomarkers and therapeutic targets.

0925 - ANTITUMOR PROPERTIES OF TWO COPPER BASED COMPOUNDS AGAINST 2D AND 3D HUMAN COLORECTAL CANCER CELL MODELS.

Maria Carolina RUIZ(1) | Karen PERELMUTER(2) | Mariela BOLLATI-FOGOLIN(2) | Ana Laura DI VIRGILIO(1) | **Ignacio Esteban LEÓN** (1)

CEQUINOR (1); INSTITUT PASTEUR MONTEVIDEO (2)

Many functions of metal ion have stimulated the development of new metallodrugs. The synthesis of new copper complexes is potentially attractive as anticancer agents; whose properties are determined by the nature of ligands bound to the metal ion. This study evaluates the action of two cooper complexes, CuII(dmp)2(CH3CN)](ClO4)2 (1) and CuII(phen)2](ClO4) (2), against human colorectal cancer cells. An in vitro cytotoxicity assay was carried out on cultured HT29, Caco-2 and LS174T cell line monolayers. To get insight over the mechanism of action, we studied the role of ROS generation, using DHR123 probe and the mitochondrial membrane potential (MMP) with DiOC6. The migration process was investigated with gelatin zymography as well. Moreover, apoptosis was studied with anexinV/IP and caspase 3 assays by flow cytometry, adding studies of morphological changes with fluorescent microscopy. Furthermore, the cytotoxicity was studied with IP on 3D cell model derived from HT29 cells. In addition, NF-κB pathway suppression was investigated. Both complexes caused significant cytotoxicity in all cell lines, proving that 1 is more active (IC50 values for HT29 are 1.45 vs. 2.76 µM, for Caco-2 2.32 vs. 6.48 µM, for LS174T 1.44 vs. 2.54 µM for 1 and 2, respectively). It can be noticed that 1 increased ROS in all cell lines and the MMP decreased with the 24 h-treatment. Flow cytometric analysis revealed that theses complexes induce apoptosis in a dose and time dependent manner. These results are validated by microscopy. Complex 1 also attenuated the secretion of the metalloproteinases 2 and 9. On cell spheroids the IC50 values were 18.32 µM for 1 and 19.12 µM for 2. Interestingly, both complexes decrease the NF-κB expression in cell monolayer and spheroids, showing an inhibition of this pathway. In conclusion, both compounds display antitumor activity; however 1 was more effective in monolayer and 3D model than 2, being a candidate for in vivo experiments.

Metabolismo y Nutrición/ Metabolism and Nutrition IV

Chairs: Luz Andreone | Mariana Tellechea

0121 - RAT MATERNAL INSULIN RESISTANCE IS ASSOCIATED WITH ABNORMAL NEUROBEHAVIORAL RESPONSE IN OFFSPRING

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It is well established that maternal diet and metabolic state during pregnancy contributes to the risk of metabolic disease in offspring. Furthermore, recent epidemiological evidence suggests that gestational factors such as increased maternal obesity and impaired glucose metabolism can likewise affect offspring neurodevelopment, increasing the risk of neuropsychiatric disorders. We aimed to investigate the influence of long-term maternal fructose intake during preconception, gestation and lactation periods on neurobehavioral development of rat offspring. Wistar rats received either 10 % fructose enriched water or regular tap water for 20 weeks before and during gestation and through lactation. On P21, all littermates were separated and housed with ad libitum access to standard food and tap water. Control and fructose-fed mother's blood samples were collected for biochemical analysis. Offspring behavior was evaluated using open field, social interaction, marble burying and T-Maze task performance tests. Data analyses were carried independently on male and female rats. Dams fed with a 10 % (w/v) beverage containing fructose showed a moderated body weight gain and significant increments in fasting glucose level, oral glucose tolerance test (OGTT) and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index. Behavioral evaluation of the offspring revealed that females exposed to maternal fructose intake were prone to have increased number of marble buried and reduced learning performance in a T-maze compared to control-diet offspring. Our findings indicate that chronic maternal metabolic stress induced by a fructose-rich diet during pre-gestational, gestational and lactational periods showed a gender-specific increase in stereotyped repetitive behavior and working memory tasks in the offspring.

0351 - CONTRIBUTION OF SPECIFIC RISK FACTORS IN ELDERLY: A COMMUNITY EXPERIENCE

María Del Rosario CUETO (1) | Mariana PENNISI(1) | Ines FERNÁNDEZ(1) | Cristina POSSIDONI(2) | Sergio GIORDANENGO(2) | Gabriel TARDUCCI(3) | Silvina Mariela VIDUEIROS(1) | Anabel PALLARO(1)

CÁTEDRA DE NUTRICIÓN. FACULTAD DE FARMACIA Y BIOQUÍMICA. UNIVERSIDAD DE BUENOS AIRES (1); HOSPITAL SAGRADO CORAZÓN DE JESÚS DE BASAVILBASO (2); UNIVERSIDAD NACIONAL DE LA PLATA, FACULTAD DE HUMANIDADES Y CIENCIAS DE LA EDUCACIÓN, IDHICS CONICET (3)

Previous studies in a group of adults who belong to the Project for Protection of Vulnerable Population against Chronic Non-communicable Diseases (PROTEGER) of Ministry of Health, demonstrated that 65% of participants presented three or more associated risk factors, such as glycemia, cholesterol, triglycerides, blood pressure, fat mass and salt intake; being fat mass increased in 86 %. In this work, the aim was to assess less frequently evaluated risk factors in a group of older adults such as fat mass and salt intake. A descriptive study was conducted in 14 women (W) (75.3 ± 9.9 y) and 10 men (M) (77.1 ± 8.8 y) who had signed an informed consent. These subjects had either attended the Primary Health Care Center Pueblo Nuevo of Basavilbaso (Entre Ríos province) or reside at nursing homes for elderly in Buenos Aires province. Body weight (BW, kg) and height (H, m) were determined to calculate body mass index (BMI= BW/H^2, kg/m^2). Fat-free mass (FFM, kg) was evaluated by deuterium dilution technique and fat mass (FM, kg) was obtained as FM= BW-FFM. Sodium, potassium and creatinine excretion were determined from spot urine and the 24 h urinary sodium excretion was estimated using the INTERSALT equation. The results showed that 93% of W and 50% of M were overweight or obese. Moreover, FM % was increased in 78.6 % of W (38.1 ± 9.3) and 60 % of M (24.1 ± 10.8). In addition, 58.3 % of participants presented salt intake higher than the WHO recommendation of 5 grams/day (6.4 ± 2.9) with a decreased K/Na ratio (0.8 ± 0.7) in 79.2 %. An increase in fat mass and salt intake was observed in this group of older adults and findings showed that both risk factors were presented in 50 % of the cases. The assessment of these less frequently evaluated risk factors would contribute as a tool for better diagnosis of the chronic non-communicable diseases.

0356 - NOVEL FMO3 MUTATIONS INVOLVED IN TRIMETHYLAMINURIA DISORDER

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Trimethylaminuria is a human autosomal recessive disorder due to mutations in the gene of the enzyme flavin-containing monooxygenase 3 (FMO3). In this condition, odorous trimethylamine (TMA) cannot be converted into its non-odorous N-oxide. Consequently, TMA accumulates and is excreted through urine and sweat being responsible of the characteristic malodor of the patients. Clinical diagnosis is based on the quantification of TMA in urine samples and is confirmed by genetic analysis which additionally contributes to risk stratification. None of them are performed in Argentina, where diagnosis is based on the body odor. The aim of this case study was to correlate the clinical diagnosis with genetic variants of FMO3. The index patient is a female (12 y.o.) with fishy malodor since she was a toddler and with an exacerbation of the condition since puberty. Symptoms are only controlled by strict diet restriction. The patient and unaffected direct relatives were analyzed to build up and evaluate the genetic pedigree. gDNA was obtained from jaw swabs. We used specific primers designed for the coding exons (2 to 9) to amplify them by PCR followed by sequencing. The mutant enzyme activity was tested using heterologous expression. The index case beard 2 SNPs of the FMO3 gene: c.472G>A (E158K) and c.923A>G (E308G). She also carried two novel mutations resulting in amino acid substitutions: P73L and F140S. All of them were in heterozygosity. The mother carried the P73L/E158K/E308G allele and the father the F140S allele. Functional analyses showed a 50 and 90 % decrease of the catalytic capacity for the mother and the father alleles, respectively (n= 3). Based on the family pedigree, we identified a compound heterozygous patient for the two novel point mutations. Functional analysis demonstrated a drastic reduction in enzyme activity for each allele, which combined with the already known changes promote the severe condition exhibited by the patient.

0428 - DECREASED HDL-MEDIATED CHOLESTEROL EFFLUX AND ANTIOXIDANT ACTIVITY IN OBESE CHILDREN AND ADOLESCENTS

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The presence of obesity during infancy is associated to the development of related complications such as dyslipidemia, a well-known risk factor for cardiovascular disease. High density lipoproteins (HDL) represent the only antiatherogenic fraction and are responsible for the promotion of cholesterol efflux from macrophages and the inhibition of low density lipoprotein (LDL) oxidation. The aim of present work was to evaluate HDL capacity to promote cellular cholesterol efflux and the activity of two HDL-associated proteins, cholesteryl ester transfer protein (CETP) and paraoxonase 1 (PON1), in 25 obese children and adolescents and 20 healthy controls. There were no differences in age or sex between the groups. Triglycerides (TG), total cholesterol (TC), HDL-C, LDL-C, apolipoproteins (apo) A-I and B, glucose and insulin were measured by standardized methods. HOMA-IR was calculated. The human monocyte line THP-1 was employed to determine cellular cholesterol efflux. CETP was analysed by a radiometric method and PON1 employing two substrates: paraoxon (PON activity) and phenylacetate (ARE activity). The obese children and adolescents showed higher triglycerides, LDL-C and HOMA-IR ($p<0.01$), in addition to lower HDL-C and apo A-I levels. Both cholesterol efflux (6.0 ± 1.6 vs. 7.6 ± 2.1 %; $p<0.01$] and ARE activity (94 ± 12 vs. 103 ± 19 µmol/ml.min; $p<0.05$) were significantly lower in the obese group compared to healthy controls. Cholesterol efflux correlated with BMI-z (r= -0.43; $p<0.05$) and TG (r= -0.28; $p<0.05$). ARE correlated with HDL-C (r= 0.38; $p<0.05$), apo A-I (r= 0.37; $p<0.05$) and PON (r= 0.48; $p<0.05$). Obese children and adolescents presented insulin resistance and a more atherogenic lipid profile in addition to lower capacity to promote cellular cholesterol efflux and ARE activity, a reflex of HDL antioxidant capacity. These findings would be indicative of functionally altered HDL particles with decreased atheroprotective potential.

0434 - ABNORMAL HDL QUALITY AND CAPACITY TO ACQUIRE PHOSPHOLIPIDS IN PATIENTS WITH RHEUMATOID ARTHRITIS: EFFECT OF TOFACITINIB AND TOCILIZUMAB.

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INFIBIOC. FAC. FARMACIA Y BIOQUÍMICA. UNIVERSIDAD DE BUENOS AIRES (1); HOSPITAL ITALIANO DE BUENOS AIRES (2); INSTITUTO DE REHABILITACIÓN PSICOFÍSICA - IREP (3); INSTITUT NATIONAL DE LA SANTÉ ET DE LA RECHERCHE MÉDICALE - INSERM (4)

Background: Patients with rheumatoid arthritis (RA) have high risk of cardiovascular disease, which may be due to dysfunctional HDL. The anti-inflammatory agents tofacitinib (janus kinase inhibitor) and tocilizumab (antibody against interleukin 6 receptor) have shown therapeutic efficacy. The aim of our work was to evaluate lipids, HDL characteristics and HDL capacity to acquire phospholipids (PL) from lipolysis of triglyceride-rich lipoproteins (TGRL), and the effect of tofacitinib and tocilizumab in RA patients. Sixteen RA female patients and 4 controls were recruited from the Rheumatology Service, BA Italian Hospital. Eight patients were treated with tofacitinib and 8 with tocilizumab. HDL subfractions were isolated by density gradient ultracentrifugation and their components were measured with commercial kits. HDL fractions were incubated with TGRL, lipoprotein lipase, and the fluorescent TopF to evaluate HDL capacity to acquire PL. Results were analyzed employing the statistical package SPSS 17.0. Plasma triglycerides (TG) were higher in RA patients than in controls, with non-significant lower HDL-C. HDL was enriched in TG and total proteins, and depleted in free and esterified cholesterol (FC and EC). In turn, TG content was higher and EC lower in HDL2b, HDL2a and HDL3a. HDL capacity to acquire PL was reduced in patients as compared with controls [56 (51-59) vs. 102 (63-104) %, respectively; $p<0.05$]. Moreover, treatment with tofacitinib or tocilizumab produced no considerable changes in lipids or lipoprotein composition. However, HDL capacity to bind released PL increased at 3 and furthermore at 6 months after treatment with tofacitinib, and at 6 months with tocilizumab. Conclusion: RA patients displayed HDL with abnormal subfraction distribution and chemical composition, which probably conditioned the deficiency to acquire PL from lipolysis of TGRL. This altered antiatherogenic function of HDL was reversed by treatment with both tofacitinib and tocilizumab.

0450 - LIPID PROFILE AND HDL-ASSOCIATED PAROXONASE IN CHILDREN WITH CELIAC DISEASE. IMPACT OF GLUTEN FREE DIET

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There is growing evidence, both in adults and children, that celiac disease (CD) could be associated with non-identified atherogenic risk factors and, consequently, with cardiovascular disease. In this context, high density lipoproteins (HDL) constitute the only antiatherogenic fraction, being the inhibition of low density lipoprotein (LDL) oxidation one of its main functions. This capacity depends on several HDL-associated components, such as the antioxidant enzyme paraoxonase (PON1). Our objectives were: 1) To assess the relationship between CD and the presence of atherogenic risk factors in recently diagnosed children compared to healthy controls, and 2) the effect of gluten-free diet (GFD). The study included 22 CD children and 22 controls. Eleven CD children completed 6 months of GFD. Triglycerides (TG), total cholesterol (TC), HDL-C, LDL-C, apolipoproteins A-I and B, glucose, haematological parameters, high sensitive C reactive protein (hsCRP), folic acid and vitamin B12 were measured by standardized methods. PON1 activity was evaluated employing two substrates: paraoxon (PON activity) and phenylacetate (ARE activity). There were significant differences in age between CD children and controls, so statistical analyses were performed adjusting by age. The CD children showed lower HDL-C, plasma iron, tranferrin saturation, folic acid and ARE activity ($p<0.05$) compared to controls. Regarding the longitudinal analysis, both HDL-C and PON1 activity increased while TG decreased after 6 months of GFD ($p<0.05$). Interestingly, after treatment HDL-C achieved by CD children resulted to be similar to the levels observed in control subjects. Conclusions: Children showed a more atherogenic lipid profile and lower ARE activity compared to healthy controls. These alterations were partially reversed after GFD, indicating a potentially beneficial effect of this treatment on both traditional risk factors and markers of HDL quality and functionality.

0671 - EFFECTS OF BODY CONDITION SCORE IN LIVER PROINFLAMMATORY STATE AND INSULIN-SIGNALING OF DAIRY CATTLE DURING THE TRANSITION PERIOD

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The transition period (last 3 weeks of prepartum until 3 weeks postpartum) is the most critical stage in the lactation of a dairy cow, characterized by lipid mobilization, proinflammatory state and insulin resistance during peripartum. The aim of this study was to evaluate the insulin-signaling pathway and certain proinflammatory factors in the liver and metabolic biomarkers in plasma during the transition period in cows with different body condition score (BCS). Sixteen grazing dairy cows from a commercial dairy farm were classified according to BCS using the 5-point scale as high BCS (HBCS, >3.5, n = 8) and low BCS (LBCS, < 3.5, n = 8). Blood and liver biopsies were sampled at -14, 4, 14 and 28 days relative to parturition. The concentrations of non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), glucose, triglycerides, insulin and liver triglycerides content were spectrophotometrically measured. Also, liver protein expression of relevant insulin signaling components: insulin receptor (IR), insulin receptor substrate 1 (IRS-1), total and phosphorylated protein kinase B (Akt and p-Akt); along with tumor necrosis factor alpha (TNF-alpha), interleukin-6 (IL-6), and nuclear factor-kB (NF-kB) were measured by Western blot. The obtained results shown that cows with HBCS had greater BHB concentration than LBCS ($p<0.05$); an interaction effect (BCS x time, $p<0.05$) for NEFA and TNF-alpha was determined; protein abundance of TNF-alpha was greater at day 4 postpartum in HBCS group ($p<0.05$). In addition, cows with HBCS had a tendency to have a greater p-Akt/Akt than those with LBCS ($p= 0.08$). These results suggest that the proinflammatory state in liver of dairy cows with HBCS during postpartum could affect the insulin signaling in agreement with reports of other researchers. This knowledge could help to understand the metabolic behavior and to optimize the health and milk production of dairy cattle during this period.

0701 - EFFECT OF PROANTHOCYANIDIN ENRICHED FRACTION OF LIGARIA CUNEIFOLIA (PLC) ON PLASMATIC LIPID PROFILE OF WISTAR RATS FED WITH A HIGH FAT DIET (HFD)

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Previously, our group demonstrated that Ligaria cuneifolia (Lc) crude extract increased blood viscosity and decreased plasma cholesterol levels in rats. In the present study, we analyzed the effect of PLc on plasmatic lipid profile in adult male Wistar rats hyperlipemic. Rats aged 70 days were fed with: standard diet (Control, C; n= 5), standard diet added with 40 % bovine meat juice during 28 days (HFD; n= 10). At the end of this time, the rats were injected i.p. each 24hr either with physiological solution (HFD, n=5, and also the C rats) or PLc 3mg/100g body weight HFD only (HFD+PLc; n= 5) during 7 days. At day 8 after treatment, rats were anaesthetized i.p. with Ketamine/Xylazine (100mg/kg and 3mg/kg, respectively) to obtain blood samples by cardiac puncture. Plasma assays: Cho (enzymatic method with cholesterol oxidase esterase) and TG (enzymatic methods) Plasma (mean ± SE): Cho (mg%): C: 138.8 ± 14.8 HFD: 203.3 ± 21.5*; HFD+PLc: 119.5 ± 28.5#; TG: C: 170.7 ±18.5 HFD: 274.7 ± 30.7*; HFD+PLc: 190.50 ± 21.65# (*$p<0.05$ vs. C; #$p<0.05$ vs. HFD). Additionally, we evaluated the total plasmatic free fatty acids (FFA) (Derivatized plasma samples were then analyzed by GC–MS) and plasmatic levels of some polyunsaturated fatty acids (PUFAs), expressed as % of Control, mean ± SE. FFA: HFD: 882.12 ± 306.27; HFD+PLc: 114.36 ± 15.72# Linolenic Acid C18:2: HFD: 59.0 ± 18.1; HFD+PLc: 82.6 ± 10.8. Arachidonic Acid C20:4: HFD: 61.7 ± 5.7; HFD+PLc: 104.7 ± 20.7 and Docosahexanoic Acid C22:6: HFD: 55.8 ± 10.7; HFD+PLc: 122.6 ± 14.2# (#$p<0.05$ vs. HFD). Our results showed that the treatment with PLc has a lipid-lowering effect in rats fed with HFD. Interestingly, we found that PLc treatment was able to restore the altered HFD-derived plasma levels of some PUFAs that are direct or indirectly associated with an obesity-related modificated lipid profile. In conclusion, we have obtained a fraction of the crude extract of Lc, which lowers the total Co and TG in plasma and restore plasma free fatty acid profile after HFD.

Farmacología/ Pharmacology III

Chairs: Alicia Consolini | Jerónimo Laiolo

0173 - DRUG COMBINATION ANALYSIS OF COMPOUNDS WITH POTENTIAL SYNERGISTIC EFFECTS IN A PRECLINICAL MODEL OF DISSEMINATED RETINOBLASTOMA

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Patients with metastatic retinoblastoma receive an empirical and aggressive chemotherapy treatment but they still have a fatal outcome. Considering this malignancy like an unmet medical need, new therapies or alternative drugs are necessary. We were previously successful in identifying FDA approved drugs with cytotoxicity effect in a cell line derived from tumor dissemination in the lymph node using High-Throughput Screening. Now, we aimed to identify and evaluate drug combinations with a synergistic effect between active drugs previously selected using a homemade decision algorithm. We evaluated carboplatin-CB, vincristine-VC, panobinostat-PN and bortezomib-BT in a 3x2 concentration matrix at the IC10, IC25 and IC50 (drug concentration that inhibits 10, 25 or 50 % of cell proliferation) to determine the most promising combinations. To confirm the activity of the selected combinations, we evaluated the dose-response curves of the combination and compared the IC50s to those calculated for individual drugs according to the combination index (CompuSyn). Mean (range) IC50s for CB, PN, BT, and VC was 95.6 µM (94.2-97.6), 67.2 nM (74.2-47.7), 5.2 nM (5.2-3.2), and 62.6 nM (56.7-68.5), respectively. The IC50s for CB and PN combined with PN at the IC25 and BT at the IC50 were 70 µM and 8.2 mM, respectively. Both combinations presented a combination index <1 meaning a synergistic effect. In this study we managed to develop a rational design of drug selection to evaluate cytotoxic activity in preclinical models and identified two drug combinations with synergistic effects in a primary cell line derived from a patient with metastatic retinoblastoma. Synergistic effect was observed between CB-PN and PN-BT in vitro. These results are the first step for an upcoming evaluation in an animal tumor model.

0189 - P-GP MODULATING EFFECT OF THE PENTACYCLIC TRITERPENOID BETULIN, ISOLATED FROM LIGARIA CUNEIFOLIA, IN MULTIDRUG RESISTANT LEUKEMIC CELLS.

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Multidrug resistant (MDR) constitutes nowadays one of the major obstacle in cancer therapy, being the efflux of drugs by P-glycoprotein (P-gp) a predominant factor. Plants are recognized as a rich source of metabolites with structural diversity being an invaluable starting point for drug discovery. With the aim of finding promising compounds to modulate MDR phenotype mediated by P-gp, 120 native and naturalized plants collected in the hills of Córdoba, Argentina, were screened on K562 human myelogenous leukemia cell line and its MDR counterpart Lucena 1. Ligaria cuneifolia was one of the most active and thus it was subjected to bioguided isolation to separate the active principle responsible for its activity. This process yielded the triterpenoid betulin (1). Intracellular doxorubicin (DOX) accumulation was determined, at non-cytotoxic concentrations determined by MTT, using flow cytometry. Fluorescence intensity radio (FIR) was calculated as the ratio between the fluorescence intensity of each cell line treated and the fluorescence intensity of its respective solvent control. The activity was confirmed by the MTT reversal assay. Compound 1 increased the accumulation of DOX at a minimum effective concentration (MEC) of 1.56 µM (FIR 1.09 ± 0.04) and increased DOX cytotoxicity by a factor of 3.83 ± 0.26 at 12.5 µM and 1.29 ± 0.08 at 0.39 µM showing no differences with respect to the same concentrations of verapamil, used as positive control ($p \geq 0.05$). Compound 1 showed no effect at the MEC on K562 cells. This is the first report regarding compound 1 as an inhibitor of the efflux mediated by P-gp. This triterpenoid could arise as scaffold to obtain novel P-gp inhibitors to use in combination with anticancer drugs for the improvement of leukemia therapies.

0190 - STUDY OF THE ANTIANGIOGENIC EFFECT OF A TERTHIOPHENE ISOLATED FROM TAGETES MINUTA.

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Angiogenesis is an essential mechanism involved in biological processes such as reproduction, development and wound healing. Imbalances between the factors that regulate angiogenesis have been linked to different diseases that affect human health. Despite the great progress achieved in antiangiogenic therapy, its limited efficacy, severe adverse effects and the development of resistance demands the continuous development of novel therapeutic agents to overcome these obstacles. In this context plant-derived metabolites continue to play a highly significant role in drug discovery. From a screening performed in our laboratory, the ethanol extract of Tagetes minuta arose as a potent antiangiogenic agent. This effect was evaluated by the tube formation assay using bovine aortic endothelial cells (BAEC). Through bio-guided chemical fractionation three compounds identified as: 5´-methyl-5-(4-hydroxybut-1-ynyl)-2,2´-bithiophene (1), 5-(4-hydroxybut-1-ynyl)-2,2´-bithiophene (2) and alfa-terthienylmethanol (3) were obtained. Among these, compound 3 showed a potent antiangiogenic activity (IC50 = 2.69 µM). The influence of 3 over cell proliferation and cell invasion induced by the vascular endothelial growth factor (VEGF) was evaluated. While no effect was observed in the MTT proliferation assays, 3D transwell experiments demonstrated that compound 3 efficiently blocked cell invasion. Additionally, molecular docking experiments showed that 3 overlaps with the tyrosine kinase inhibitor sorafenib (a potent antioangiogenic agent) at the catalytic cleft of VEGF receptor 2 interacting with key aminoacids such as Glu885, Phe1047 and Cys919, extending over Val916 into the adjacent allosteric hydrophobic back pocket resembling a type II inhibition. These studies contribute to position 3 as a potential candidate to be used in antiangiogenic therapy itself or as a lead compound for the development of analogues with improved activity.

0375 - COMPARATIVE XENOBIOTIC BIOTRANSFORMATION IN PRECISSION-CUT LIVER SLICES FROM SWINE AND CATTLE

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Species differences in the pattern of metabolism of foreign compounds is a key issue for veterinary practitioners. Comparative studies on the in vitro hepatic metabolism of xenobiotics among different farm animals requires the use of consistent and robust methodologies. The aim of the current work was to validate the technique of precision-cut liver slices (LSs) for comparative studies on xenobiotic metabolism in swine and cattle. Swine (n= 3) and bovine (n= 6) liver samples were obtained from local abattoirs. LSs

(around 20 mg wet weight) were produced by operating a Brendel-Vitron™ tissue slicer filled with oxygenated ice-cold Krebs buffer. LSs were cultured (up to 6 h) in Williams' Medium E at 37 °C under a humidified atmosphere (95% O_2: 5% CO_2). Tissue viability was assessed by histopathology and the measurement of intracellular reduced (GSH) and oxidized (GSSG) glutathione. The assayed enzyme activities were: benzydamine N-oxidase (BZ N-ox, flavin-containing monooxygenase), ethoxyresorufin O-deethylase (EROD, cytochrome P450 1A1) and methoxyresorufin O-demethylase (MROD, cytochrome P450 1A2). The S-oxidation of the anthelmintic drugs albendazole (ABZ) and fenbendazole (FBZ) was also assessed. HPLC analysis was performed and the rates of appearance of the metabolites in the incubation medium were calculated. Similar BZ N-ox and EROD activities were observed in both species. Conversely, MROD was 2.6-fold higher ($p<0.05$) in swine (12 ± 9 pmol/h) compared to cattle (4.6 ± 2.5 pmol/h). The S-oxidation of ABZ was 1.5-fold higher ($p<0.05$) in swine (3.3 ± 0.7 nmol/h) compared to cattle (2.2 ± 0.5 nmol/h), but the opposite occurred for FBZ S-oxidation. In both species, as observed in other in vitro systems like liver microsomes, ABZ S-oxidation resulted 5-fold (cattle) and 13-fold (swine) higher ($p<0.05$) compared to FBZ S-oxidation. The work described here shows that LSs are a useful tool to obtain relevant information on species-specific differences in xenobiotic metabolism.

0493 - GA-RXODE METHOD, A NEW PROCEDURE WITH SEVERAL USES IN PHARMACOMETRICS

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Background: In the "Big Data era" it seems paradoxical that few drugs have a complete and available set of pharmacometric data. Combining two R written routines, "Genetics Algorithms" (GA) and "Running simulations from Ordinary Differential Equations based models" (RxODE), we have developed a procedure to improve those data from the available ones. The aim of present work was to describe and validate GA-RxODE method, an informatic instrument running in any computer, capable to optimize raw data in general. We have applied the method on pharmacokinetic (PK) raw data of clarithromycin sustained release formulation study and validated it by population PK modelling in SAEMIX, an R written non-linear mixed effects algorithm. Applying GA-RxODE we have obtained a final construct called token data or sim(ulated)-data. From reconstructed sim-data concentration-time curves of clarithromycin we have generated several population PK models: Complete Model (CM) including the original number of determinations (11) as the clarithromycin study, and Partial Models (PMs) containing 4 to 10 determinations. We have expressed the values as median (interquartile range) and used one- or two-way ANOVA to analyze them. The uncorrected-to-zero correlation between variances (raw concentration and the corresponding sim-data) exhibited a slope of 0.6026 ± 0.087; r^2= 0.842; $p<0.001$, indicating sim-data maintained the original structure with less variability. In addition, Cmax analysis, CM= 1.91 (1.54-2.28) mg/l vs PM with only 4 values= 1.73 (1.41-2.04) mg/l, was non-significant indicating a good agreement between models. Conclusions: By optimizing raw data, maintaining their structure and reducing their variability, the derived sim-data can be suitable to any further PK analysis. Furthermore, the procedure can be considered valid because the CM was non-different from PMs. This places GA-RxODE method as a proper tool to be applied in Pharmacometrics.

0635 - POPULATION PHARMACOKINETIC MODELING OF PYRIMETHAMINE (PYR) DURING THE TREATMENT OF CONGENITAL TOXOPLASMOSIS IN PEDIATRICS.

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Infection with Toxoplasma gondii, is one of the most widespread zoonoses in the world. Particularly risky is congenital form (CT) with a global risk of transmission by this route of about 40 %, reaching 90 % in the last month of pregnancy. Children with CT receive treatment, usually PYR and sulfadiazine, to prevent morbidity. The current therapy in pediatric patients is protocolized, but due to the absence of pediatric formulations the drugs are prepared in the hospital pharmacy in the form of syrup and at the moment, pharmacological parameters of these drugs have not been corroborated in patients, especially PYR. The objective of this study was to evaluate the pharmacokinetics of PYR in the treatment of pediatric toxoplasmosis, from a population approach (Pop-PK). The study was approved by the institutional ethics committee of the HNRG, including the informed consent for the use of the samples. Residual blood samples (taken for routine clinical procedures) were obtained from 12 pediatric patients undergoing treatment with PYR (0.77-2.7 mg/kg/day), and followed-up in the Parasitology service of the Ricardo Gutiérrez Children's Hospital (HNRG). PYR was quantified on 60 serum samples by high performance reverse chromatography coupled to tandem mass spectrometry (Shimadzu Nexera and Sciex qTrap6500). For the analysis of Pop-PK and the evaluation of pharmacokinetic models, Monolix® was used. Akaike´s information criteria and Bayesian information criteria were selected as statistical criteria for the selection of the best model. The results obtained in the Pop-PK modeling proposed a one-compartmental (Vp= 1.05 L) model with first-order absorption (Kap= 0.843 h^{-1}) and linear elimination (Kep= 0.00531 h^{-1}), with weight-dependent distribution volume (β= 0.349 L/Kg). Other more complex models did not result in an improvement in fit and were discarded. Pharmacokinetic studies reported for PYR that differ in populations, set of drugs used and treatment times, propose similar results when comparing Ka and Ke for the pediatric population, but were found to be significantly different from those evaluated for the adult population.

0742 - DIFLOXACIN PLASMA PHARMACOKINETICS, TISSUE DISPOSITION AND WITHDRAWAL PERIOD IN BROILERS

Bernardo SOBRECASAS(1) | Natalia URZÙA(1) | Jimena MESSINA(1) | Marìa TONINI(1) | Guillermo PRIETO(1) | Marìa Emilia ERRECALDE(1) | Carlos LUDERS(2) | Rosendo LIBOÀ(1) | **Carlos ERRECALDE** (1)

UNIVERSIDAD NACIONAL DE RÍO CUARTO (1); UNIVERSIDAD CATÒLICA DE TEMUCO (2)

Difloxacin (DFX) is a fluoroquinolone used only in domestic animals that develops bactericidal actions by selective blocking of the DNA-gyrase enzyme in G+, G- and mycoplasma microorganisms. The background of DFX in mammals indicates high tissue distribution. This study seeks to establish in broiler chickens, if plasma and tissue levels are equivalent and establish a withdrawal period (Pr) in different tissues. Chickens (n= 70) Cobb line, approximately 45 days old and 1.9 ± 0.2 kg weight, randomly selected from a stock of 1200 birds. 14 batches of 5 animals each were formed, who received an oral single dose of 20 mg/kg of DFX after fasting for 12 and 3 hours before and after application, respectively. Subsequently, each batch was sacrificed at pre-established times, samples of blood, by exanguination, and tissue samples were obtained until 120 hours post application. DFX was quantified by HPLC using C18 column, mobile phase consisting of water, acetonitrile and triethylamine (790: 200: 10 v/v/v) adjusted to pH 3.0 and fluorometer reading set at 295 nm excitation and 490 nm emission. In plasma and each tissue, DFX concentration averages by time were analyzed with the non-compartimental pharmacokinetic software PK Solution. Pr was

estimated with the WT1.4 software, according to the 300 µg/kg MRL established for muscle, skin and lung of, 600 µg/kg for kidney and 1,900 µg/kg for liver. Due to its fat-soluble characteristics and poor plasma protein binding, difloxacin oral application experiences rapid absorption, high tissue distribution and prolonged permanence in the body, generates ratios in muscle, skin, liver, kidney and lung versus plasma of 3.9, 3.1, 32.8, 166.3 and 7.7, respectively and a Pr of 3 days for muscle, liver and skin and 5 days for kidney and lung, compatible with the productive cycles of broilers.

0834 - ENROFLOXACIN DISPOSITION IN EDIBLE TISSUES OF RAINBOW TROUT IN DIFFERENT APPLICATION MODELS

Carlos ERRECALDE (1) | Natalia URZÙA(1) | Guillermo PRIETO(1) | Carlos LUDERS(2) | Jimena MESSINA(1) | Rosendo LIBOÀ(1) | Miguel MANCINI(1) | Carlos ERRECALDE(1)

UNIVERSIDAD NACIONAL DE RÍO CUARTO (1); UNIVERSIDAD CATÒLICA DE TEMUCO (2)

Enrofloxacin (EFX) is a second generation fluoroquinolone for veterinary use only. It is used in some countries as a therapeutic for diseases of productive interest. The objective of the study was to determine EFX concentrations in muscle and skin of rainbow trout, administered orally and in immersion baths at two different models and establish withdrawal period. Rainbow trout of 190 ± 25 g of weight were randomly selected, distributed in 3 batches of 45 fish each, batch A received an oral dose of 10 mg/kg, for the immersion bath, batch B was administered a dose of 20 mg/L for 2.5 hours and batch C 100 mg/L for 0.5 hours. Fishes were sacrified at different times from 0.25 to 120 hours post-application. Sample pretreatment was performed with trichloroacetic acid, mechanically homogenized and kept at rest for 12 hours, centrifuged and 150 µL of the supernatant was added with methanol, deionized water and internal standard, obtaining the solution to elute. A C-18 column, fluorescence detector and mobile phase composed of water, acetonitrile, triethylamine was used. The pharmacokinetic analysis was performed using the PK-Solutions 2.0 software and withdrawal time calculation was performed by EMEA W.T 1.4 program. In all batches, tissue concentrations of EFX were detected up to 120 hrs. In batch A concentrations were ≤ 1.7 µg/g in skin and muscle, while in batch B and C the maximum concentrations for muscle and skin were 0.5 and 0.7 µg/g respectively, reached at different times, and with a elimination half-life less than batch A. Withdrawal periods were 14 and 46 days in batch A, 4 and 18 days in batch B and 5 and 11 days batch C, for muscle and skin, respectively. According to the results, immersion baths provides therapeutic concentrations, and compatible withdrawal periods with the production cycle.

0969 - LIQUID BIOPSY FOR T790M DETECTION IN PATIENTS WITH NON-SMALL CELL LUNG CANCER IN A PUBLIC HOSPITAL IN CORRIENTES CAPITAL

Melina Noelia LORENZINI CAMPOS | Maria de Los Milagros SUSSINI | Maria Carla ZIMMERMANN

LABORATORIO DE MEDICINA GENOMICA Y MOLECULAR. FACULTAD DE MEDICINA. UNNE

Access limitations to an early diagnosis influences high rates of lung cancer mortality in the northeast region of Argentina. Lack of specialists and equipment plus long distances to medical centers that perform such studies are some of the reasons. Non-small cell lung cancer (NSCLC) constitutes 85% of all lung cancer cases and shows the highest mortality. Solid biopsy has been demonstrated to be unsafe for the patient and has low sensitivity to detect tumor dynamics, drug sensitivity and residual lesions. In addition, treatment has evolved into customized strategies based on histological and molecular subtypes. Due to T790M mutation in the EGFR gene is actionable, the objective of the present study was to optimize its detection in NSCLC by liquid biopsy in patients of the University Hospital in Corrientes. To this purpose, an observational cross-sectional study was performed in patients at any stage of NSCLC who attended to the pneumonology service between June 2018 and July 2019. Circulating DNA extraction was carried out using QIAmp® MinElute® ccfDNA kit. T790M was evaluated by performing AS-PCR and 2% agarose electrophoresis. As a result, positive control was amplified however none of the patients carried the mutation. Thus, it was possible to develop a method for T790M detection in Corrientes, which will be accessible to patients in the region.

Nefrología/ Nephrology

Chairs: Elisabet Oddo | Claudia Silberstein

0184 - DETECTION OF SHIGA TOXIN TYPE 2 BINDS TO MICROVESICLES IN THE PLASMA SAMPLE OF A PATIENT WITH SHIGELLOSIS

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LABORATORIO DE FISIOPATOGENIA, IFIBIO-HOUSSAY (UBA-CONICET), FACULTAD DE MEDICINA-UBA (1); HOSPITAL NACIONAL "PROF. DR. ALEJANDRO POSADAS" (2)

Shiga toxin type 2 (Stx2) are mainly produced by Shiga toxin–producing Escherichia coli (STEC) that it has been reported to be highly pathogenic and to be associated with hemorrhagic colitis hemolytic and uremic syndrome (HUS). At this moment, an early method of diagnosis of HUS is not exist. Our laboratory developed a method of detection of Stx2 bind to microvesicles. The aim was to detect plasma Stx2 bounded to microvesicles (MVs-Stx2) in a patient with Shigellosis and systemic complications. A 10-year-old girl was admitted to the hospital because of mucous diarrhea with bloody stretch marks over two days. Her laboratory admission showed normal hematocrit, hemoglobin and platelet count. Renal function was conserved without hematuria and proteinuria. Coproculture sample was positive for Shigella flexneri. Two days after admission, hematocrit (30.7 %), hemoglobin (9.8 g/dl) and platelet count (187,000/mm^3) were decreased. Take into account this results it was suspected a development of HUS. A peripheral blood smear was performed and did not show schistocytosis. We obtained blood samples in order to detect the presence of plasma Stx2. Samples were sequentially ultracentrifuged to obtain microvesicles (MVs)-enriched suspension. Then, MVs carrying Stx2 were analyzed by flow cytometry. Data are expressed as the percentage of positives MVs-Stx2. From the controls, a cut-off range for MVs-Stx2 was established (1.02-1.90 %, n= 5). A significant higher percentage of MVs-Stx2 (13.6 %) was detected. The patient was not present renal function alterations during this time. These results indicate that the systemic alterations observed in this patient could be explain by the effects of circulating Stx2, like happens in patients with incomplete HUS (absence of kidney disorders) Based on this, it is essential to incorporate Stx2 detection to patients with bacterial infection such as Shigellosis.

0306 - AQUAPORIN-2 AS A TARGET IN A MODEL OF CHRONIC KIDNEY DISEASE.

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Chronic kidney disease (CKD) is one of the leading sanitary problems in Argentina. Vasopressin (VP), the main regulator of Aquaporin-2 (AQP2) expression, was suggested as a contributor to the development of CKD. However, the mechanisms of VP contribution remain unknown. We characterized a model of CKD induced by adenine administration and found increased renomedullary AQP2 expression. The aim of this work is to study the effect of Tolvaptan (T; 30 mg/kg/day in the food), a V2 receptor antagonist, on renal parameters and on AQP2 expression in this model of CKD. Male Sprague-Dawley rats were divided in 4 groups: C (Control; fed with standard rat chow powder); CKD (0.25% adenine in food); CKD + T and T; n=3/4 for each group. After 2 weeks, animals were kept in metabolic cages to collect 24 h urine. Blood samples were obtained and the kidneys were processed for further determinations. Results: Media ± SEM; ANOVA followed by Bonferroni was performed. Renal (w/100 g bw): C= 0.36 ± 0.01; CKD= 0.46 ± 0.02 ***##; CKD + T= 0.43 ± 0.02; T= 0.38 ± 0.01. Plasmatic (P) Urea (g/l): C= 0.57 ± 0.08; CKD= 0.82 ± 0.12 *##; CKD + T= 0.58 ± 0.12; T= 0.39 ± 0.03 Urinary (U) urea (g/l): C= 31.67 ± 4.23; CKD= 47.44 ± 7.76*#&; CKD + T= 30.42 ± 1.36; T= 33.66 ± 1.50. P Creatinine (mg/l): C= 6.19 ± 0.31; CKD= 8.86 ± 0.35*#; CKD + T= 6.94 ± 1.11; T= 5.40 ± 0.16. Creatinine Clearance (ml/min): C= 2.01 ± 0.14; CKD= 1.50 ± 0.06*#&; CKD + T= 1.68 ± 0.24; T= 2.60 ± 0.30 AQP2 expression (WB): C= 1.00 ± 0.11; CKD= 1.54 ± 0.19*#&; CKD + T= 0.91 ± 0.12; T= 1.01 ± 0.06. cAMP renomedullary concentration (pmol/mg protein): C= 2.92 ± 0.04; CKD= 5.73 ± 1.7 *#&; CKD + T= 1.32 ± 0.44; T= 1.51 ± 0.32. *p < 0.05 vs. C; ***p<0.005 vs. C; #p < 0.05 vs. T; ##p < 0.01 vs. T; & p < 0.05 vs. CKD + T. Tolvaptan administration prevented the increase in AQP2 expression and improved some of the P and U parameters, suggesting that AQP2 increased expression could be at least one of the mechanisms by which VP is involved in the development of CKD.

0307 - FROM BRAIN TO KIDNEY: CENTRAL AT1 RECEPTORS AND SYMPATHETIC NERVOUS SYSTEM INTERACTION IN SODIUM EXCRETION MECHANISMS

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FACULTAD DE CIENCIAS QUÍMICAS UCC- IIBYT-CONICET-UNC (1); DEPARTAMENTO DE FARMACOLOGÍA, FACULTAD DE CIENCIAS QUÍMICAS, IFEC-CONICET, UNC. (2)

Central angiotensin II through AT1 receptors (AT1-R), closely interact with sympathetic nervous system (SNS) in the maintenance of renal sodium equilibrium under normal and pathological conditions. Our aim was to unmask the brain AT1-R role in the renal sodium excretion mechanisms and the interaction with the SNS. For these purposes, male Wistar rats with renal nervous ablation/sham and implanted with bilateral cannulae in lateral ventricle, received normosodic (0.4 %) or hypersonic (4 %) diet in metabolic cages for 5 days. The surgical procedures were performed under ketamine/xylaxine (75/5 mg/kg i.p.) anesthesia. The urine was daily collected and water intake was register along the experiment. On day 6 the animals received saline/losartan (AT1-R antagonist 4ug/1 µl) intracerebrally and sacrificed 12 hours later. The parameters analyzed were; in urine: volume, sodium, potassium, water, creatinine and osmolarity to evaluate kidney function; at brain: c-Fos expression in paraventricular (PVN), supraoptic (SON) and subfornical (SFO) nucleus and vasopressin by immunohistochemistry. The data were analyzed by factorial ANOVA. The effects of central AT1-R and the interaction with SNS were observed on water intake and sodium and water excretion. Renal sodium excretion and water intake are under central AT1-R activation depending on renal nervous integrity. AT1-R blockade blunted the increased c-Fos expression induced by hypersodic diet in vasopressinergic neurons (PVN and SON). We conclude that SNS regulates the complex interaction between central angiotensin II, through AT1-R, and vasopressinergic neurons at SON and PVN under sodium overload conditions.

0309 - POSTNATAL INHIBITION OF ENDOTHELIN SYSTEM AND SALT SENSITIVITY GENERATION IN ADULTHOOD: PARTICIPATION OF AVP PATHWAY

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Previous results from our group showed that postnatal inhibition of Endothelin (ET) system generates salt sensitivity (SS) in male adult rats. These animals have an impaired ability to eliminate water and sodium overload, with increased AQP2 and α-ENaC expression and increased blood pressure. It was shown that both transporters are regulated by vasopressin (AVP) through V2 receptors (Stockand JD. Kidney Int. 2010;78(9):849-56) and that adenylyl cyclase 6 (AC6) mediates AVP-stimulated ENaC activity in the kidney (Roos KP et al. J Am Soc Nephrol. 2013;24:218-27). The aim of this work was to investigate the participation of AVP system in the mechanism of SS in this experimental model. We evaluated: V2 receptor expression and AC6 by real time PCR and cAMP production in the renal medulla of adult male Sprague-Dawley rats fed with a normosodic (NS) or hypersodic (HS) diet (the animals had been treated during their postnatal period with a dual ET receptor antagonist [ERA]: bosentan 20 mg/kg/day). Four experimental groups were studied: control males with NS diet (CNS), control males with HS diet (CHS), ERA males with NS diet (ERANS) and ERA males with HS diet (ERAHS). Two-way ANOVA was used for statistics. V2 receptor mRNA expression was significantly lower in ERANS vs. CNS ($p<0.05$) and in ERAHS vs. CHS ($p<0.05$); AC6 mRNA expression increased in ERAHS vs. CHS group ($p<0.05$). Besides, ERANS group had a higher level of AC6 expression than CNS ($p<0.05$). There was a tendency to increase cAMP production (expressed as pmol cAMP/g protein) in ERAHS vs ERANS rats meanwhile that tendency was not seen in CHS vs CNS. The increased renomedullary expression of AQP2 and α-ENaC in ERAHS rats would not be due to a greater level of V2 receptor expression. The increased expression of both transporters in ERAHS rats could be mediated, at least in part, by increased AC6 expression and activity and cAMP production.

0320 - CHARACTERIZATION OF CHRONIC KIDNEY DISEASE IN RATS TREATED WITH LITHIUM

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UBA, FAC MED, DEPTO PATOLOGÍA, CPEA; HOSPITAL ALEMÁN; CONICET (1); UBA, FAC MED, DEPTO PATOLOGÍA, CPEA; HOSPITAL ALEMÁN (2); UBA, FACULTAD DE FARMACIA Y BIOQUÍMICA, CÁTEDRA DE QUÍMICA GENERAL E INORGÁNICA (3); UBA, FACULTAD DE MEDICINA, DEPARTAMENTO DE FISIOLOGÍA, LABORATORIO DE ENDOCRINOLOGÍA (4); UBA, FACULTAD DE MEDICINA, DEPTO DE PATOLOGÍA, CENTRO DE PATOLOGÍA EXPERIMENTAL Y APLICADA (5); UBA, FACULTAD DE MEDICINA, DEPTO DE CIENCIAS FISIOLÓGICAS (6); UNIVERSIDAD FAVALORO, FUNDACIÓN INECO, INCYT; CONICET (7)

Lithium (Li) is the drug of choice for long-term prophylactic treatment of bipolar disorder. Chronic kidney disease is one of the complications of prolonged use of Li, which may be more frequent than previously thought. The aim of this study was to evaluate renal damage in rats treated with Li for 3 and 6 months. Forty Wistar male rats were divided into 4 groups (G): control (C) fed ad libitum standard diet or experimental (E) fed the same diet containing 60

mmol Li/kg diet. C and E rats were sacrificed after 3 or 6 months of experiment (3 or 6). Lithemia, serum creatinine (SC), Clearance of creatinine (CrCl) and fractional excretion of sodium (FENa) were measured. Kidney samples were processed for: histopathology; oxidative stress (TBARs) and immunohistochemistry (Aquaporin-2; AQP2). Lithemia (mmol/L) reached therapeutic values in both EG (E3= 0.69 ± 0.14 and E6= 0.63 ± 0.13). SC (mg/dl) was higher in both EG (C3= 0.37 ± 0.05 vs. E3= 0.46 ± 0.11; t= -2.22, p= 0.043; C6= 0.36 ±0.04 vs. E6= 0.46 ± 0.09; Z= -2.26, p= 0.024). CrCl (mL/min) was lower in both EG (C3= 2.84 ± 0.43 vs. E3= 1.33 ± 0.55; t= 6.13, p<0.001; C6= 3.27 ± 0.48 vs. E6= 1.19 ± 0.59; Z= -3.24, p= 0.001). FENa (%) was within normal values in all G, but it was significantly higher in both EG (C3= 0.07 ± 0.01 vs. E3= 0.51 ± 0.22; t= -5.62, p<0.001; C6= 0.20 ± 0.06 vs. E6= 0.85 ± 0.99; Z= -2.87, p= 0.004). No significant difference was found in SC (p= 0.982), CrCl (p= 0.642) and FENa (p= 0.908) between EG. Histological damage was observed in the cortical collecting tubules (CCT), positive with AQP2, in EG (hypertrophy and hyperplasia in some CCT and atrophy in others) being of greater magnitude in E6G. A significant increase in TBARs (nmol/mg) was observed in both EG (C3= 0.36 ± 0.08 vs. E3= 0.58 ± 0.14; Z= -2.62, p= 0.009 and C6= 0.32 ± 0.07 vs. E6= 0.60 ± 0.12; Z= -3.26, p= 0.001); no significant difference was found between EG (p= 0.958). Although chronic Li treatment produces kidney damage that alters functional, morphological and oxidative stress parameters; functional and oxidative stress changes remain constant in EG while CCT damage is worst in E6G.

0362 - POSTNATAL INHIBITION OF ENDOTHELIN SYSTEM AND RENAL DEVELOPMENT IN RODENTS: NITRIC OXIDE AND SUPEROXIDE AS MOLECULAR PLAYERS

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In a previous work we had shown that Endothelin (ET) inhibition during the postnatal period with bosentan, a dual receptor antagonist (ERA), produces an imbalance between proliferation and apoptosis. We had also shown a tendency to increase TBARS, suggesting an increased oxidative stress. Bearing in mind that oxidative stress is an important stimulus for apoptotic intrinsic pathway (Vakifahmetoglu-Norberg; Biochem Biophys Res Commun. 2017; 482: 426-31), an increase in oxidative stress could be a link between perinatal adverse impacts and diseases in adulthood. Besides, the modulation of cell signaling by nitric oxide (NO) and superoxide (O_2^-) is associated with apoptotic cell death in inflammatory kidney diseases (Pautz; Kidney International. 2002; 61: 790–6). The aim of this work was to evaluate in 7 days old rats (male and female controls and ERA-treated rats): pro and antiapoptotic molecules Bax and Bcl-xl; NO and O_2^- production in mitochondria isolated from renal tissue, NO/O_2^- ratio and nitric oxide synthase (NOS) activity in different renal structures estimated by NADPH-diaphorase technique. Four experimental groups were studied: control males (Cm), males treated with bosentan (ERAm), control females (Cf) and females treated with bosentan (ERAf). Two-way ANOVA was used for statistics. We found a significant increase in Bax/Bcl-xl ratio in both ERAm ($p<0.01$) and ERAf ($p<0.05$) vs their respective controls and a clear tendence to decrease NO/O_2^- ratio in both ERAm (0.10 ± 0.02 vs. 0.06 ± 0.01) and ERAf (0.08 ± 0.01 vs. 0.06 ± 0.01) vs their respective controls. We found a decrease in NADPHd activity in immature cortical renal structures only in ERAm ($p<0.05$) and in macula densa in both ERAm and ERAf vs their controls ($p<0.05$). The inhibition of ET during the postnatal period stimulates the intrinsic pathway of apoptosis in the kidney and this may be consequence of the imbalance in NO/O_2^- ratio in some renal structures.

0413 - BLOOD PRESSURE REDUCING EFFECT ON THE EXPRESSION OF PROTEINS LINKED TO SODIUM TRANSPORT IN PERIPHERAL BLOOD LYMPHOCYTES IN A RAT MODEL OF SALT-SENSITIVE HYPERTENSION

Sandra Gabriela VLACHOVSKY | Luis Alberto DI CIANO | Pablo J. AZURMENDI | Elisabet ODDO | Nora Paula GOETTE | Elvira E ARRIZURIETA | Claudia SILBERSTEIN | Fernando Raul IBARRA

INSTITUTO DE INVESTIGACIONES MÉDICAS ALFREDO LANARI - UNIVERSIDAD DE BUENOS AIRES

Recent investigations have highlighted the role of the adaptive immune system in the genesis of high blood pressure. In previous work we described a model of salt-sensitive hypertension in ovariectomized Wistar rats in which we studied the expression of proteins related to sodium transport in peripheral blood lymphocytes (PBL). We showed that proteins such as Na^+, K^+-ATPase (NKA), cotransporter Na^+, K^+, $2Cl^-$ (NKCC1), dopamine receptor 1 (D1DR) and $CD4^+$ T-cell surface protein responded differently to a high sodium intake (HS) in intact female (IF) or ovariectomized (oVx) rats. The aim of the present work to determine whether the changes in the protein expression in PBL occur in response to the increase in blood pressure or secondary to the HS intake. For this purpose, IF and oVx rats, with normal sodium (NS) or HS intake, were treated with hydralazine (HZ), a peripheral vasodilator. Half of rats were ovariectomized at 60 days of life and at 145 days the IF and oVx were divided into two subgroups with either NS (0.24 % NaCl) or HS (1 % NaCl in drinking water). At day 150 systolic blood pressure (SBP, tail-cuff method) was recorded and blood samples were taken for PBL separation (ficoll). Then, rats started with HZ treatment in drinking water (20 mg/kg/day). At day 157, SBP records and PBL samples were repeated. Protein expression was analyzed by Western Blot. Results: SBP (mmHg), IF NS: 131 ± 4.5 vs. IF NS HZ: 117 ± 3.62, IF HS: 125 ± 3.87 vs. IF HS HZ: 117 ± 6.77, oVx NS: 128 ± 4.03 vs. oVx NS HZ: 113 ± 3.92, oVx HS: 148 ± 4.81* vs. oVx HS HZ: 112 ± 3.82; * p <0.05 oVx HS group vs. all other groups. HZ reduced SBP in all groups ($p<0.05$). While NKCC1 expression decreased significantly with HZ only in oVx HS group, no changes were observed in the expression of NKA, D1DR or $CD4^+$ pre and post treatment with hydralazine in any of the other groups. NKCC1 was sensitive to the reduction of blood pressure. The altered expression of NKA, D1DR and $CD4^+$ may correspond to variations in sodium intake.

0433 - ELIGLUSTAT PREVENTS AGAINST THE CYTOTOXIC EFFECTS OF SHIGA TOXIN TYPE 2 IN HUMAN RENAL TUBULAR EPITHELIAL CELLS

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IFIBIO HOUSSAY (UBA-CONICET) DPTO DE CS FISIOLOGICAS, FACULTAD DE MEDICINA, UBA (1); HOSPITAL GENERAL DE NIÑOS PEDRO DE ELIZALDE (2)

In Argentina, post-diarrhea Hemolytic Uremic Syndrome due to Shiga toxin-producing Escherichia Coli is a common cause of acute renal failure in children. Shiga toxin type 2 (Stx2) binds to the globotriaosylceramide (Gb3) receptor on the surface of renal cells. We have previously shown that the compound C-9 (Genzyme), an inhibitor of glucosylceramide synthase (GLI-1), decreased Gb3 expression and prevents the cytotoxic actions of Stx2 in renal cells. The aim of the present work was to study whether Eliglustat (EG, MedKoo Biosci, USA), a new potent inhibitor of GLI-1, prevents the cytotoxic effects of Stx2 on primary cultures of human renal tubular epithelial cells (HRTEC) and HK-2, a human renal proximal tubule cell line. HRTEC were developed from nephrectomies performed at

the Hospital de Niños Pedro de Elizalde. Cells were pre-incubated with or without EG (1-1000 nM, 6-24 h), followed by co-incubation with EG and Stx2 (10 ng/ml, 24-72 h). Cell viability and proliferation were measured by neutral red and bromodeoxyuridine uptake, respectively. Apoptosis was evaluated by acridine orange/ethidium bromide staining. Treatment with Stx2 significantly decreased cell viability and proliferation and increased cell apoptosis in HRTEC and HK-2 compared to control cells ($p<0.01$). The cytotoxic dose of Stx2 to kill 50% of cells at 72 h was 1 ng/ml for HRTEC and 10 ng/ml for HK-2. Pre-incubation of HRTEC and HK-2 with EG, from a dose 10 nM for 24 h and 100 nM for 6 h, significantly prevented the effects of Stx2 on cell viability, cell proliferation and apoptosis ($p<0.05$). However, C-9 prevented the Stx2 damage at doses 100 times higher than EG. In conclusion, EG protects the human renal tubular epithelium against the activity of Stx2, being more effective than C-9. Decrease Gb3 expression by compounds like Eliglustat could be a novel substrate inhibition therapy to neutralize Stx2 action in renal cells.

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0689 - THE CAMP CONTENT IN WHOLE URINE AND EXTRACELLULAR VESICLES (EVS) AS POSSIBLE MARKERS OF ADPKD PROGRESSION. A PRELIMINARY REPORT

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ADPKD is characterized by an intracellular calcium depletion that belongs to deficient activity of the polycystin 1 or 2 complex which, in turn, stimulates cAMP production by adenylate cyclases and inhibit its degradation by phosphodiesterases. However, the behavior of the cAMP in the disease progression is not completely elucidated. We had previously shown that urine in ADPKD is enriched on cAMP when compared to controls. On the other hand, EVs secreted in urine (namely urinary exosomes) play a pivotal role in cell-to-cell communication and are an emerging tool as renal disease progression biomarkers. Urine exosomes appears to be an accessible tool to measure intracellular parameters such as cAMP. Our objective was to evaluate the levels of urinary cAMP over time in ADPKD at a three-year follow-up. In addition, we analyzed whether urinary exosomes contain cAMP in ADPKD as well as in healthy volunteers. Urinary cAMP was measured in a 3-years follow-up of 15 ADPKD (36 ± 1 years, 7 women) and the data were compared with total kidney volume (TKV), GFR and urine osmolality. Urinary EVs were obtained from 4 ADPKD patients and 4 healthy controls by precipitation method. Quantification of cAMP was performed by radioligand binding assay. The results show that the urinary cAMP varies timely in ADPKD by age ($r= 0.72$, $p<0.0001$), together with known progression markers (TKV and GRF). Moreover, cAMP related to osmolal excretion ($r=-0.4340$, $p<0.02$), another early manifestation of the disease. The cAMP concentration of EVs was higher in urine from controls than in ADPKD patients ($p= 0.0017$) but this difference disappeared when the amount was corrected by urinary creatinine. Taken together, these data could propose the utility of cAMP as a marker of ADPKD progression in a longitudinal study. Furthermore, the cAMP content in EVs could reflect the activation of the renal vasopressin-V2 receptor axis and its role in the cell to cell communication.

0710 - PODOCYTURIA IN FABRY NEPHROPATHY

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Fabry is X-linked recessive disease due to lysosomal alpha-galactosidase deficiency that produce a defect in the degradation of globotriosylceramide (Gb3). Nephropathy is associated with lysosomal inclusions at the level of podocytes and tubular cells. The renal manifestations such as proteinuria by the alteration in the filtration barrier is appear. Fabry disease currently has no cure, but an enzyme replacement treatment has been achieved thus preventing the continuous accumulation of Gb3. The aim was to detect an early biomarker of renal alteration in order to include the patients in replacement therapy. Cross-sectional studies were realized. Fabry patients (British Hospital Buenos Aires) were studied. Control patients recruited among potential kidney donors were used. A mid-stream freshly voided urine sample was collected. Renal biochemical parameters were determined. Smears were performed to study podocyturia by the expression of synaptopodin and the colocalization with podocalixyn (S/P) by indirect immunofluorescence. Podocytes were counted in 10 randomly chosen ×20 fields and the average was considered as the final count for each subject. The detection of urine CD80 was performed by ELISA. We detected before a development of proteinuria an increase (33 %) in podocyturia in Fabry patients, 13/48 colocalized synaptopodin and podocalyxin (27 %) versus controls (40%). When the colocalization was analyzed according to therapy, untreated Fabry individuals showed colocalization only in 2/15 patients (13 %). The ratio urinary concentration of CD80/creatinine was significantly higher in Fabry patients (45 ng/g) vs controls (17 ng/g). Our results suggest that alterations in surface podocytes and the expression of CD 80 could be related with the mechanisms involved in the detachment of podocytes. Podocyturia may be used as an early biomarker of kidney damage in Fabry disease.

0743 - RENAL EFFECTS OF SHIGA TOXIN TYPE 2 IN PREGNANT AND NON-PREGNANT FEMALE RATS.

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Shiga toxin-producing Escherichia coli cause acute renal failure and Hemolytic Uremic Syndrome. Previous studies showed that Shiga toxin type 2 (Stx2) intraperitoneally (i.p.) injected in rats during the early gestation period produces kidney damage. After a renal injury, tubular epithelial cells have the capacity of proliferate and repair. The aim of the work was to study the effects of Stx2 on damage and tubular proliferation in kidneys of pregnant rats during the early gestation period, and compare with non-pregnant rats. Pregnant Sprague-Dawley rats, at the eighth day of gestation, were inoculated ip with a sublethal dose of Stx2 (PS) (0.5 ng/g body weight) or diluent (PC). Non-pregnant rats were injected with the same dose of Stx2 (NPS) or diluent (NPC). Rats were placed in metabolic cages, urine samples were collected, and rats were euthanized 1 to 4 days post-injection (dpi). The kidneys were removed for histopathological observations and Ki67 expression, as proliferation marker, was evaluated by immunofluorescence. Tubular necrosis was observed in renal cortex of PS and NPS rats from 2 dpi, which increased significantly at 4 dpi, with respect to PC and NPC rats ($p<0.05$). Medullar tubules of both NPS and PS did not show significant necrosis. However, a significant increase in Ki67 expression was observed in tubular cells of renal medulla of NPS (8.0 ± 2.0 %) and PS (6.4 ± 0.4 %) with respect to NPC (0.7 ± 0.6 %) and PC (0.7 ± 0.1 %) ($n= 4$, $p<0.05$), indicating an increase in

medullar and not cortical tubular proliferation. NPS showed a significant increase in urinary flow compared to NPC (36.2 ± 2.6 ml/d vs. 17.0 ± 3.5 ml/d) that was not observed in pregnant rats. Our results showed a similar increase in tubular proliferation in renal medulla of NPS and PS rats. We propose that Stx2 may produce a milder damage in medulla than in cortex, allowing proliferation and repair of the tubular epithelium.

0916 - EARLY DETECTION OF RENAL DAMAGE DURING HEMOLYTIC UREMIC SYNDROME

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Hemolytic uremic syndrome (HUS) is a thrombotic microangiopathy associated with Shiga toxin-producing E. coli (STEC). Its clinical features are hemolytic anemia, thrombocytopenia and renal failure. HUS is the most frequent cause of renal failure in children. After the acute episode 25% of children suffer from some degree of reduced renal function. Our aim was to detect the presence of urine podocytes and to study the relation with evolution of renal injury to chronicity in a HUS model in rats. Adult male Sprague-Dawley rats (200 g) were randomly divided into two groups. The experimental group was injected intraperitoneally with culture supernatant of E.coli O157 125/99 (E.coli wild type CLADO 8). Control group was injected with culture supernatant of E.coli that did not express Stx2. Rats were sacrificed at 1 week. Functional, histological and immunocytochemical studies were performed in both groups. The functional studies showed a decrease in glomerular filtration volume at 1 week without proteinuria. We could observe an increase in urine podocytes from experimental group. Our results suggest that the detection of podocyturia could be used as a biomarker of renal damage in HUS. More experiments will be needed to analyze the evolution of the injury.

Biología celular y molecular de procesos fisiológicos y patológicos / Biology V

Chairs: Fernando Correa | Nicolás Surkin

0787 - ROL OF ESTROGEN-RELATED RECEPTOR ALPHA AND ESTROGEN RECEPTOR ALPHA ON TRANSCRIPTIONAL REGULATION OF ACYL-COA SYNTHETASE 4 IN BREAST CANCER CELLS

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Acyl-CoA synthetase 4 (ACSL4) overexpression plays a causal role in the aggressiveness of triple negative breast cancer (TNBC). In turn, a negative correlation has been established between ACSL4 and estrogen receptor alpha (ER) expression. Our hypothesis is that transcriptional regulation is involved in the differential expression of ACSL4 in breast cancer cells. We observed that TNBC cells (MDA-MB-231 and Hs578T) exhibit greater activity of ACSL4 promoter than ER+ cell lines (MCF-7 and T47D) ($p<0.001$). We described that estrogen-related receptor alpha (ERRalpha), a transcription factor involved in breast cancer aggressiveness, is involved in ACSL4 expression in TNBC cells. By site directed mutagenesis, we observed that ERRalpha activates ACSL4 promoter in MDA-MB-231 ($p<0.001$). ChIP assays showed that ERRalpha interacts with the ACSL4 promoter in MDA-MB-231 but not in MCF-7 cells ($p<0.001$). ERRalpha silencing diminished ACSL4 protein expression ($p<0.01$), mRNA level ($p<0.05$) and promoter activity ($p<0.001$) only in MDA-MB-231. XCT-790, an inverse agonist of ERRalpha, was able to downregulate ACSL4 expression ($p<0.01$) on MDA-MB-231 by reducing the transcriptional activity of the promoter ($p<0.001$). Furthermore, the combination of inhibitors of ACSL4 and ERRalpha produced a synergistic decrease in MDA-MB-231 cell proliferation ($p<0.001$). Moreover, ER restoration in MDA-MB-231 and Hs578T cells reduces ACSL4 promoter activity. This restoration also downregulates ACSL4 protein expression ($p<0.01$) and mRNA levels ($p<0.01$). However, the promoter sequence lacks ER elements and in fact, we did not observe any interaction of ER and ACSL4 promoter by ChIP analysis on MCF-7 cells. This indirect effect could be produced at least in part by the downregulation that ER exerts on ERRalpha expression ($p<0.05$). The results presented here demonstrate the role of ERRalpha and ER in the transcriptional mechanism that leads to different expression of ACSL4 in human breast cancer cells of different aggressiveness.

0804 - CLN8 DEFICIENCY, ASSOCIATED WITH CLN8 DISEASE OF NEURONAL CEROID LIPOFUSCINOSIS, INCREASES LYSOSOMAL PH AND ALTERS THE TRANSFERRIN RECEPTOR DISTRIBUTION IN HIPPOCAMPAL NEURONAL MODEL

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CLN8 protein, whose mutations cause CLN8 disease, is an endoplasmic reticulum (ER)-resident transmembrane protein that travels between ER and Golgi apparatus. It carries soluble lysosomal proteins and regulates the activity of I2PP2A, a PP2A phosphatase inhibitor. In this work, we aim to study the effects of CLN8 deficiency on lysosomal pH and protein distribution. HeLa cells and rat hippocampal neurons of 7 d. i.v. were transfected with pYFP, pCLN8wt or pshCLN8 plasmid to modulate the expression of CLN8. To study lysosomal pH, HeLa cells were co-transfected with the plasmid pALP (mApple-LAMP1-pHluorin), which locates the pHluorin (whose fluorescence is sensitive to pH) and the mApple protein on the inner and outer side of the lysosomal membrane, respectively. The intensity ratio (IpHluorin/ImApple) was taken as indicative of the luminal pH. To study protein distribution, neurons were co-transfected with pLAMP1, pTfR (transferrin receptor) or pTMEM106b. The protein distribution was expressed as polarity index (Idendrites/Iaxon). Images were taken by confocal microscopy and analyzed using ImageJ-Fiji and Motion Tracking softwares. The intensity ratio in CLN8-deficient HeLa cells was 1.3 ± 0.1 (mean ± SEM), in CLN8wt cells 1.01 ± 0.05, and in control cells 0.9 ± 0.1 ($p<0.01$). Respect to protein distribution in neurons, the index of LAMP1 in control cells was 1.8 ± 0.2 and 1.5 ± 0.1 in CLN8-deficient cells. For TfR, the index in control neurons was 4.5 ± 0.4, and 2.5 ± 0.2 in CLN8-deficient cells ($p<0.001$). For TMEM106b, the index in control neurons was 1.4 ± 0.1 and 1.5 ± 0.1 in CLN8-deficient cells. CLN8 deficiency increases the lysosomal pH, which affects the normal development of lysosomal metabolism. We suggest that this effect indirectly alters the distribution of some proteins, as we observed for TfR, possibly also affecting iron metabolism, observed in CLN8 disease. Further studies are needed to understand the pathophysiology of CLN8 disorder.

0829 - OPTIMIZATION OF RECOMBINANT ZIKA VIRUS NS1 PROTEIN SECRETION FROM HEK293 CELLS

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Zika virus (ZIKV) is a mosquito-borne flavivirus that has gained global attention after the 2015 outbreak in Brazil, where devastating congenital neurodevelopmental defects were associated with maternal ZIKV infection. Sensitive, accurate and cost-effective diagnostic tests are urgently needed to detect ZIKV acute infection to improve patient care and to control future outbreaks. Nonstructural 1 (NS1) glycoprotein results in an excellent diagnostic marker since it is released in a hexameric conformation from infected cells into the patient's bloodstream early in the course of the infection. The aim of this study consisted of establishing a stable and optimized recombinant ZIKV NS1 (rZNS1) mammalian expression system in order to purify the hexameric protein, which can potentially be used in the development of diagnostic tests. Stable rZNS1-His-expressing HEK293 cells were generated through lentiviral transduction followed by dilution cloning, obtaining 1E4-C9 clone, which presented the highest intracellular and secreted rZNS1-His protein levels by Western blot assays. Optimization of rZNS1-His protein secretion in 1E4-C9 HEK293 cells was accomplished with 50 nM rapamycin treatment followed by serum-free media incubation for 9 days. Nickel affinity-purified rZNS1-His hexamer was recognized by anti-NS1 antibodies in ZIKV patient's serum by indirect ELISA (iELISA) and Dot blot assays, and showed the ability to induce a humoral response in immunized mice as assessed by iELISA. The obtained recombinant protein is a reliable biological tool that can potentially be applied in the development of diagnostic tests to detect ZIKV in infected patients during the acute phase.

0833 - DEMETHYLATION OF H3K4ME3 IS A NECESSARY STEP TO ALLOW DNA DOUBLE STRAND BREAKS REPAIR.

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Human genome is highly susceptible to DNA damaging agents. Double-strand breaks (DSBs) are considered the most cytotoxic form of DNA damage. As consequence to DSB, DNA damage response (DDR) initiates repair mechanisms including homologous recombination (HR) and non-homologous end joined (NHEJ). Epigenetic involves mechanisms that affect DNA-based processes such as DSB repair through changes in the chromatin. Particularly, Jumonji (JmjC) histone lysine demethylases (KDM) play roles in DNA repair pathways. Our aim is to study if demethylation of histones is a required step during the DSB repair. The efficiency of repair by NHEJ and HR were assayed with a plasmid-based reporter system. DSB were induced by using ionizing radiation on H1299 cancer cell line. DNA repair was determined by immunofluorescence against gH2AX and 53BP1. Demethylation abrogation was performed using pharmacological inhibitors (JIB-04, PBIT, GSK-J4) or performing siRNA knockdown (KD) experiments. Rescue experiments were performed by electroporating H1299 cells with a plasmid codifying for JARID1B. Demethylases activity was assayed using an Epigentek commercial kits. CHIP-qPCR experiment was performed using a Millipore commercial Kit. Histone methylation levels were assayed by western blot (WB). The efficiency of repair by NHEJ and HR is inhibited by JIB-04, a JmjC pan-inhibitor. In addition, treatment with JIB-04 and inhibitors of H3K4me3 KDM (PBIT) but not of H3K27me3 KDM (GSK-J4) delay the resolution of DSB marked by gH2AX and 53BP1 foci ($p<0.001$). Similarly, DSB foci resolution is delayed by KD of JARID1B, a target of JIB-04 and PBIT inhibition, but not other members of the H3K4me3 JmjC KDM sub-family ($p<0.001$). Overexpression of JARID1B, rescues the DNA repair defects induced by JIB-04 ($p<0.001$). In addition, irradiation of H1299 induce an increase of global H3K4me3 demethylase activity ($p<0.05$). Finally, CHIP-qPCR and CHIP-WB experiments showed that JIB-04 leads to specific accumulation of H3K4me3 but not H3K9me3 at DSB marked by gH2AX ($p<0.05$). Our study suggests that demethylation of H3K4me3 is a required step during the DDR to DSB.

0877 - INVOLVEMENT OF KRÜPPEL LIKE FACTOR 6 IN REDOX AND ENDOPLASMIC RETICULUM HOMEOSTASIS

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An imbalance in the antioxidant response, endoplasmic reticulum (ER) homeostasis, and unfolded protein response (UPR) has been associated with several pathologies. Under physiological conditions, different cell types have different levels of UPR components, due in part to their metabolic and protein biosynthetic activity. Placental and tumor cells share many properties such as a high demand for protein synthesis and adaptation to a changing oxygen microenvironment. Krüppel like factor 6 (KLF6) is a transcription factor early activated by hypoxia and other stressors, and although its role as a tumor suppressor has been well established, its function largely depends on the cellular context. Herein, we analyzed KLF6 contribution in redox and ER homeostasis in the non-tumoral trophoblast-derived cell line HTR8/SVneo as well as in T98G, HepG2 and MDA-MB 231 tumoral cell lines. Downregulation of KLF6 by siRNA generated an increase in reactive oxygen species (ROS) in all the cell types analyzed, detected by flow cytometry through the H2DCFDA probe. The expression level of BiP, Ero1alpha, PDI, calnexin and calreticulin chaperones as well as the activity of Ire1alpha and PERK pathways, involved in ER homeostasis, were evaluated in KLF6-silenced cells. Results revealed that these UPR components were differentially modulated by KLF6 in each cell line. In addition, components involved in redox balance were analyzed in HTR-8/SVneo cells. At this regard, KLF6 silencing did not modify the canonical ROS-activated Nrf2 pathway as measured by its cytoplasm to nucleus translocation and HO-1 expression. Neither the peroxiredoxins nor the catalase protein levels and its activity were altered. Moreover, the mitochondrial membrane potential determined by flow cytometry through JC-1 dye was not affected. Our results suggest that KLF6 is involved in redox and ER homeostasis modulating UPR components in a cell type dependent-manner.

0882 - STIMULATION OF INFLAMMATORY MICROENVIRONMENT BY A HUMAN APOLIPOPROTEIN A-I NATURAL VARIANT

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The cascade of molecular events leading to human apolipoprotein A–I (apoA–I) amyloidosis is not completely understood, not even the pathways that determine clinical manifestations associated to systemic protein deposition in organs such as liver, kidney and heart. Among more than twenty natural variants of apoA-I, it was shown that the substitution of an Arg in position 173 by a Pro in the sequence of apoA-I (R173P) induced heart amyloidosis. The mechanisms determining its pathogenicity are not clear. In this work we gained deep insight into cellular events probably elicited by the soluble conformation of R173P. WT apoA-I and R173P were obtained by molecular biology techniques. Human umbilical vein

endothelial cells (HUVEC) or a human monocyte-derived cellular line (THP-1) were treated with 1.5 µg/ml for 24 h. Immunofluorescence of NFkB was used to evaluate endothelial activation; and enzyme immunoassays (ELISA) were performed to evaluate tumor necrosis factor (TNF) alpha; and interleukin (IL)-1ß. We detected that the natural variant R173 induced NFkB translocation into the nucleus of endothelial cells. Moreover, the incubation of R173P with THP-1 resulted in the release of tumor necrosis TNF- alpha ($p<0.001$), and IL-1ß ($p<0.0001$), without affecting cell viability. On the other hand, WT apoA-I did not show the mentioned events. These findings suggest that at least part of the pathological mechanisms of R173 variant may be to promote an inflammatory microenvironment which could in turn result in endothelial dysfunction.

0892 - INVOLVEMENT OF LXR LIGANDS IN CONTROLLING PROLIFERATION OF MAMMARY EPITHELIAL CELLS

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LXRs are transcription factors activated by cholesterol metabolites whose target genes participate in the de novo synthesis of triglycerides and cholesterol reverse transport in many tissues. Two different LXR isoforms has been described: LXRa and LXRb. Genetic and pharmacological studies defined them as key factors connecting cellular processes such as lipid metabolism, inflammation and cellular proliferation and death. Recent evidence suggests that LXR activation and consequent intracellular cholesterol decrease have antitumoral effect. However, it was also demonstrated that LXR activation favors Warburg effect, relevant in tumor cells. In this sense, our goal was to evaluate the role of LXRs on breast cancer cell proliferation and migration. Here, we report that both LXR isoforms are expressed in ER + breast cancer (BC) cell line MCF7 and in ER - BC cells MDA-mb-231. The expression of LXRb; isoform was inhibited in MDA-mb-231 upon treatment with the commercial LXR agonist GW39065 (1 µM) while LXRa; levels remained unaffected. Cell viability results assessed by MTT assays suggest that in MCF-7 cells treated with or without estradiol (10nM), GW39065 (1 µM) decreases cell proliferation upon 24 h treatment. However, this effect was not observed in MDA-231 cells. On the other hand, wound healing assays suggested that the LXR agonist increases MDA-231 but does not affect MCF-7 cell migration. LXR signaling would arise as a relevant pathway in controlling breast cancer cells proliferation, but its involvement would dependent on each cellular context.

0912 - HRAS EXPRESSION IS REGULATED AT MRNA LEVEL BY CA2+ ENTRY THROUGH CA2+ RELEASE ACTIVATED CA2+ (CRAC) CHANNELS

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The store-operated calcium entry (SOCE) channel is the major Ca^{2+} entry pathway in nonexcitable cells. Store-operated Ca^{2+} influx is essential for maintaining ER Ca^{2+} content at a precise level and functions in various physiological processes such as gene transcription, cell-cycle progression, and apoptosis. SOCE is a cellular mechanism linking the calcium depletion of the endoplasmic reticulum (ER) to the activation of plasma membrane (PM) Ca^{2+}- permeable channels. The mains components of SOCE are Orai1 and STIM1 proteins. Upon the stimulation of cell surface receptors, like EGFR, depletion of ER Ca^{2+} results in triggering STIM1 translocation to ER-plasma membrane junctions where they bind and activate Orai1, the pore subunit of the Ca^{2+} release-activated Ca^{2+} (CRAC) channel, and resulting in refilling of ER stores. Previously we showed by FRET that Hras protein interacts with Orai1 showed in Ca2+ imaging experiments with FURA2 that there was a reduction of SOCE in HEK293 cells trasnsfected with HRas, Nras or Kras. We also showed that a dominant positive mutant of Hras (Hras-Q61L) did not reduced SOCE but that the dominant negative mutant of Hras (Hras-S17N) reduced SOCE as did the wt Hras. All these results confirmed that SOCE was being regulated by the Ras signaling cascade(s). We next went on to test if SOCE could regulate the Ras signaling casdade. To this end we used wild type MEFs and MEFs lacking expression of Orai1 (O1KO MEFs) and amplified by RTPCR the mRNA of cFOS as a marker of Ras activity. In parallel we transfected Hras and/or Orai1 both in HEK293 cells and in WT and O1KO MEFs. Finally, we loaded MEF wt and MEF-OIKO with the Ca^{2+} quelator BAPTA-AM, and analized the impact on Hras and cFOS at mRNAs. Our preliminary results showed that Ca^{2+} entry through Orai1channels (CRAC channels) reduced mRNA of cFOS in WT compared to O1KO MEFs cells. On the other hand, the increasment of mRNA of cFOS in O1KO compared to WT MEFs due to the transfection of Hras confimed the dependence of cFOS changes on the Hras system. In a final experiment, we incubated WT and O1KO MEFs cells with BAPTA-AM and by RTPCR we found a reduction in the mRNAs of both Hras a cFOS. Taken toghether our results showed that Ca2+ entry through Orai1channels reduces the mRNA and/or activity of HRas. We hipothetize that influx of Ca^{2+} through Orai1channels may be acting as negative feedback control on an excessive Ca^{2+} entry by mediating the inactivation of the Ras signaling cascade

0922 - INCREASED LYSOSOME EXOCYTOSIS IN SH-SY5Y CELLS OVEREXPRESSING THE P5-ATPASE ATP13A2

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The P-type ion pumps are membrane transporters energized by ATP-hydrolysis which are classified into five subfamilies termed P1-P5. The substrate transported by P5-ATPases is still unknown. ATP13A1-ATP13A5 genes that belong to this group have been identified in humans. Mutations of the ATP13A2 gene were associated with neurodegenerative diseases like Parkinson's disease, neuronal ceroid llipofuscinosis (CNL12), hereditary spastic paraplegia (SPG78) and amyotrophic lateral sclerosis. ATP13A2 is localized in lysosomes and late endosomes. Dysfunction of this protein diminishes the lysosomal degradation, the autophagic flux and the exosome externalization. Lysosomal exocytosis (Ly-exocytosis) is now arising as a potential therapeutic target in diseases characterized by the accumulation of undigested material. We have shown that ATP13A2 expression modifies the lipid homeostasis in a way that seems to switch the endo-lysosomal system towards endo-lysosome secretion. By measuring the activity of the lysosomal enzyme ß-hexosaminidase in the culture medium, we found that SH-SY5Y cells stably expressing the ATP13A2 (SH-SY5Y-ATP13A2) have an increased Ly-exocytosis. As this process is upregulated by increasing lysosomal Ca^{2+} levels, we evaluated the Ca^{2+} kinetics by following the fluorimetric measurement of the calcium sensor Fura-2 AM in preloaded SH-SY5Y cells. We found that SH-SY5Y-ATP13A2 cells are able to diminish the cytoplasmic calcium content more efficiently than control cells overexpressing an inactive ATP13A2 pump. Considering that Ly-exocytosis is dependent upon cytoskeleton dynamic, we evaluated the cytoskeleton structure by fluorescence microscopy. Preliminary results show that SH-SY5Y-ATP13A2 have a more prominent cortical actin cytoskeleton distribution than control cells. Altogether these results suggest that ATP13A2 overexpression may be favoring Ly-exocytosis in SH-SY5Y cells.

0928 - CELLULAR AND MOLECULAR ALTERATIONS IN FIBROBLASTS FROM MUCOPOLYSACCHARIDOSIS IIIA PATIENTS

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Mucopolysaccharidosis type III (MPS III) -also known as Sanfilippo syndrome- is a lysosomal storage disease (LSD) caused by genetic defects in the enzymes responsible for heparan sulfate (HS) degradation. Stored glycosaminoclycans (GAGs) not only affect lysosomal function, but also interfere with several intracellular processes that are still poorly understood. The aim of this study was to deepen in the knowledge of the cellular alterations involved in MPSIIIA with focus on lysosomes and mitochondria. For this purpose, we employed GM00643 and GM00879 MPSIIIA human fibroblast cell lines and GM00498 (age- matched control) from Coriell Institute for Medical Reseach. SGSH activity was decreased 90 ± 5 and 89 ± 3% ($p<0.001$) in GM00643 and GM00879 cells, respectively. We did not observe changes in LAMP-1 levels in cell lysates analyzed by western blotting. On the other hand, GM00879 cells showed lysosomal membrane permeabilization, evidenced by both a diffuse staining of Lysotracker Red DND-99 (14 ± 4 % of the cells, $p<0.001$) and the release of cathepsin D to the cytosol. Mitochondria were fragmented in MPSIIIA cells, presenting "donut" structures. OPA1 was increased and no changes were detected for DRP1 in mitochondria- enriched fractions. Considering the fission of the mitochondrial network and the occurrence of lysosomal damage, we next analyzed autophagy activation. We did not detect differences in LC3-II levels among cell lines. Inhibition of lysosomal degradation by Bafilomycin A1 increased LC3-II expression supporting the functionality of the autophagic flux. Interestingly, Parkin levels were increased for GM00643 and GM00879 fibroblasts (2.2 ± 0.2 and 3.4 ± 0.8 fold), suggesting that damaged mitochondria is being targeted for degradation. Our results provide new evidence regarding the mechanisms involved in lysosomal and mitochondrial damage in MPSIIIA patient cells and may contribute for the design of future therapies.

0933 - INTERACTION OF NOTCH AND IGF-1 PATHWAYS IN THE MAC-T BOVINE MAMMARY EPITHELIAL CELLS

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We previously described the involvement of IGF-1 system as well as the expression and activation of the Notch pathway in the pubertal development of the heifers' mammary gland, with a possible role of Notch in cell proliferation and angiogenesis. We therefore aimed to study the effect of Notch pathway inhibition with DAPT, and the IGF-1 treatment on the MAC-T bovine mammary epithelial cell line, in an attempt to elucidate those pathways interaction. Cells were cultured with 50 µM DAPT to inhibit Notch pathway during 24, 48 or 72 h, or with DMSO as control. We determined that 72 h DAPT treatment decreases the NOTCH4 receptor expression by Western Blot ($p= 0.047$), and triggers a trend to decrease the HES1 target gene expression ($p= 0.08$), indicating the effectiveness of DAPT in Notch pathway inhibition. We then performed MTS and wound healing assays to functionally study the effect of DAPT on MAC-T cells. The viability of MAC-T cells decreased with 72 h DAPT treatment ($p= 0.02$) and cell migration was altered with 24 h DAPT. Interestingly, mRNA IGFR1 expression increased with 72 h DAPT treatment by RT-qPCR ($p= 0.03$). When MAC-T cells were stimulated with 10 ng/ml IGF-1 during 24 h we observed an increment in cell viability by MTS assay and cell migration was altered respect to the control, determined by wound healing assay. In addition, we observed a trend to increase the expression of the Notch target gene HEY1 with 10 ng/ml IGF-1 treatment, by RT-qPCR. To study IGF-1 and Notch pathway interaction we treated the MAC-T cells with 50 µM DAPT during 24, 48 and 72 h and 10 ng/ml IGF-1 during 24 or 48 h. We observed that IGF-1 reversed the decrease in cell viability induced by DAPT (72h DAPT-24h IGF-1). These results suggest that IGF-1 and Notch pathways may interact to regulate cell proliferation and migration in the bovine mammary cells. Further studies are needed to deepen the knowledge of the regulation of mammary gland development.

0960 - EFFECT OF PROTEIN RESTRICTION AND CONGENITAL VIRAL INFECTION IN BRAIN DEVELOPMENT DURING EMBRYONIC LIFE

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U.E. ESTUDIOS EN NEUROCIENCIAS Y SISTEMAS COMPLEJOS, ENYS CONICET (1); UNIVERSIDADE FEDERAL DE RIO DE JANEIRO (2)

During prenatal life, different environmental factors could affect normal brain development. It has been demonstrated that maternal malnutrition impacts on offspring neural development, although some authors have stated that brain growth is relatively preserved in comparison to other organs and tissues (brain sparing effect). As well, one of the consequences of maternal malnutrition is the impairment of the immunological condition, which could lead to more risk to be affected by different kinds of infections. Here, we evaluated the combined effect of maternal protein restriction and congenital Zika virus (ZIKV) infection in a mouse model. Wild-type mouse dams were exposed to a severe low-protein or a standard diet and they were infected with ZIKV during the peak of embryonic neurogenesis or exposed to a sham injection. Three days post-infection, dams were sacrificed and embryos were analyzed. Using immunohistochemistry labeling in brain embryos, we identified microglial cells (Iba1+) to assess the immune local response to viral infection in cases of nutritional deprivation. Also, proliferation processes in the ventricular and subventricular zone were analyzed using anti-ph3 antibody as a marker in order to infer if neurogenesis was affected by the interaction of protein restriction and ZIKV infection. We found that the embryos from dams that were undernourished and exposed to infection present abundant Iba1+ cells in the lateral ventricles, while this sign is absent in the other groups. Also, the quantification of ph3+ cells in this group revealed that there is a reduction of the dividing cells in the subventricular zone, while in the ventricular zone no significant differences were found ($p<0.05$). Our results highlight how protein restriction could enhance the effects of congenital ZIKV infection altering normal brain development, which has implications for human populations potentially affected by both factors.

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Chairs: Mónica Frungieri | Vanesa Hauk

0095 - MELATONIN AMELIORATES CHEMOTHERAPY-INDUCED OVARIAN DAMAGE

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Melatonin (MEL) is a lipofilic molecule which can act as a potent reactive oxigen species scavenger. MEL has been shown to protect tissues from severe oxidative stress. Premature ovarian failure (POF) is characterized by the depletion of ovarian follicles in young women, which may be caused by chemotherapy. Current treatments for POF, mainly hormone therapies, are not completely effective. One of the mechanisms underlying chemotherapy-induced POF is a strong increase in oxidative stress, which results in an aged ovarian phenotype. The present study proposes the application of MEL as a strategy to protect the ovary in patients undergoing chemotherapy. To induce POF, cyclophosphamide (CTX, 75mg/kg, i.p.) was applied in F1 mice (C57XBalbC 8 weeks old) on day 1. MEL (15 mg/kg, i.p.) was applied on days 1, 6 and 11. Sacrifices were made at day 15. The ovaries were isolated for histological analysis and protein extraction for Western blot assays. For all data analysis ANOVA followed by Tukey test were performed. An ovarian morphological analysis showed that CTX increased the % of primary and atretic follicles and reduced the % of antral follicles ($p<0.05$). MEL increased the % of antral follicles and decreased the % of atretic follicles compared to CTX ($p<0.05$). These results were corroborated by IHC for anti-Müllerian hormone (AMH), where it was found that CTX diminished the % of follicles expressing AMH, while MEL restored this value to control levels. CTX increased the BAX/BCL-2 ratio ($p<0.05$) while MEL restored this value to control levels. BAX/BCLX-L ratio remained unchanged. Given the antioxidant properties of MEL, the expression of catalase (CAT) and SOD1 were measured. CAT expression was unchanged, while SOD1 expression was reduced in CTX compared to control ($p<0.05$) but not in CTX+MEL compared to control. In conclusion, MEL administration might be a potential therapeutic agent for ovarian protection and fertility preservation in patients undergoing chemotherapy.

0157 - A MATERNAL DIET ENRICHED IN OLIVE OIL REGULATES MATRIX METALLOPROTEINASES ACTIVITY IN MATERNAL AND CORD BLOOD AND IN TERM PLACENTAS FROM WOMEN WITH GESTATIONAL DIABETES MELLITUS.

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CENTRO DE ESTUDIOS FARMACOLÓGICOS Y BOTÁNICOS (CEFYBO-CONICET), FACULTAD DE MEDICINA, UBA (1); HOSPITAL GENERAL DE AGUDOS DR. IGNACIO PIROVANO (2); UMYMFOR (CONICET-UBA), DEPARTAMENTO DE QUÍMICA ORGÁNICA, FACULTAD DE CIENCIAS EXACTAS Y NATURALES (3)

Matrix metalloproteinases (MMPs) are proteolytic enzymes involved in placental development, but markers of a pro-inflammatory state when produced in excess. Previous studies have established that gestational diabetes mellitus (GDM) induces a pro-inflammatory intrauterine environment. We hypothesized that a diet enriched in olive oil (OO) regulates MMP-2 and MMP-9 activities in placentas and in maternal and cord blood from women with GDM. Fifty control (C) and GDM women were enrolled after signing an informed consent (protocol approved by the Ethics Committee of Hospital Pirovano, Buenos Aires). All of them followed the WHO diet for pregnancy, and a group of GDM women received a 26 mL-OO addition daily from week 24-28 of gestation until delivery (GDMO group). At delivery, placental villous explants and maternal and cord blood were obtained and stored at -80ºC for zymography analysis. Fatty acids (FAs) profile was evaluated in cord blood by GC-FID. MMP-9 activity was increased in the placentas and in maternal blood of the GDM group (133 and 81 % respectively, $p<0.05$ vs. C), alterations prevented by the diet enriched in OO ($p<0.05$ vs. GDM). No changes were found in the MMP-2 activity among the three evaluated groups. Regarding cord blood, MMP-9 and MMP-2 activities were both increased in the GDM group compared to C (90 and 21 % respectively, $p<0.05$ vs. C), increases prevented by the OO addition ($p<0.05$ vs. GDM). These changes occurred without major changes in fatty acid composition in the cord blood from the three experimental groups. The diet enriched in OO modulates MMP-9 activity in term placentas and in maternal and cord blood, indicating the capability of this diet to module the pro-inflammatory intrauterine environment induced by GDM. The lack of changes in FFA profile in cord blood suggest that the unsaturated fatty acids provided by the diet exert anti-inflammatory effects within the placenta and fetal organs, with possible benefits for the offspring's later life.

0174 - PATERNAL DIABETES INDUCED BY INTRAUTERINE PROGRAMMING AFFECTS LIPID METABOLISM IN THE PLACENTA

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CENTRO DE ESTUDIOS FARMACOLÓGICOS Y BOTÁNICOS (CEFYBO-CONICET), FACULTAD DE MEDICINA, UBA

Maternal diabetes programs diabetes in the adult offspring with alterations in peroxisome proliferator activated receptor (PPAR) pathways that regulate lipid metabolism. However, little is known about the paternal contribution to long-term offspring's metabolic health. The aim was to evaluate the regulation of lipid metabolism in the placenta of male fetuses from healthy pregnant rats that were mated with male diabetic rats. Control (C) and type 2 diabetic male rats (D, diabetes obtained by intrauterine programming, glycemia 140-190 mg/dL) were mated with control female rats. On day 21 of gestation, the pregnant and male rats were euthanized. The placenta, the fetuses and fetal plasma were obtained for evaluation. In paternal and fetal, the levels of plasma glycemia, triglyceridemia and cholesterolemia were evaluated by commercial kits. Lipid levels (by TLC) and mRNA levels of genes involved in lipid metabolism (by qPCR), were measured in the placenta of male fetuses from C and D rats. In the paternal plasma of D rats the levels of triglycerides and cholesterol were increased (39 and 21 %, respectively; $p<0.05$), as well as in fetal plasma of D group (39 and 21 %, respectively; $p<0.05$). Fetal weight was increased in D group (15 %; $p<0.05$), and placental weight was similar in both groups. The levels of triglycerides, cholesterol and free fatty acids were increased (46, 43 and 27, respectively; $p<0.05$) in the placenta of D group. The mRNA levels of PPARalpha and its co-activator PGC1alpha were increased in the placenta of D group (168 and 214 %, respectively; $p<0.001$). The mRNA levels of insulin-sensitive fatty acid transporter (FATP1) and endothelial lipase (LIPG) were also increased in the placenta of D group (95 and 106 %, respectively; $p<0.05$) when compared to C. Conclusion: Paternal diabetes induced by intrauterine programming leads to placental alterations in the lipid metabolism that could be related to the increase in the levels of lipids in fetal plasma. These metabolic alterations may lead to adverse consequences to the adult offspring.

0256 - REGULATION OF MTOR PATHWAY IN THE DECIDUA FROM DIABETIC RATS BY MATERNAL DIETS ENRICHED IN SUNFLOWER AND CHIA OIL DURING EARLY POSTIMPLANTATION

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Embryo development defects induced by maternal diabetes may start at early pregnancy when histotrophic nutrition occurs through the decidua. Leukine inhibitor factor (LIF) and insulin growth factor binding protein 1 (IGFBP1) participate in decidualization and development. Mammalian target of rapamycin (mTOR) pathway senses nutrient availability and is regulated by polyunsaturated fatty acids (PUFAs). The aims were to evaluate mTOR pathway, LIF and IGFBP1 gene expression in the decidua of diabetic rats at day 9 of pregnancy, and the effect of diets enriched in sunflower and chia

oil (enriched in n-6 and n-3 PUFAs respectively) at early postimplantation on these parameters. Diabetes was induced by streptozotocin (50 mg/kg) in female Wistar rats 2 weeks before mating. On days 7, 8 and 9 of pregnancy diabetic rats received a standard diet or diets enriched in 6% of sunflower or chia oil. In the decidua of 9-days-pregnant rats mRNA levels of LIF, IGFBP1 and mTOR were measured by RT-qPCR and protein levels of 4EBP1, rpS6 and AKT (proteins downstream mTOR pathway) were measured by Western blot. The mRNA levels of LIF, IGFBP1 and mTOR were reduced in the decidua of diabetic rats ($p<0.001$, 71 %; $p<0.05$, 34 %; $p<0.05$, 54 %; respectively). PUFA-enriched diets prevented the reduced IGFBP1 mRNA levels and sunflower enriched diet also prevented the reduced LIF and IGFBP1 gene expression. Phosphorylated protein levels of 4EBP1, rpS6 and AKT were reduced in the diabetic group ($p<0.05$, 27 %; $p<0.05$, 40 %; $p<0.05$, 14 %; respectively), with no changes in total protein levels. PUFA-enriched diets restored the phosphorylated/total levels ratio of these proteins. Conclusion: the inhibition of the mTOR pathway and the low expression of LIF and IGFBP1 suggest impairments in decidualization and regulation of histotrophic nutrition at early postimplantation in diabetic rats. These alterations were prevented by PUFA-enriched diets suggesting benefits of these dietary treatments for early postimplantation development in maternal diabetes.

0312 - CYTOPROTECTIVE ROLE OF HUMANIN AGAINST OXIDATIVE STRESS IN GRANULOSA CELLS

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INBIOMED- INSTITUTO DE INVESTIGACIONES BIOMÉDICAS. UBA-CONICET (1); CENTRO DE INVESTIGACIONES ENDOCRINOLÓGICAS "DR. CÉSAR BERGADÁ" (CEDIE)-CONICET (2)

Humanin (HN) exerts a cytoprotective action in the presence of pro-oxidative agents in several tissues. Previously, we reported that inhibition of endogenous HN increases apoptosis in the human granulosa-like tumor cell line (KGN). Also, we demonstrated that HN decreases ROS production in KGN cells exposed to pro-oxidative environment. In the present study, our aim was to evaluate the anti-apoptotic action of HN, as a cytoprotective mechanism against oxidative stress in granulosa cells. To explore this aim, we assessed the percentage of TUNEL positive KGN cells incubated with HN (1 µM) for 30 min and H_2O_2 (150 µM) for further 1 h. Our results demonstrated that HN decreased the percentage of KGN TUNEL-positive cells induced by H_2O_2 (C: 0.3, HN: 0.7, H_2O_2: 2.1, H_2O_2+HN: 0.7, *$p<0.01$ vs respective control without H_2O_2, ^$p<0.01$ vs. respective control without HN; X^2 test). Also, we examined the effect of HN on apoptosis in granulosa cells from ovaries of prepuberal and adult rats incubated in pro-oxidative environment. Thus, we incubated each ovary from prepuberal or adult rats with HN (1 µM) for 30 min and H_2O_2 (150 µM) for further 1 or 2 h, respectively. Contralateral ovaries from rats of each treatment were used as respective controls. We determined the number of apoptotic granulosa cells per follicle by TUNEL. In prepuberal ovaries, we observed that HN decreased the number of apoptotic granulosa cells per follicle induced by H_2O_2 (C: 2.5 vs. HN: 4.0 ns.; C: 1.6 vs H_2O_2: 7.0 $p<0.05$; H_2O_2: 9.9 vs. H_2O_2+HN: 6.5 $p= 0.075$. paired t test). In adult rats, HN seemed to decrease the number of apoptotic granulosa cells per follicle induced by H_2O_2 (C: 6.2; HN: 9.0; H_2O_2: 34.8; H_2O_2+HN: 18.3). To conclude, our results suggest that HN protects granulose cells against oxidative stress, at least in part, through an anti-apoptotic mechanism.

0390 - MATERNAL ADMINISTRATION OF SODIUM BUTYRATE PREVENTS MACROSOMIA AND LIVER LIPID OVERACCUMULATION IN FETUSES FROM OBESE RATS

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Maternal programming of metabolic alterations is considered a cause for the worldwide increase in obesity. Fetal macrosomia precedes the programming of metabolic derangements. Moreover, fetal liver lipid overaccumulation is a clear predictor of fatty liver later in life. Sodium butyrate (SB), a short chain fatty acid produced by the gut microbiota, plays an important role in metabolic homeostasis. It stimulates adipose lipolysis, liver lipid oxidation, glucose homeostasis and protects gut barrier function. Our aim was to evaluate whether maternal administration of sodium butyrate (SB) prevents the development of macrosomia and liver lipid overaccumulation in fetuses from obese rats. Methods: Female Wistar rats were fed with standard or saturated fat diet (28 % fat) since they were 6 week-old (FD rats). After 8 weeks, they were mated with control males. SB was given orally (3 %) to FD rats during pregnancy (FD+SB rats). Control, FD+SB, and FD rats were euthanized at 21 days of gestation. Fetuses were explanted, weighed and maternal plasma obtained. Plasma glucose, triglycerides (TG) and cholesterol levels were assessed by colorimetric assays. Fetal livers were explanted, weighed and saved for lipid levels evaluations by TLC (TG, Cholesterol (Ch) and Cholesterol Esters (Ch. E.) were assessed). Maternal TG were increased in plasma from FD rats (36 %vs. CT, $p<0.05$) and the administration of SB restored the control values in FD+SB rats (31 % vs. FD, $p<0.05$). FD fetuses were heavier than controls (7 %, $p<0.05$) and their livers showed lipid overaccumulation (TG: 140 % and Ch. E.: 144 %, $p<0.05$ vs. Control). SB prevented fetal macrosomia (7 %, $p<0.05$ vs. FD) and liver lipid overaccumulation (TG: 48 % and Ch. E.: 47 %, $p<0.05$ vs. FD). Conclusions: Butyrate is able to prevent fetal macrosomia and liver lipid overaccumulation, this will probably influence the metabolic health of the offspring in later stages of their life.

0414 - PLACENTAL AUTOPHAGIC AND EPIGENETIC MODIFICATIONS ASSOCIATE WITH FETAL GROWTH RESTRICTION.

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Intrauterine growth restriction (IUGR) is a condition whereby a fetus is unable to achieve its genetically determined potential size. Most of the adaptive responses of the placenta to support restricted fetal growth are thought to result in changes in epigenetic regulation. Furthermore, IUGR is associated with placental insufficiency, where altered trophoblast cells turnover and function contribute to reduced feto-placental growth. The aim of this work was (1) to explore placental histone methylation and acetylation profiles in a mice model of dexamethasone-induced IUGR and (2) to compare the differences in autophagy and apoptosis between normal and IUGR placentas. Pregnant Balb/c mice received 8 mg/kg (s.c.) of dexamethasone between days 14 and 15 of pregnancy. The control group was sham-treated with saline. Prenatal glucocorticoid treatment not only induced IUGR but also decreased placental weight. Placental tissue from pregnant animals was dissected on gestational days 15 to 18 and processed for western blot analysis. Term placentas (day 18) from IUGR fetuses showed increased levels of Histone 3 acetylation at Lys9 (H3K9) ($p<0.05$). We did not observe significant changes on the other epigenetic marks like H4K16 acetylation, dimethylation of H3K27 and trimethylation of H3K9, nor were histone deacetylase SIRT1 levels altered. Mice with IUGR presented higher placental levels of LC3B-II ($p<0.05$) on day 18 and lower Bcl2 protein levels ($p<0.05$) on day 15 compared with controls. There were no

significant changes in mTOR and Bax protein levels between groups. In summary, dexamethasone-induced IUGR is associated with placental changes in epigenetic marks, particularly we found an increase in H3K9 acetylation. In addition, dexamethasone treatment led to a decrease in the anti-apoptotic protein Bcl2 in placentas on day 15 of pregnancy. Furthermore, signs of augmented autophagy were found in placentas at term.

0437 - ENRICHMENT OF MATERNAL ENVIRONMENT PROTECTS THE OFFSPRING THROUGH CHANGES IN THE AMNIOTIC FLUID.

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CENTRO DE ESTUDIOS FARMACOLÓGICOS Y BOTÁNICOS (CEFYBO), UNIVERSIDAD DE BUENOS AIRES-CONICET

Maternal lifestyle affects both pregnancy outcome and maternal health. We previously demonstrated that the exposition to an enriched environment (EE), a non-invasive stimulus of the sensory pathway combined with voluntary physical activity, prevented from preterm birth induced by the administration of bacterial lipopolysaccharide (LPS) in a mouse model. Furthermore, mothers exposed to EE presented less perinatal death when compared to control environment (CE, standard cages) and EE also reverted some of the deleterious effects of the LPS during development. The amniotic fluid (AF) exerts several functions during pregnancy. It protects the fetuses by not only cushioning it from outside pressures but also having immunological functions. The aim of this work was to analyze physiological changes in the AF, associated to the protective effects of the EE on the offspring exposed to LPS. Animals were housed in EE (or CE) cages during 6 weeks and then mated with CE males. On day 15 of pregnancy, LPS was administered and 8h later, amniotic fluid was collected to evaluate several cytokines expression and cellular profile by flow cytometry. We found higher levels of IL-10, an anti-inflammatory cytokine, in AF from EE exposed females when compared to controls ($p<0.05$). It was not modified in any group by LPS treatment. In contrast, LPS induced a significant increase of IL-6 levels ($p<0.05$) (a pro-inflammatory cytokine) in AF from both groups. However, it was 3.6 times higher in CE exposed group when compared to EE. Furthermore, IL-22, involved in protective response against inflammation, was significantly increased by LPS in both groups ($p<0.05$), but it was 6.7 times higher in EE group. We analyzed the presence of B cells in the AF and found a higher percentage of this population in EE exposed mice compared to controls ($p<0.05$). Our results suggest that the enrichment of maternal environment modulates the AF components and response to systemic LPS-administration, protecting the offspring.

0523 - DIRECT EFFECT OF METFORMIN ON HEALTHY OVARIAN CELLS.

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INSTITUTO DE BIOLOGÍA Y MEDICINA EXPERIMENTAL (IBYME-CONICET)

Metformin (MET) is an oral antihyperglycemic drug introduced in the treatment of polycystic ovary syndrome (PCOS) to manage hyperglycemia. PCOS is a common disorder that affects women in reproductive age. MET improves ovulation, pregnancy and live birth rates in patients with PCOS. The mechanism by which MET of these effects are not fully understood. MET primary mechanism of action is through the activation of the AMP-activated protein kinase (AMPK), which acts as an energy sensor within the cell. The aims of the present work were to analyze a possible effect of MET on healthy rat ovary and on granulosa cells (GCs) in culture. Methods: For in vivo experiments, 21 d old female Sprague Dawley rats received MET (300 mg/kg) dissolved in the drinking water for 15 days (MET group). The control group received drinking water alone. Rats were killed on day 16 and the ovaries removed. Proteins were extracted for western blot analysis. For in vitro experiments, Sprague Dawley rats (21 d) were injected subcutaneously with diethylstilbestrol (1mg/rat) daily for three days. GCs were isolated by percoll gradient. GCs were stimulated with MET (0.01 ng/ml) with or without the organic cation transporter (OCT) inhibitor cimetidine (CIM). Cells were harvested 48 h later and proteins extracted. One Way ANOVA or t-test were used. p-AMPK was increased in the rat ovaries ($p<0.05$) and in GCs after stimulation with MET ($p<0.05$) while VEGF was decreased ($p<0.05$). Inhibition of OCTs by CIM reversed these effects ($p<0.05$ compared with MET). No changes in Angiopoietin 1 and 2 were found either in vivo or in vitro. Our results suggest that MET acts directly on ovarian cells regulating cell metabolism and VEGF expression, entering the cells through OCTs. Our findings are relevant to optimize PCOS fertility treatment and to explore direct ovarian MET actions in other female pathologies. These results provide new evidence to explain the effect of MET on infertility treatments.

0691 - ROLE OF VALOSIN CONTAINING PROTEIN (VCP/P97) IN MOUSE SPERM CAPACITATION

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Capacitation is a process that prepares mammalian sperm to undergo an exocytotic event called acrosome reaction (AR) which in turn, is an essential step of fertilization. The study and characterization of the proteins involved in these events is extremely important in order to understand the dynamics of the whole process. In the present work we evaluated the role of Valosin Containing Protein (VCP/p97) in mouse sperm. We found that VCP is localized in the equatorial segment and along the flagellum. In addition, we observed that VCP is cleaved and released during AR. In contrast to human sperm, VCP is not phosphorylated in tyrosine residues. To elucidate how VCP is involved in the capacitation process we used a pharmacological approach. Mouse sperm were incubated in capacitating conditions with or without VCP inhibitors. Several aspects of the capacitation such as phosphorylation of PKA substrates, tyrosine phosphorylation, AR or motility were evaluated. In these experiments, we used four VCP inhibitors: NMS-873, DBeq, CB-5083 and ML-240. By Western blot, we observed no significant differences in the levels of phosphorylation of PKA substrates. Surprisingly, we noticed that all four inhibitors completely abolished tyrosine phosphorylation although this inhibition could be bypassed by using cAMP analogs. Next, we evaluated AR using transgenic EGFP sperm and flow cytometry. We observed that the AR induced by progesterone is strongly inhibited by NMS-873. Finally, we study sperm motility using CASA with different concentrations of this inhibitor and in neither of these, the motility was significantly changed. Taken together, these results indicate that VCP plays an important role in mouse sperm capacitation and if inhibited, these cells cannot undergo AR. On the other hand, motility does not appear to be modified by VCP inhibition.

0853 - DEVELOPMENT OF A LC-MS/MS METHOD TO MEASURE SIMULTANEOUSLY 10 SEXUAL STEROIDS IN PEDIATRIC ENDOCRINOLOGY

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CENTRO DE INVESTIGACIONES ENDOCRINOLÓGICAS "DR. CÉSAR BERGADÁ" (CEDIE)-CONICET (1); JENCK (2)

Mass spectrometry (MS) allow the determination of a panel of steroids in small sample volume with superior specificity than immunoassays, important advantages in pediatric samples. To develop LC-MS/MS method to measure concomitantly Cortisol (F), Androstenedione (d4-A), Dehydroepiandrosterone (DHEA),

Progesterone (P4), 17OH-Progesterone (17OHP4), Pregnenolone (P5), 17OH-Pregnenolone (17OHP5), Testosterone (T), 17ßEstradiol (E2) and Dihydrotestosterone (DHT) in pediatric serum samples. An UHPLC-MS/MS system (Shimadzu-ABSciex QTRAP®6500) in Multiple Reaction Monitory mode (MRM) was used. Calibration curves covering pediatric ranges were used to test linearity. Internal standards were used to calculate recovery. Precision and bias were evaluated with human serum quality controls (Biorad). F, d4-A, DHEA, P4, 17OHP4, P5, 17OHP5, T, and E2 could be measured by APCI+ ionization; measurement of DHT resulted to be more sensitive by ESI+ ionization. Two MRM transitions per analyte (qualitative and quantitative), compound and source parameters were optimized. A 13-min (APCI) and 8-min gradient (ESI) allowing the separation without interferences were used. Sample preparation shown recoveries between 90 and 105 %. All curves shown linearity within the concentration range studied (r>0.99). LLD (signal/noise ratio= 3) and LLQ (signal/noise= 10) were: F: 0.013, 0.044 ug/dl, d4-A: 0.41, 1.37 ng/ml, DHEA: 0.37, 1.22 ng/ml, P4: 0.025, 0.08 ng/ml, 17OHP4: 0.005, 0.018 ng/ml, P5: 0.23, 0.76 ng/ml, 17OHP5: 1.95, 6.49 ng/ml, T: 0.56, 1.87 ng/ml, E2: 20.5, 69 pg/ml, DHT: 0.13, 43 pg/ml, respectively. CV % lower than 5 % were obtained in the reportable pediatric range for F, d4-A, T, DHEA, 17OHP4 and P4. A sensitive and selective clinical research LC-MS/MS method has been developed for concomitantly measurement of 10 sexual steroids in serum. Estradiol measurement performance must be improved in terms of sensitivity to be applied in pediatric setting.

0888 - REGULATORY TRANSCRIPTIONAL DOMAIN OF KRÜPPEL-LIKE FACTOR 6 (KLF6) IS NECESSARY FOR PLACENTAL TROPHOBLAST CELLS FUSION

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CENTRO DE INVESTIGACIONES EN BIOQUÍMICA CLÍNICA E INMUNOLOGÍA (CIBICI-CONICET). UNC.

Placental development and maintenance require the fusion of villous cytotrophoblasts (vCTB) to generate the syncytiotrophoblast layer involved in the placental barrier function, synthesis of critical proteins and nutrient exchange between the mother and the fetus. The Krüppel-like factor 6 (KLF6) is highly expressed in placenta and Klf6-/- mice die at day E12.5 showing impaired placenta development. Previously, we have demonstrated that KLF6 modulates the expression of the cell cycle inhibitory protein p21, of Syn-1, and ß -hCG, and it is required for cell–cell fusion in human primary vCTB as well as in the BeWo trophoblast-derived cell line. More importantly, KLF6 is sufficient to trigger cell fusion in BeWo cells. Herein we analysed the KLF6-dependent mechanisms involved in KLF6-induced fusion. Stable BeWo cell lines containing a KLF6 mutant that lacks the acidic domain required for its transcriptional activity (KLF6deltaac-BeWo), the full-length KLF6 protein (KLF6-BeWo), or the empty-vector (EV-BeWo) were generated. KLF6deltaac-BeWo cells were treated or not with 30 µM forskolin and BeWo-KLF6 cells were transduced with KLF6deltaac lentivirus particles and cell fusion was analysed by immunofluorescence after 72 h. Transcript and/or protein levels of Syn-1, ß-hCG, Cx43, ABCG2, Gal-1, and GRP78 were measured by qRT-PCR and Western blot, respectively. KLF6-BeWo cells were treated with a specific p21 siRNA or a scramble siRNA as control. KLF6 overexpression induced the formation of syncytial structures and increased the expression of molecules involved in this process. While, KLF6deltaac did not promote syncytialization, impaired forskolin-induced cell fusion, and decreased Syn-1 and ß-hCG expression. Moreover, cell fusion was reduced in KLF6-BeWo cells transduced with the deletion mutant lentivirus or silenced for p21. These results suggest that KLF6 induces vCTB syncytialization through a mechanism that involves its regulatory transcriptional domain in a p21-dependent manner.

Endocrinología/ Endocrinology IV

Chairs: Florencia Labombarda | Mariela Urrutia

0112 - COMBINED STAT5B AND IGFALS HETEROZYGOUS GENETIC VARIANTS IN A PATIENT WITH PARTIAL GROWTH HORMONE INSENSITIVITY

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CENTRO DE INVESTIGACIONES ENDOCRINOLÓGICAS "DR. CÉSAR BERGADÁ" (CEDIE)-CONICET (1); HERITAS-CONICET (2)

Monogenic causes of growth hormone insensitivity (GHI) include defects in genes encoding the GH receptor itself (GHR), the signal transducer and activator of transcription (STAT5B), the insulin like-growth factor type I (IGF1) and the acid-labile subunit (IGFALS). Affected children could be severely growth retarded, although mutations in heterozygous state in some of these genes were associated to a milder clinical phenotype. We aimed to characterize the molecular defect in a patient with short stature and partial GHI. Patient and methods: The boy was born at term adequate for gestational age from non-consanguineous normal-stature parents. At 2.2 years, he presented proportionate short stature (height -2.77 SDS), wide forehead and normal mental development. Whole-exome analysis and functional characterization (dual luciferase reporter assays, immunofluorescence and, western immunoblot) were performed. Biochemical and endocrinological evaluation revealed partial GH insensitivity with normal stimulated GH peak (7.8 ng/ml), undetectable IGF1, and low IGFBP3 levels. Two heterozygous variants in the GH-signaling pathway were found: a novel heterozygous STAT5B variant (p.K632N) and a hypomorphic IGFALS variant (p.R548W). In vitro studies demonstrated that mutation p.K632N impairs STAT5b phosphorylation, nuclear translocation and transcriptional activity. Conclusions: In short children with GHI, genetic testing is recommended. We present compelling evidence to support p.K632N as a novel inactivating STAT5b variant. The combination of heterozygous IGFALS and STAT5B variants contributes to the patient's partial GHI phenotype.

0195 - NEUROPROTECTIVE EFFECTS OF OXALIS ERYTHRORHIZA IN DIABETES MELLITUS

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In the last decades, the exploration of phytomedicine became an important issue to find new therapeutic treatments. In the Cuyo region (Argentine), Oxalis erythrorhiza Gillies ex Hooker et Arnott (Oxalidaceae; Oe) is popularly consumed as a "medicinal plant" for hepatic and heart complains even when these effects lack scientific support. Recently, chemical characterization of aereal parts of Oe revealed the presence of compounds with potential antidiabetogenic properties. Otherwise, the diabetes mellitus represents an important health concern, as involved chronic hyperglycemia, oxidative stress state and neurodegenerative changes. Previously, we showed that Oe decoction improves the increase in neurofilaments (NF200) observed in the hypothalamus of rats with experimental diabetes. In this work, adult male rats, controls (C, i.p. veh) or diabetics (D, i.p. STZ 30mg/Kg) drunk Oe

decoctions at 5 % (COe5/DOe5), 10 % (COe10/DOe10), or water (CW/DW) by 4 weeks. nNOS and NF200 expressions levels were studied by WB in hippocampus and cerebral cortex. Compared to CW, DW presented raises in nNOS and NF200 expressions in hippocampus (32.50 and 33.50 % respectively; $p<0.05$) and cerebral cortex (33.50 and 53.50 % respectively; $p<0.05$). DOe5 presented lower nNOS and NF200 values than DW (14 and 22 % respectively; $p<0.05$) in the hippocampus, and NF200 in cerebral cortex (28 %; $p<0.05$). Otherwise, compared to DW, DOe10 reduced the NF200 expression only in the hippocampus (25 %; $p<0.05$). These results suggest that Oe could have metabolic and neuroprotective effects on these brain areas. Thus, a more extended treatment will be necessary to propose Oe like new source of therapeutic phytocompounds.

Supported by CONICET-PIP00243, PIO-SECITI2250, CICITCA UNSJ and PIO-CONICET SECITI 022 grants.

0258 - ORAL TREATMENT WITH METFORMIN PREVENTS THE EFFECTS OF THE EXPERIMENTAL METABOLIC SYNDROME ON THE PROLIFERATION AND DIFFERENTIATION OF MURINE DENTAL PULP PROGENITOR CELLS.

Ayelen Daniela LENZI | María Virginia GANGOITI

LABORATORIO DE OSTEOPATIAS Y METABOLISMOS MINERAL (LIOMM), FACULTAD DE CIENCIAS EXACTAS, CICPBA-UNLP

Metabolic syndrome (MS) affects different systems, including bone and mesenchymal stem cells, although the effects on dental pulp progenitor cells (DPPC) have not yet been studied. We previously demonstrated that oral metformin induces osteogenic effects in normal rats, promoting osteoblastic differentiation of bone marrow progenitor cells. The hypothesis of this work is that the induction of MS could be associated with alterations in the osteo/ odontogenic potential of DPPC, and consequently the tissue regeneration capacity could be diminished. In addition, we propose that metformin could partially or totally prevent these alterations. DPPC were isolated from adult rats. Animals received water ad libitum, control (C) or 20 % fructose (F) for 3 months. In addition, half of the animals were treated with 100 mg/kg/day of metformin (CM and FM) in the drinking water. Animals were weighed and sera were analyzed. An increase in weight, glycemia and triglyceridemia was observed in group F (vs. C) compatible with the development of MS, which tended to normalize by metformin treatment in the FM group. DPPC were grown in DMEM-20% FBS medium (basal condition) until confluence and sub-cultures in multiwell plates for experiments. Proliferation (violet crystal) and actin cytoskeleton (Alexa fluor phalloidin) of the basal cells were evaluated, and it was found that the DPPC-F had a lower growth rate and an alteration in the organization of their cytoskeleton in comparison with the DPPC-C. The DPPC isolated from CM and FM groups presented similar behaviors to the control cells. For each experimental group, the activity of alkaline phosphatase (enzymatic assay), the production of type 1 Collagen (Sirius red) and the accumulation of extracellular mineral (Alizarin red) were evaluated after 7, 14 and 21 days of differentiation in osteogenic medium (basal medium supplemented with ascorbic acid and ß-glycerophosphate). After osteogenic induction, the osteo/odontogenic potential of DPPC-F suffered a significative decrease. These cells expressed lower levels of ALP, produced lower amounts of type 1 collagen and mineralization nodules than obtained from C rats. Metformin administration (FM group) completely prevented the fructose-induced decrease in all of these parameters. Additionally, possible changes in the expression of the two major transcription factors involved in osteogenic and adipogenic differentiation, Runx2 and PPARgamma, as well as characteristic differentiation markers such osteocalcin and dentinsialoprotein, were evaluated by RT-PCR. It was found that DPPC-F presented a lower Runx2/PPARgamma ratio than de DLPC-C, whereas DPPC-CM and DPPC-FM presented similar values of ratios than control. At last, the expression of osteo/odontogenic markers decreased in DPPC obtained from group F, while treatment with metformin partially prevents these effects. These results confirm that the fructose-induced MS in rats decreases the proliferative and osteo/odontogenic potential of dental pulp progenitor cells. They also show that these deleterious effects can be completely or partially prevented by prolonged treatment with oral metformin.

0282 - MITOCHONDRIAL PHOSPHOENOLPYRUVATE CARBOXYKINASE IS EXPRESSED IN PP- BUT NOT BETA-CELLS FROM HUMAN PANCREATIC ISLETS

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Mitochondrial phosphoenolpyruvate carboxykinase (M-PEPCK) is a cataplerotic enzyme that positively modulates glucose-stimulated insulin secretion in rodent beta-cells. However, M-PEPCK expression has not been addressed in human beta-cells. We hypothesized that M-PEPCK is expressed in human beta-cells, and used immunohistochemical techniques to prove it, comparing with rodent pancreas. For this end, pieces of pancreas from Sprague Dawley male rats, C57BL/6 beta-cell-ablated male mice, and cancer patients were fixed in formalin. Multiple immunofluorescence analysis of M-PEPCK and hormones specifically secreted by each cell population in the Langerhans' islets revealed the expression of M-PEPCK in insulin-producing beta-cells in both rat and mouse pancreas, as previously reported. However, we detected higher levels of M-PEPCK immunoreactivity in another endocrine cell population: PP-cells that secrete pancreatic polypeptide (PP). In fact, the same analysis in beta-cell-ablated mice (due to exclusive expression of human receptor for diphtheria toxin in beta-cells, and death of beta-cells upon treatment of mice with diphtheria toxin) showed that M-PEPCK reactivity and co-localization with PP is still high in the pancreas lacking beta-cells. Most significantly, we observed that M-PEPCK co-localizes with PP but not with insulin in biopsies from cancer patients. We conclude that M-PEPCK is not expressed in beta-cells from human pancreas, suggesting that extrapolation of data about M-PEPCK function in rodent pancreas should be reconsidered. In contrast, higher levels of M-PEPCK in PP-cells from both human and rodent pancreas suggest that this enzyme plays a role in PP secretion. This is the first study where the expression of M-PEPCK is addressed in human pancreas. Further studies are needed to understand M-PEPCK contribution to PP-cell metabolism. We postulate that M-PEPCK modulates PP production and/or secretion, contributing to nutrient homeostasis. This hypothesis is currently under investigation.

0288 - THE HYPOTHALAMIC EXPRESSION OF GONADOTROPIN-RELEASING HORMONE IS MODULATED BY THE COMBINED ACTION OF GLUTAMATE AND ESTRADIOL IN THE SOUTH AMERICAN PLAINS VIZCACHA (LAGOSTOMUS MAXIMUS).

Victoria FIDEL | Pablo Ignacio Felipe INSERRA | Sofia PROIETTO | Santiago Andrés CORTASA | Alejandro Raúl SCHMIDT | María Clara CORSO | Alfredo VITULLO | Julia HALPERIN | Verónica Berta DORFMAN

CENTRO DE ESTUDIOS BIOMÉDICOS, BIOTECNOLÓGICOS, AMBIENTALES Y DE DIAGNÓSTICO-UNIVERSIDAD MAIMÓNIDES

Lagostomus maximus shows peculiar reproductive features like reactivation of the reproductive axis during pregnancy with follicular recruitment and ovulation. Previously, we showed that hypothalamic expression of gonadotropin-releasing hormone

(GnRH) of vizcacha correlates with serum estradiol (E2), estrogen receptors (ER) and N-methyl-D-aspartic acid receptor (NMDAR) expression during gestation. In addition, we observed that glutamate (GLU) down regulates GnRH delivery through NMDAR. Here we investigated the interaction between E2 and NMDA over GnRH expression in the hypothalamus of vizcachas in order to analyze their role on GnRH expression during gestation. We developed two approaches: 1- Hypothalamic explants of non-pregnant adult female vizcachas incubated with: a) GLU with or without (±) GLU receptors antagonists, b)NMDA ± NMDA-R antagonist CGP, c) E2 ± ERa or ERb antagonists, d) E2 ± NMDA ± CGP. GnRH mRNA levels were studied by RT-PCR; n=5/group. 2- Non-pregnant adult vizcachas ovariectomized (OVX) and treated with E2 (5 ug/kg); n=5/group. GnRH and NMDAR1 hypothalamic expression was evaluated by immunohistochemistry. We determined significant induction of GnRH mRNA expression by E2 and REa agonist related to control and REb agonist ($p<0.01$). In the contrary, we observed a significant decrease in GnRH mRNA levels induced by NMDA, and it was canceled by CGP ($p<0.005$). The combination of E2 with NMDA did not induce significant changes. On the other hand, hypothalamic protein expression of GnRH and NMDA showed significant increments of both neuromodulators in arcuate nucleus and medial eminence of OVX+E2 related to OVX and SHAM ($p<0.05$). These results suggest that GnRH expression is modulated by the combined action of E2 and GLU with opposite effects. This could represent a fine system of regulation of hypothalamic function over the reproductive axis of the female vizcachas.

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0297 - SIMILAR EFFECTS OF ESTRONE AT VASCULAR AND BONE TISSUES: ¿RISK OR BENEFIT?

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It is known that the decrease in estrogen levels during menopause negatively affects bone tissue and vascular function. We have previously shown that E_1 modulates the cellular events involved in atherogenesis such as vascular smooth muscle cells (VSMC) proliferation and migration, and monocyte/platelet adhesion to endothelium. Vascular calcification represents the advanced stage of atherogenesis, where VSMC transdifferentiation on bone like cells plays a crucial role. The aim of this work was to compare the effect of 10 nM E_1 (48 h) on murine calvarial osteoblasts (OB), and on vascular smooth muscular cells transdifferentiated into osteoblasts (VSMC-OB) by incubation in a procalcification medium (10 mM glycerophosphate) for 21 days. We focused our attention on cell differentiation. E_1 increased cell proliferation on both cellular types, either using MTT assay (39 vs. 27% a/each C, OB vs. VSMC-OB, $p<0.05$) or cell counting technique (41 vs. 28 % a/each C, $p<0.05$; OB vs. VSMC-OB). On OB, E_1 treatment stimulated ALP activity (4.64 ± 0.32 vs. 3.37 ± 0.25; E_1 vs C; $x10^{-2}$ IU/mg prot., $p<0.001$), calcium deposition (40.2 % a/C, $p<0.05$; alizarin red staining) and collagen in extracellular matrix visualized by Sirius red staining (21 % a/C, $p<0.05$). Similar results were obtained when CMLV-OB cells were employed. E_1 enhanced ALP activity (3.72 ± 0.25 vs. 3.00 ± 0.14; E_1 vs. C, $x10^{-2}$ IU/mg prot, $p<0.001$) and the number and size of calcification nodules in extracellular matrix (56 % a/C, $p<0.05$). Simultaneously, E_1 decreased calcium content in culture medium (735.0 ± 30.5 vs. 468.2 ± 21.1; C vs. E_1, µg Ca/mg prot., $p<0.001$). Indeed E_1 enhanced extracellular collagen deposition in CMLV-OB cells (19 % a/C, $p<0.05$). The results presented in this work show a similar action of E_1 in both cellular systems. The data suggest an opposite physiological relevance: a beneficial action at bone level favouring osteoblastogenesis and, a deleterious one at vascular homeostasis, promoting vascular calcification.

0308 - ESTROGEN RECEPTOR AND ENDOTHELIAL DYSFUNCTION

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Estrogen receptor (ER) plays key role on vascular homeostasis. Alterations of ER signaling could conduct to vascular dysfunction, impairment of angiogenesis and platelet aggregation and activation. Preeclampsia (PE) is a pregnancy disease that exhibits these features. We have previously reported that the ER activation by estrone (E1), second natural estrogen, stimulates vasodilators synthesis (NO and PGI2) and inhibits platelet aggregation. This work presents basic (1) and clinical (2) results about the association of ER and vascular dysfunction. 1) Endothelial cells (EC) were incubated with E1 10 nM for 24 h. (2) We tested the existence of association between polymorphisms of ESR1 with PE in a high-risk pregnant women population. Polymorphic variants were studied by RFLP-PCR, employing PvuII and XbaI and resolved by electrophoresis in agarose gels. The in vitro treatment of EC with E1 shows that the hormone stimulated EC growth (91 % a/C, $p<0.001$) and VEGF synthesis. ICI 182780 (RE antagonist), suppressed E1 action. In pregnancy women, the frequencies determined by PvuII was 29 % 1 (C/T); 59 % 2 (T/T) and 12 % 3 (C/C). When XbaI was used the distribution was 35.3 % A (A/G); 2 % B (A/A) and 62.7 % C (G/G). The main haplotype determined was 2C. In contrast, in young women without PE risk the main was 1A. PE pregnant women exhibits a significant reduction in plasmatic NO levels respect to the whole pregnant population (0.15 ± 0.024 vs. 0.29 ± 0.040 mM NO/plasma resp.) Results shows that E1 and ER have relevant action on cellular processes involved in endothelial dysfunction. Since the distribution of ER genotypes is different among young women with and without risk of endothelial dysfunction, it could suggest a possible clinical utility of these markers as PE risk predictors.

0403 - ONCOGENIC POTENTIAL OF PROLONGED GH-ADMINISTRATION IN ADULT MICE LIVER

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Growth hormone (GH) is given to children with growth impairment and to adults under catabolic states, even if they are not GH-deficient. Chronic exposure to elevated GH levels induces pro-oncogenic liver pathology; GH-overexpressing transgenic mice develop liver tumors at advanced ages. We had evaluated the effect of 5-week GH-treatment, given with the hepatic tumor inductor diethylnitrosamine (DEN), on tumor formation in growing male mice liver. GH did not promote tumor formation, nor did GH given with DEN increase the number of hepatic lesions in growing mice. The aim of this study was to assess if the same prolonged GH pharmacological treatment would induce any alterations when given to 5 month-old mice. Livers (n= 7-9) were collected at 48 weeks of age and visually inspected. GH-treatment alone did not induce visible lesions. DEN treatment induced liver tumor formation, whereas combination with GH did not promote further tumor development. Microscopical evaluation revealed that only DEN-treated groups exhibited dysplastic foci, although non-significant differences were attained with GH-treatment. The number of hepatocytes per microscopic field was increased in the dysplastic foci compared to the surrounding tissue ($p<0.05$), denoting smaller cell size. Mice treated with GH exhibited a significantly lower hepatocyte count per microscopic field ($p<0.05$),

indicating cell enlargement, regardless of DEN treatment. Proliferating cell nuclear antigen (PCNA) was determined by immunohistochemistry to assess hepatocellular proliferation. GH-treated groups exhibited a non-significant increase of PCNA positive nuclei. Increased cell proliferation was also observed inside dysplastic foci, although differences were significantly only for animals that did not receive GH treatment ($p<0.05$). Therefore, similar to that observed in growing mice, prolonged GH-administration to adult mice per se does not promote tumor formation, nor is it fostered by tumor inductor adjuvant treatment.

0585 - BENZOPHENONES 2 (BP2) AND 3 (BP3) AFFECT CELLULAR ADAPTIVE RESPONSES IN THE PANCREATIC BETA CELL LINE MIN6B1 IN THE PRESENCE OF THE AUTOPHAGY INHIBITOR CHLOROQUINE

Florencia SZULAK | Marina Olga FERNANDEZ | Damasia BECU DE VILLALOBOS | **Eleonora M. SORIANELLO**

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Benzophenones used as ultraviolet light blockers in plastic packaging of food and in sunscreens, are considered endocrine disruptors. In addition, autophagy is a mechanism of degradation and recycling of cellular components essential for cell homeostasis. In pancreatic beta cells autophagy has a fundamental role in relieving endoplasmic reticulum (ER) stress caused by misfolded proteins, including insulin. Our research focused on studying the effect of BP2 and BP3 on mouse pancreatic beta cell line MIN6B1 function in the presence of the autophagy inhibitor Chloroquine (CQ). The results showed that basal insulin secretion was inhibited by the lysosomotropic compound CQ (10 µM), and also by BP3 (10-5 M) both when incubated alone and in the presence of CQ. In addition, CQ triggered an adaptive response involving induction of genes related to lysosomal biogenesis, Lamp2, or autophagy, Sqstm1/p62. Interestingly, BP3 significantly reverted the induction of Lamp2 and showed a strong tendency to counteract the induction of Sqstm1/p62 by CQ, in addition to decreasing the mRNA levels of the autophagy marker Ulk1 both basally and in the presence of CQ. BP2 (10-5 M) only reverted the induction of Lamp2. Interestingly, these effects failed to alter the protein levels of LC3II or SQSTM1/p62, or the autophagic flux itself. Regarding ER stress markers, BP3 decreased the transcription of Xbp1 and its spliced form, and counteracted the induction of Chop and Grp78/Bip triggered by CQ. Likewise, BP2 partially reverted the induction of Grp78/BiP mRNA by CQ. We conclude that benzophenones, mainly BP3, and to a lesser extent BP2, counteract adaptive responses related to autophagy, lysosomal biogenesis and reticulum stress, in a condition of lysosomal stress and autophagy block caused by CQ. Since BP3 also inhibited basal insulin secretion, we suggest that both BP2 and BP3 alter the function of the pancreatic beta cell.

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0642 - BLOCKING GABAB RECEPTORS (GABABR) FROM BIRTH TO WEANING INDUCES PROFOUND CHANGES IN THE GONADOTROPIC AXIS IN ADULT MICE

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We have previously shown that administration of GABAB antagonist (CGP55845) to neonatal mice from postnatal day (PND) 2 to weaning (PND21) significantly decreased arcuate nucleus (ARC) kisspeptin (Kiss1) expression and increased dynorphin (Pdyn) expression in both sexes on PND21. Here our aim was to evaluate the effect of this sustained inhibition of GABABR signaling on the gonadotropic axis in adulthood. Neonatal Balb/c males (M) and females (F) were injected with CGP55845 (1 mg/kg, s.c., CGP) or saline from PND2-21, three times/day (8 AM, 1 PM, 6 PM) and sacrificed in adulthood. Serum samples and gonads were collected for hormonal measurements (RIA). Brains were frozen and 500 µm slices were obtained on a cryostat. ARC and anteroventral periventricular nucleus (AVPV) micropunches were obtained. Kiss1, Pdyn, neurokinin B (Tac2), tyrosine hydrolxylase (Th), progesterone receptor (Pgr) and GnRH (Gnrh1) mRNA expression was assessed in the micropunches by qPCR. Body weight (BW) and AGI (anogenital distance/BW) were evaluated on PND 7, 14 and 21. Puberty onset was determined by vaginal opening (VO) or preputial separation (PS). CGP significantly increased BW on PND21 in F ($p<0.02$) while BW was decreased by CGP at all ages studied in M ($p<0.001$). CGP significantly decreased AGI in both sexes (F: $p<0.001$; M: $p<0.02$). CGP decreased Kiss1 ($p<0.01$), Tac2 ($p<0.02$), Pdyn ($p<0.04$) and Pgr ($p<0.01$) in ARC of both sexes. In AVPV from CGP-treated F, Th was significantly decreased ($p<0.01$) and a near significant decrease in Pgr was observed ($p<0.07$). FSH was increased in CGP-treated M ($p<0.04$). In addition, CGP increased gonad progesterone ($p<0.01$) and testosterone ($p<0.002$) in F. These results demonstrate that sustained inhibition of GABABR signaling during a critical postnatal period of development and maturation of the gonadotropic axis profoundly alters many parameters of this axis in adulthood. Supported by: CONICET, ANPCYT, UBA, Fund. René Barón and Fund. Williams.

0676 - PRENATAL D-AMPHETAMINE EXPOSURE INFLUENCES PROLACTIN SYNTHESIS AND SECRETION IN RESPONSE TO STRESS AND ESTROGEN IN ADULTHOOD.

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The dopaminergic system, a main regulator of prolactin (PRL) secretion, is closely involved in the responses to stress and amphetamine. The ovarian steroids modulate PRL secretion and several aspects of stress responses. Previously, we found that prenatal exposure to amphetamine (PEA) induced changes in pituitary mRNA D2R expression during adulthood. Our present aim was to study PEA effects and the influence of estrogen (E2) on pituitary D2R expression (protein) and their correlation with pituitary PRL content (mRNA and protein levels) in adult OVX rats in response to stress. Moreover, we also explored the role of E2 in response to stress on the expression of de pTH-Ser 40 in the medium basal hypothalamus (MBH) of OVX adult rats in PEA animals. For these purposes, female Wistar rats were treated daily with D-amphetamine 2.5 mg/kg i.p./saline during days 15 to 21 of pregnancy. Their female offspring were OVX at day 60; 15 days later treated with estrogen/oil (E2; 2 x 5 µg/rat/24 h) and exposed to immobilization stress during 30 min. Blood and tissue samples were collected for corticosterone by RIA and pituitary D2R and PRL content by real time PCR and Western blot (WB) determinations. pTHSer-40 expression was determined by WB in MBH extracts. Data were analyzed using two-way ANOVA and Student´s-t test. Pituitary D2R expression (protein) was diminished only in PEA OVX+E2 rats ($p<0.05$) and no effects of stress were observed. E2 increased PRL mRNA levels in control and PEA OVX rats ($p<0.05$) and prevented the effect of stress. Stress diminished PRL pituitary content (protein) in control rats with E2 and the PRL protein in PEA rats independent of E2 treatment ($p<0.05$). Moreover, stress decreased MBH p-TH in PEA OVX+E2 rats ($p<0.05$). Thus, prenatal treatment with D-amphetamine sensitizes the hypothalamus-pituitary system affecting PRL synthesis and secretion in response to stress and E2.

0717 - PROLONGED EXPOSURE TO GROWTH HORMONE (GH) AND IMPAIRED INSULIN SIGNALING IN THE HEART: EVIDENCE FROM GH-TRANSGENIC AND LIVER IGF1-DEFICIENT (LID) MICE

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We have previously reported that 7-month-old transgenic mice overexpressing growth hormone (GH-Tg) exhibit impaired insulin signaling in the heart, which could be related to the cardiomegaly they exhibit. Liver IGF1-deficient (LID) mice, which have a marked reduction in circulating IGF1 levels with a consequent increase in serum GH concentration, also display impaired insulin signaling in the heart. However, cardiomegaly was not observed in the LID mice studied, which were 2-4 months old. This could be due to the difference in age of the animals studied or to the IGF1 circulating levels, which are increased in GH-Tg and decreased in LID mice. Therefore, the first objective was to assess if GH-Tg mice of 2-4 months old display the same alterations previously described for older animals, in order to compare the results with those obtained for LID mice. Moreover, downstream signaling mediators that had not been studied were also analyzed. The second aim of this work was to evaluate possible mechanisms implicated in the impaired insulin signaling. Mice received an insulin injection, the heart was removed after 5 min, and immunoblotting and ELISA assays of tissue extracts were performed. Insulin-induced phosphorylation of the insulin receptor and downstream signaling mediators including IRS1, Akt, GSK3ß and AS160 were decreased in the heart of GH-Tg and LID mice compared to normal controls. This was associated with higher basal phosphorylation of MAPK p38 and Erk1/2 in LID mice, and only of p38 in GH-Tg mice ($p<0.05$). Phosphorylation of IRS1 on Ser307, an inhibitory modification, was increased in GH-Tg and decreased in LID mice ($p<0.05$). Young adult GH-Tg mice displayed cardiomegaly, in contrast to LID mice ($p<0.05$). We conclude that although exposure to GH impairs insulin signaling in the heart, the molecular mechanisms involved and the associated cardiomegaly may vary depending on the circulating levels of IGF1.

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Chairs: Ricardo Cabrera | María Laura Palumbo | Carlos Pomilio

0080 - EVIDENCE OF IMPAIRED MICROGLIAL AUTOPHAGY IN ALZHEIMER'S DISEASE: FROM IN VITRO MODELS TO PATIENTS.

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by the presence of extracellular amyloid plaques, mainly composed by Amyloid-ß peptides (Aß). Microglial activation plays a crucial role in the clearance of Aß, and other studies suggest that microglial autophagy may also participate in this function. Our aim was to study whether microglial autophagy could be affected during AD progression, using in vivo and in vitro models. The hippocampus of aged PDAPPJ20 mice –a validated animal model of AD– showed hyper reactive Iba1+ cells around plaques. Eighty nine % of microglial cells exhibited the lysosomal phagocytic marker CD68 in control aged mice, which is decreased to 76 % in AD ($p<0.05$, n= 4), also showing a significant three-times accumulation of ubiquitinized proteins (n= 4, $p<0.001$) located inside autophagosomes as evidenced by co-localization with p62. We also exposed BV2 microglial cells to fibrillar Aß peptides during a short or a long period of time. A short exposure promoted a significant increment in autophagic flux, as evaluated by WB against LC3 (n= 5, $p<0.05$). When Aß exposure was longer -a condition closer to a chronic disease like AD-, autophagic flux was impaired, suggesting an accumulation of autophagosomes, which was also verified by transfection with a plasmid carrying LC3-GFP-mCherry (0.08 M2 index in control vs. 0.16 in treated, n= 30 cells, $p<0.01$). In addition, the long exposure to Aß caused a 2.3-times increment in the percentage of cells exhibiting membrane permeability to lysotracker staining (9.1 ± 2.0 OD vs. 15.7 ± 3.7, $p<0.01$), which is compatible with lysosomal dysfunction. Taken together, our results suggest that fusion between autophagosomes and lysosomes may be impaired during the progression of experimental AD. Moreover, preliminary data analyzing human brain tissue showed the presence of LC3 puncta on microglial cells from AD patients, emphasizing that autophagy deficiency on glial cells may contribute to pathophysiology of AD.

0166 - PARTICIPATION OF THE CD44 RECEPTOR AND GENES IMPLICATED IN HYALURONAN METABOLISM IN COGNITIVE DEFICIT OF CHRONICALLY STRESSED MICE.

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We demonstrated that chronic mild stress (CMS) induce cognitive deficit and a decrease in hippocampal neurogenesis in Balb/c mice. Furthermore, the glycosaminoglycan hyaluronan (HA) is a key component of the extracellular matrix (ECM) in the brain. HA plays an important role in physiological and pathological process, like neuronal development, synaptic plasticity and neurodegeneration. HA is synthesized by HA synthases (HAS1-3) and degraded by hyaluronidases (Hyal1-3). CD44 is the main receptor for HA and it is related to neurological disorders. The aim of this work was to study the effects of the CMS on learning and memory in female C57BL/6J CD44+/- and the expression of CD44 and genes implicated in the metabolism of HA in the hippocampus of female Balb/c mice. We found a decrease in learning and memory in CD44+/- CMS mice respect to WT and CD44+/- control mice in Y-maze (% spontaneous alternation: $p<0.05$). CD44+/- CMS mice showed a reduced habituation capacity in open field test at 24 h compared to 0h in the number of crossings (% habituation 0 vs. 24 h= WT control: 39 %, p= 0.04; WT CMS: 48 %, p= 0.02; CD44+/- control: 61 %, p= 0.01 CD44+/- CMS: 25 %, p= ns). In female Balb/c mice, we found a poor performance in the Y-maze in CMS mice respect to control mice (p= 0.01). Mice exposed to CMS have a significant decrease in the mRNA levels of Hyal1 (p= 0.04), Hyal2 (p= 0.04) and HAS1 (p= 0.01) and a significant increase in mRNA level of HAS2 (p= 0.01) compared to control mice, analyzed by RT-qPCR. We did not find a significant difference in CD44 and Hyal3 mRNA levels in stressed mice. These results indicate that the reduced expression of CD44 in C57BL/6J mice exposed to chronic stress is related to cognitive deficit. In CMS Balb/c mice, impairment in learning and memory could be related to a differential expression of the genes involved HA synthesis and catabolism. This finding could provide new tools in order to understand the function of the ECM in cognitive deficit.

0188 - THE ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTOR SUBTYPE 3 MODULATES

THE EXPRESSION OF CD44 RECEPTOR IN ASTROCYTES.

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The glutamatergic transmission has been postulated as the pathologic base of several degenerative disorders. The prevention of excitotoxicity by subtypes 2/3 metabotropic glutamate receptors (mGluR) activation is considered promising in psychiatry. The astrocytes present mGlu3R and remove the glutamate from the synaptic space through specific transporters, thereby avoiding excitotoxicity resulting from glutamate excess. Furthermore, strong positive correlations were found between CD44 (transmembrane receptor for the glycosaminoglycan hyaluronan (HA)) and genes of the glutamate–glutamine cycle typically expressed in astrocytes. Following damage in the central nervous system, HA (main component of the extracellular matrix in the brain) is synthesized at high levels by reactive astrocytes. HA is synthesized by HA synthases (HAS 1-3) and degraded by hyaluronidases (Hyal 1-3). The aim of this work is to study the expression of the CD44, HAS and Hyal and the HA levels in cortical astrocytes of rats in presence of LY379268 (agonist of mGlu3R). The cortices of postnatal P0-2 Wistar rats were cultivated during 3 weeks to obtain a culture of astrocytes, which were treated with (+LY) and without (-LY) 0.1 µM LY379268 during 24 h. We found a decrease in the expression of CD44 in astrocytes +LY ($p = 0.033$, $n=4$) respect to –LY analyzed by RT-qPCR. However, no significant differences were observed in the Hyal 3 expression between astrocytes +LY and –LY. Hyal 1-2 and HAS 1-3 mRNA levels were undetectable. The HA levels in astrocytes supernatants +LY and –LY did not show significant differences by ELISA. We conclude that the activation of mGlu3R by LY379268 in astrocytes modulates the expression of CD44 by decreasing its levels. However, it did not affect HAS, Hyal or HA levels. This astrocyte-mediated mechanism could play an important role in the neurodegenerative disease promoting minor adhesion to the extracellular matrix and contribute to improving their excitotoxicity function.

0340 - MULTIPLE STRATEGIES FOR BRAIN DRUG DELIVERY: IN VITRO AND IN VIVO ANALYSIS OF P-GLYCOPROTEIN INHIBITION BY NANOCARRIERS COVERED WITH POLOXAMER 188, AND GLUCOSE QUANTUM DOTS FUNCTIONALIZATION EFFECTS OVER PREFERENTIAL BRAIN DISTRIBUTION

Johanna CATALÁN (1) | Pilar CONTRERAS(2) | Nicolás ÓRDENES-AENISHANSLINS(3) | Fernanda PONCE(4) | Pablo M. GONZÁLEZ(2) | José M. PÉREZ-DONOSO(3) | Javier O. MORALES(5) | Mariela Fernanda PÉREZ(1)

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The pharmacological treatment of central nervous system (CNS) disorders has limited efficacy due to the blood brain barrier. Thus, drugs with therapeutic potential are not being used mainly because of pharmacokinetic limitations. For example, loperamide (Lop) is a µ-opioid agonist that poorly penetrates the CNS since it is a hydrophobic drug and a P-glycoprotein (Pgp) substrate; however, it has potential neuroprotective effects. In this work, we evaluated the capacity of nanocarriers of Eudragit® RS covered with poloxamer 188 (P188) loaded with Lop (NP-Lop) for active delivery to the CNS. NP-Lop were developed to increase Lop aqueous dispersion, and P188 was used for Pgp inhibition. Finally, under fasting conditions, preferential brain delivery was assessed by the conjugation of Quantum-Dots (QDs) with glucose, since glucose transporters (GLUTs) exhibit a relative brain overexpression during hypoglycemia. NP-Lop effects over Pgp were evaluated by uptake and transport assays in MDCK-MDR1 cells. Central distribution was assessed by evaluating Lop supraspinal analgesic effects in mice submitted to a hot plate test. Glucose functionalization effects over QDs biodistribution was evaluated by determining the fluorescence of mice organs using a multi-modal in vivo imaging system. All treatments were intravenously adminstered. Confidence interval was set at 95 %; statistical analysis was performed by ANOVA and Holm-Sidak or Tukey posthoc tests. NP-Lop increased Lop uptake 2.1 times, while Lop efflux was decreased 10 times, regarding Lop in solution. Maximum possible effect in the hot plate test obtained with NP-Lop was 7.2 higher than the observed for Lop in solution. QDs conjugated with glucose showed to be preferentially distributed to the brain under fasting conditions. Conclution: NP-Lop decrease Lop efflux and favour its transport towards the CNS. Glucose conjugation may induce a preferential brain distribution by GLUTs specific interactions.

0515 - AT2 ANTAGONIST (PD123319) PRENATAL TREATMENT MODIFY THE EXTERNAL GRANULAR CELL LAYER IN POSTNATAL CEREBELLUM DEVELOPMENT.

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The renin angiotensin system (RAS) inhibitors administrated particularly during second and third trimester were associated with fetopathy. Fetal development is dependent of complex prenatal expression and function of the RAS. AT2 receptor has been related to neuronal differentiation during postnatal fetal development. AT2 receptors were localized only in the cerebellum Purkinje cells (PC). The granule cells (GC) are most neuronal cell types in the brain. Proliferation, differentiation, migration, and synaptogenesis were regulated by PC. The high interaction between PC and GC shapes cortex cerebellum. The aim was to investigate the effect of prenatal AT2 blockage in postnatal cerebellar cells development. Pregnant Wistar rats on the 13th day of pregnancy were implanted mini-osmotic pumps subcutaneously with AT2 antagonist (PD123319) and vehicle. Morphological studies by indirect immunofluorescence analysis on P0, P3, P5, P7 and P15 ($n= 24$ slices/each day) were performed. The external granule cell layer (EGL) length was significantly increased on treated animals at P5 ($p<0.001$). Proliferative GC was found in the outer part of the EGL (oEGL) and significant increased length at P5 in treated animals ($p<0.001$). The differentiated/premigratory GC were localized in the inner part of the EGL (iEGL at P0 ($p<0.05$), P5 ($p<0.01$) and P7 ($p<0.05$)) in treated pups respect to control. The present study demonstrates important modify in cerebellar granule cells proliferation and migration processes in pups treated development. In addition, we observed that the proliferation processes are modifying others cells layers next the EGL. The results suggest a relevant participation of AT2 receptors in the cortex cerebellar organization.

0541 - ALTERATIONS IN GLUCIDIC METABOLISM AND BEHAVIOUR INDUCED BY A HIGH-FAT DIET IN C57BL/6J MALE MICE. EFFECTS OF CHRONIC MILD STRESS AND PHARMACOLOGICAL TREATMENT WITH METFORMIN OR FLUOXETIN.

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We previously studied in mice the effects of a high-fat diet (HFD) and chronic mild stress (CMS) in the development of cognitive impairment and metabolic dysregulation. HFD caused both hyperglycemia and glucose intolerance while CMS only caused the latter. Both treatments reduced spatial and working memory, yet no synergy had been observed between them. Metformin (MET) is a medicament for type 2 diabetes and Fluoxetin (FLX) is a drug used as treatment for stress-related symptoms. The objective of this research is to analyze the effects of MET and FLX in the way how HFD and CMS develop metabolic and conductual alterations. 4 weeks-old mice were fed with standard diet (SD) or HFD. 8 weeks later, groups were subdivided and exposed to CMS. Some received either FLX or MET in drinking water in doses of 15 mg/kg/day and 250 mg/kg/day, respectively. FLX treatment began concurrently with CMS, while MET was administrated 4 weeks after CMS initiation. These treatments lasted until the subjects were 32 weeks-old. Behaviour and metabolic tests were performed during the final weeks of treatment. Our results show that MET had no effect on glucidic metabolism, and while mice under HFD+CMS had glucose intolerance ($p<0.01$), those with HFD+CMS+FLX did not ($p>0.05$). Concerning behaviour, HFD and HFD+CMS reduced working memory in the Y-maze test compared to SD (HFD, $p= 0.02$; HFD+CMS, $p= 0.04$). MET reverted these effects, and FLX corrected behaviour only if CMS was present (HFD+FLX vs. SD, $p<0.01$). In the spatial object recognition test mice under HFD, CMS and HFD+CMS had lower discrimination index compared to the control group (HFD, $p<0.05$; CMS and HFD+CMS, $p<0.01$). These deficiencies were corrected with MET, but FLX only normalized groups under CMS (HFD+FLX vs. SD, $p<0.05$). We conclude that MET protects spatial and working memory against HFD and CMS, despite losing its metabolic effect, and FLX prevents metabolic and conductual alterations caused by CMS, yet has no effect against HFD.

0543 - STUDY OF CB1 CANNABINOID RECEPTORS INVOLVEMENT IN THE ANXIETY LIKE-BEHAVIOUR AND MEMORY CONSOLIDATION ASSOCIATED TO ACETIC ACID-INDUCED VISCERAL PAIN IN CB1 KNOCKOUT ADOLESCENT MICE

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In previous studies we evaluated the interaction between the CB1 cannabinoid receptors and the endogenous opioid system studying the antinociceptive effect of morphine (MOR) by using the acetic acid (AA)-induced writhing test in CB1 knockout (KO) adolescent male and female mice. The aim of the present study was to evaluate the motivational (anxiety like-behaviour) and cognitive (memory consolidation) responses associated to the AA-induced visceral pain model in adolescent CB1 KO mice of both sexes. The anxiety like-behaviour associated to visceral pain was measured by the elevated plus maze (EPM). CB1 KO and WT mice were pre-treated with MOR (1, 3 or 9 mg/kg i.p.) or saline (SAL) injection, 20 minutes before the AA (1.5 %, 10 ml/kg i.p.) or SAL administration. Immediately after, the time spent and number of entries to the open arms were registered during 20 min. The memory consolidation associated to visceral pain was determined using the novel object recognition (NOR) test and expressed as a differentiation index (DI). During the training phase of NOR test, CB1 KO and WT mice were placed in an open field with two identical objects for 9 minutes and right after, mice were pretreated with MOR (1, 3 or 9 mg/kg) or SAL injection 20 minutes before AA or SAL administration. On the test phase, 24 hs after, one of the two identical objects was randomly replaced by a different object. Time spent exploring either novel or familiar object, were measured and the DI was calculated. In the EPM test only MOR 1 mg/kg attenuated the AA-induced anxiogenic like effect expressed as the % of time in the open arms in CB1 KO ($p<0.05$), but not in WT male mice. In the NOR test no significant changes were observed in any of the experimental groups. Our results suggest that CB1 receptors could modulate the anxiety-like behaviour associated to AA-induced visceral pain in males. On the other hand, memory consolidation process would not be affected by the AA-induced visceral pain.

0586 - BEHAVIORAL AND BIOCHEMICAL TESTS IN AN ANIMAL SCHIZOPHRENIA MODEL.

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In this study, behavioral and biochemical tests were performed on an epigenetic model of schizophrenia, with the aim of revealing alterations characteristic of the disease. For this purpose, Swiss albino mice were administered with methionine 5.2 mmol/kg for 30 days. After this period, they were subjected to a task of object recognition, based on the etiological paradigm of novel preference. The procedure consists of exposing a lot of 5 animals to a first session with two identical objects placed in an open field during 10 min and then 24 h later these animals were exposed again to two objects, one of which was in the first session and the other with which the animal has not been. The results showed that the animals touched the object with their noses 84 ± 14 times (control) 60 ± 13 times (treated) in the first session and 57 ± 14 times (control) and 72 ± 21 times (treated) in the second session, without any significant differences among the groups. However, control animals interacted more often with the novel object (65 %) than 46 % of the treated animals, indicating that the animals subjected to the schizophrenia model showed changes in the memory involved in the recognition of the objects. We have previously observed that lipid peroxidation of the cerebral cortex increased in animals subjected to the epigenetic model. In this case, the determination of substances reactive to 2-thiobarbituric acid was carried out by the modified method of Yokohawa (Yokohawa et al. 1973) subsequent to the administration of chlorpromazine 3 mg/kg, vapreotide (somatostatin analogue) 100 mcg/kg or both produced significant reductions of 43, 48 and 60%, respectively; in the cerebral cortex lipid peroxidation. In addition, no changes were observed in the control animals. These results suggest that certain modifications of the disease would be represented in this model allowing its use for the testing of new antipsychotic agents.

0678 - DIAZEPAM-INDUCED TRANSCRIPTIONAL REGULATION OF GABAA RECEPTOR ALPHA1 SUBUNIT GENE VIA L-TYPE VOLTAGE-GATED CALCIUM CHANNEL ACTIVATION IN RAT CEREBROCORTICAL NEURONS

Nelsy MEDINA | María Florencia FOITZICK | Lucía IGLESIAS GARCÍA | **María Clara GRAVIELLE**

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GABA-A receptors are targets of different pharmacologically relevant drugs, such as barbiturates, benzodiazepines, and anesthetics. In particular, benzodiazepines are prescribed for the treatment of anxiety, sleep disorders, and seizure disorders. Prolonged activation of GABA-A receptors by endogenous and exogenous modulators induces adaptive changes that lead to tolerance. For example, chronic administration of benzodiazepines produces tolerance to most of their pharmacological actions, limiting their usefulness. The mechanism of tolerance is still

unknown. We have previously demonstrated that chronic diazepam administration in rats result in tolerance to the sedative and anxiolytic effects which is accompanied with a decrease in the interactions between GABA and benzodizepine binding sites (uncoupling) in cerebral cortex. The aim of this work was to investigate the molecular mechanism of benzodiazepine tolerance in an in vitro model of primary neuronal cultures from rat cerebral cortex. The exposure of cultured neurons to diazepam for 48 h produced a 40 % uncoupling ($p<0.05$) which was prevented in the presence of nifedipine, an inhibitor of L-type voltage-gated calcium channels (L-VGCCs). Benzodiazepine treatment also induced a 45 % decrease ($p<0.05$) in GABA-A receptor alpha1 subunit mRNA levels ($p<0.05$) that was inhibited by nifedipine. We hypothesized that the adaptive changes of GABA-A receptors induced by sustained exposure to benzodiazepine are mediated by a signaling pathway that involves activation of L-VGCCs. Results from calcium mobilization and nuclear run-on assays suggested that benzodiazepine exposure produces an increase in the calcium influx through L-VGCCs that activates an intracellular signaling cascade finally leading to the transcriptional repression of alpha1 subunit gene expression. Insights into the mechanism of benzodiazepine tolerance will contribute to the design of new drugs that can maintain their efficacies after long term treatments.

0723 - ELECTROPHYSIOLOGICAL AND MOLECULAR EVALUATION OF TWO NMDAR ANTAGONISTIC PEPTIDES, POSSIBLE CANDIDATES IN NEUROPROTECTION PROCESSES.

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Glutamate (E), an excitatory amino acid of the central nervous system (CNS), acts through different receptors, among which is the N-methyl-D-aspartate receptor (NMDAR). The NMDAR is responsible for allowing the flow of Ca2 + ions into the postsynaptic neuron, playing an important role in synaptic plasticity. One of the most relevant pharmacological features of NMDARs is the high specificity of their antagonists, making them important targets for drug design. The aim of this work is to synthesize and evaluate in vitro the neuroprotective effect of two additional peptides (EAR-17 and EAR-19). The methodological design has three general aspects as follows: In-silico design and molecular coupling of the two peptides with the GluN2B subunit of the NMDAR. Synthesize the two peptides by solid phase and subsequently characterize, purify and determine their secondary. Electrophysiological evaluation in tsA 201 (HEK293T) and hippocampal neurons. The neuroprotection of peptides EAR-17 and EAR-19 in the oxygen-glucose deprivation model (OGD), for which primary cultures of hippocampal neurons were used to perform a molecular approach through the activation of caspase-3 and calcium imaging techniques. As a result of the docking of the peptides EAR-17 and EAR-19 with the GluN2B subunit of the NMDAR, it was found that the affinity of the peptides varies according to the receptor conditions. In addition to the interactions established between the peptides and the GluN2B subunit, there are dipole to dipole electrostatic interactions, among which the hydrogen bond predominates. In general, the process of synthesis and purification has been optimal, allowing to have pure peptide species, with a reaction efficiency greater than 50 %. This great efficiency confirms a synthesis process without the presence of adducts of the peptides. The electrophysiological evaluation confirmed the antagonistic effect of the EAR 17 and EAR 19 peptides on the current evoked by the NMDAR. For each peptide, the IC 50 was established, finding that the EAR 19 has more affinity for the NMDAR. In the EPSC assay, EAR-17 and EAR-19 decreased in the postsynaptic current on the hippocampal neurons, without recovering the initial current of the EPSCs although they are repeated continuously in the same period of time. The vehicle has no action on hippocampal neurons. In neuroprotection, the OGD-model in hippocampal neurons activated the path of cell death by caspases, which is a consequence of the irregular entry of calcium mediated by the NMDAR. At the same time, we observed significant changes when the OGD was performed with or without the presence of the peptides, with significant differences observed if the peptides were added at the beginning of the OGD or in the OGD recovery process. In this study, we identified two peptidic molecules called EAR-17 and EAR-19 that have the possibility of being antagonists of the GluN2B subunit of the NMDAR and therefore the possibility of modulating the permeability to the calcium ion inducing a neuroprotection effect. We suggest continuing the evaluation of EAR-17 and EAR-19 with other in-vitro and in-vivo approaches.

0793 - NEW FINDINGS FOR OLD PLAYERS: IMIDAZOLIUM SALTS AS PROTECTIVE DRUGS IN C. ELEGANS MODELS OF STRESS AND NEURODEGENERATION

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Imidazolium salts are attractive pharmacological agents that have been linked to a wide range of biological effects, including antitumoral, antimicrobial, anthelmintic and anti-inflammatory. In this study, we aim to evaluate the role of these compounds as antioxidant and anti-aging agents. We synthesized imidazolium salts and analyzed their ability to improve oxidative stress (OS) resistance. We used an established model in biomedical research, the free-living nematode C. elegans, and exposed them to the oxidizing agent $FeSO_4$. We identified a derivate, 1-Mesithyl-3-(3-sulfonatopropyl) imidazolium (MSI), that enhances animal resistance to OS. To delineate MSI roles, we split this work into two goals: i) to describe MSI action mechanisms and, ii) to evaluate MSI role in neurodegenerative models. To gain insight into its mechanism of action, we evaluated MSI ability to activate DAF-16 (FOXO in vertebrates), a transcription factor relevant for cytoprotective defense mechanisms. Unexpectedly, our experiments revealed that MSI stress protection was not dependent on DAF-16. These results support the idea that other transcription factors (such as SKN-1 (Nrf-2 in vertebrates), HSF-1), could be involved in MSI protection. We are currently performing experiments to identify the role of these molecular players, in MSI-induced stress resistance. The second goal is held by the theory that links OS to aging and neurodegeneration. We are currently evaluating if MSI increases lifespan, healthspan, and improves biological markers of neurodegeneration in a C. elegans model of Alzheimer disease. This strain expresses Aß1-42 in muscle and shows age-dependent protein aggregation and paralysis. Our preliminary results show that MSI delays paralysis in this strain. Additional research is needed to underpin the protective role of MSI and to determine if these effects can be extrapolated in other neurodegenerative scenarios.

0968 - EVALUATION OF SERUM CYTOKINES LEVELS AS POTENTIAL PERIPHERAL MARKER OF MILD COGNITIVE IMPAIRMENT IN WOMEN.

María Micaela CASTRO (1) | Romina Alejandra PAVÓN(2) | Mario Oscar MELCON(2) | Natalia Erica MENITE(1) | Ana Maria GENARO(3) | María Laura PALUMBO(1)

CIT NOBA-UNNOBA-UNSADA-CONICET. CIBA-UNNOBA (1); FINEP (2); BIOMED-UCA-CONICET. DEPTO. DE FARMACOLOGÍA, FACULTAD DE MEDICINA-UBA. (3)

Mild cognitive impairment (MCI) is a transitional stage between cognitive changes of normal aging and early-stage dementia. MCI is recognized as a pathological condition that typically precedes Alzheiner´s disease. A fraction of 20-40 % of MCI individuals will progress to dementia within 3 years following the initial diagnosis. Previously, we demonstrated that the cognitive deficit observed in Balb/c mice exposure to chronic mild stress was correlated with a decrease of IFN-g and an increase of IL-4 in hippocampus and peripheral lymph nodes. The aim of this work was evaluated the levels of cytokines IFN-g, IL-1b, IL-4 and IL-6 in serum of subjects with MCI and control as a possible peripheral marker of cognitive deficit. In this pilot study participated six female subjects (aged 60-70 years) from Junin´s city. The participants were randomly selected taking into account the inclusion and exclusion and criteria according to the protocol approved by the COENOBA and the Central Ethics Committee of the Province of Buenos Aires (exp. 2,919-1,593/17). The subjects were evaluated by neuropsychological tests following the diagnostic criteria of Petersen. Criteria: the control group showed scores in all cognitive functions evaluated in normal range for patient age and schooling (Z score between -1 and 1). The MCI group (MCI of the amnesic type) showed values equal or less than Z -1.5 in the memory tests. The cytokines levels (pg/ml) were measure in serum by ELISA. We found an increase in IL-4 level (C: 0.7 ± 0.4; MCI: 7.0 ± 1.7; $p<0.05$) in MCI respect to control subjects. We did not find a significant difference in IFN-g (C: 51.4 ± 11.5; MCI: 38.3 ± 2.1), IL-1b (C: 13.9 ± 1.7; MCI: 19.0 ± 2.3) and IL-6 (C: 5.7 ± 1.6; MCI: 7.3 ± 2.7) between the MCI group compared to control group. We conclude that changes in these cytokines serum levels could be related to the cognitive deficit observed in MCI subjects.

Medicina Regenerativa y Terapia celular/ Regenerative Medicine and Cell Therapy I

Chairs: Andrea Fellet | Daniel Grasso

0125 - REJUVENATION BY PARTIAL CELL REPROGRAMMING: TRANSFER OF THE YAMANAKA GENES TO FIBROBLASTS FROM YOUNG AND OLD RATS

Priscila CHIAVELLINI (1) | Marianne LEHMANN(1) | Martina CANATELLI MALLAT(1) | Gustavo R. MOREL(1) | James CLEMENT(2) | Rodolfo G. GOYA(1)

INIBIOLP (1); BETTERHUMANS INC. (2)

Cyclic partial cell reprogramming is an emerging avenue of research based on the transient expression of pluripotency genes to generate progressively deeper rejuvenated cells that retain their cell identity. Unlike conventional reprogramming, it can rejuvenate cells ex vivo and in vivo. To this end, we have constructed a regulatable bidirectional adenovector expressing the green fluorescent protein (GFP) and Oct4, Sox2, Klf4 and c-Myc (OSKM) genes. Here, we characterized primary fibroblast cultures derived from young (2 mo.) and old (28 mo.) rats and determined their age-related differences assessing hallmarks as beta-galactosidase, H3K9me3, telomere length, 53BP1, ROS, and H2A. Then, we implemented partial reprogramming by short-term transduction of young and old fibroblasts with our OSKM adenovector and determined whether the above parameters tend to reverse to younger levels in the old cells. Cell aging and identity markers were determined by immunocytochemistry (ICC). Fibroblasts were transduced with our STEMCCA HD adenovector, letting the OSKM genes act for 5 days, and their markers compared with non-treated cells. Cell markers showed the expected age-related alterations. After 5 days of OSKM gene expression, some cell marker levels showed in the old cells a trend to change back to younger levels. Markers of cell identity were not affected by the OSKM treatment. Expression of the pluripotency markers NANOG and SSEA was not detected in the treated cells. Partial reprogramming emerges as a powerful tool for the implementation of in vitro and in vivo rejuvenation keeping cell type identity unchanged.

0179 - REGULABLE ADENOVECTORS HARBORING YAMANAKA GENES AS A NOVEL TOOL FOR IMPLEMENTING REGENERATIVE THERAPY IN THE AGING BRAIN

Marianne LEHMANN | Martina CANATELLI MALLAT | Priscila CHIAVELLINI | Gustavo MOREL | Rodolfo GOYA

INIBIOLP

Biological restoring of aging hallmarks by partial cell reprogramming is an emerging avenue of research. In this context, regulable pluripotency gene expression systems are the most widely used at present. A regulable bidirectional adenovector expressing green fluorescent protein (GFP) and oct4, sox2, klf4 and c-myc genes (OSKM) was constructed. Gene expression is controlled by a Tet-Off system. The promoter is placed between OSKM arranged as a bicistronic tandem (hSTEMCCA tandem) and the GFP gene. Separately, a constitutive cassette expresses the regulatory protein tTA. In order to generate the virus, linearized plasmidic DNA was transfected in HEK293 Cre cell line, and subsequently infected with H14 helper adenovector, which provides in trans all the viral genes. Given that H14 has its packaging signal flanked by loxP sites and that Cre recombinase is produced by the cell line, H14 is unable to package its DNA. The newly-generated vector was expanded by 5 iterated co-infections of the above cells and the adenovector purified by ultracentrifugation in a CsCl gradient. Titer was 1.2×10^{12} physical viral particles/ml. As expected, GFP fluorescence in vector-transduced rat fibroblast cultures declined with the dose of doxycycline (DOX) present in the medium. Immunocytochemical analysis of transduced cells confirmed the expression of the 4 Yamanaka genes. Three days after vector injection in the hypothalamus of rats, a significant level of fluorescence was observed in the region. Addition of 2 mg/ml DOX to the drinking water reduced GFP expression. This adenovector constitutes a promising tool for implementing non-integrative partial cell reprogramming.

0198 - MODULATION OF MESENCHYMAL STEM CELLS BY HYALURONAN AND SULFATED DERIVATIVES IN OSTEOSARCOMA

Antonella ICARDI (1) | Ina SEVIC(1) | Fiorella M SPINELLI(1) | Daiana L VITALE(1) | Matias VALENZUELA ALVAREZ(2) | Luciana GUTIÉRREZ(2) | Mariana Gabriela GARCIA(3) | Marcela BOLONTRADE(2) | Laura ALANIZ(1)

CIT NOBA-UNNOBA-UNSADA-CONICET. CIBA-UNNOBA (1); INSTITUTO DE MEDICINA TRASLACIONAL E INGENIERÍA BIOMÉDICA (IMTIB) CONICET (2); IIMT (3)

The treatment and reconstruction of bone in osteosarcoma, after tumor resection, remains a challenge. A promising therapy is the therapeutic use of mesenchymal stem cells (MSCs). Since hyaluronan (HA) is a component of the extracellular matrix, MSCs can interact with HA affecting the differentiation of these cells in therapeutic applications. Aim: To evaluate the therapeutic effect in osteosarcoma of MSCs derived from umbilical cord (hUC-MSCs) treated with HA or its sulfated derivatives (sHA). Also, the role in tissue remodeling and as well as in tumor behavior. Viability and apoptosis assays by MTS and AnnexinV/IP by flow cytometry were used to determine the dose of HA or sHA to treat hUC-MSCs. Mixed lymphocytes cultures (MLC) were performed to evaluate the effect of HA or sHA on the immunogenicity of hUC-MSCs. The effect of these biomaterials on the bone regenerative capacity of hUC-MSCs was analyzed by differentiation assays. Finally, the effect of conditioned media (CM) of hUC-MSCs treated with HA or sHA on the Saos-2 was analyzed by cellular viability analysis. hUC-MSCs exhibited low immunogenicity under MLC conditions and retained their immune properties after treatment with HA or sHA. HA or sHA

reduced differentiation towards adipogenic lineage in vitro, in a dose-dependent manner and associated with the HA sulfation levels. Regarding the capacity for hUC-MSCs osteogenic differentiation, HA treatments showed a tendency to increase in comparison to control. The CM derived from hUC-MSCs had a pro-tumor effect, but the effect decreased when the cells were treated with HA or sHA by decreasing cell viability. Conclusion: The hUC-MSCs are permissive for allogeneic transplantation. However, our results suggest HA and sHA treatments reduced the ability of hUC-MSCs to differentiate towards the adipogenic lineage. Besides, in tumor context favor the tumor behavior of Saos-2, but this effect diminished with HA or sHA treatments.

0203 - P53 ABSENCE INCREASES HEME OXYGENASE 1 LEVELS EVIDENCING A CROSSTALK BETWEEN DIFFERENT STRESS PATHWAYS IN PLURIPOTENT STEM CELLS

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INSTITUTO DE QUÍMICA BIOLÓGICA DE LA FACULTAD DE CIENCIAS EXACTAS Y NATURALES (IQUIBICEN)

Embryonic stem cells (ESCs) are pluripotent stem cells (PSCs) derived from the inner cell mass of blastocyst. PSCs are widely exploited to model early embryo development and are a great promise in the regenerative medicine field. It is well known that ESCs possess a fine tuned reactive oxygen species (ROS) balance. This is highly relevant for genome integrity maintenance required for pluripotency and differentiation, and consequently for proper development. Heme Oxygenase 1 (HO-1) is an antioxidant protein essential to redox homeostasis and it was suggested to have also an unknown nuclear function, different from its enzymatic activity. On the other hand, it was proposed that p53 influences ROS balance besides its classical function in response to DNA damage. Although the relationship between these proteins has been explored, little is known about their connection in PSCs. To explore the crosstalk between HO-1 and p53 in PSCs, in this work, we focused in HO-1 gene regulation by p53 in ESCs. We have previously found that HO-1 protein levels were increased in a p53-/- ESC line generated in our lab. Notably, RNA levels were not altered suggesting that HO-1 protein stability is regulated by p53 in ESC. To delve into the mechanism involved we evaluated the effect of protein inhibition by cycloheximide in HO-1 gene modulation, both in wild type (wt) and p53-/- ESCs and found higher HO-1 half-life in the knockout cell line. Remarkably, HO-1 transcription increased in response to hemin treatment, a widely used pharmacological HO-1 gene inductor, and to differentiation stimulus in p53-/- cells similar to wt. These results evidence different levels of HO-1 gene regulation in ESCs. We are currently studying if the differential HO-1 protein stability found involves a p53-dependent proteasome-mediated proteolysis. A p53-HO-1 crosstalk could evidence a link between different stress response pathways relevant to ESC survival and differentiation.

0260 - A NEW STRATEGY TO EXPRESS THE GRANULOCYTE MACROPHAGE COLONY-STIMULATING FACTOR IN MESENCHYMAL STROMAL CELLS BY IN VITRO TRANSCRIBED MRNA.

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INSTITUTO DE INVESTIGACIONES EN MEDICINA TRASLACIONAL, CONICET-UNIVERSIDAD AUSTRAL

In the last years, mesenchymal stromal cells (MSCs) have been used for therapeutic purposes, and particularly in cancer, to deliver anti-tumoral agents due to their capacity to home to tumors. Most of the strategies to engineer MSCs are based on virus-mediated gene transfer. However, the use of in vitro transcribed mRNA (IVT mRNA) is gaining attention as a promising tool to induce the expression of therapeutic agents by MSCs. It has also been demonstrated that the granulocyte macrophage colony-stimulating factor (GM-CSF) reduced tumoral growth by improving the antitumor immune response. The aim of this work was to express GM-CSF in MSCs by IVT mRNA with the final purpose to treat liver tumors. To this end, mRNAs (of GM-CSF or DsRed as control) were in vitro transcribed, modified to be more stable within the cell, and then transfected into human umbilical cord derived MSCs. GM-CSF production by MSCs was assayed by ELISA and DsRed expression by flow cytometry. Different quantities of mRNA were used and we observed that low amount of mRNA (0.2 µg mRNA/40.000 cells) was efficient to express DsRed (57.1 ± 3.9 %) and GM-CSF (2.48 ± 0.23 µg/ml/1x10^6 cells) in MSCs. Since the use of MSCs for therapeutic purposes relies on their migration capacity and their low immunogenicity, we evaluated their migration capacity (by a modified Boyden chamber) and their surface markers (CD90, CD44, CD34, CMHII, CD80 and CD86) by flow cytometry. We observed that MSCs expressing IVT mRNA of GM-CSF have the same migratory capacity than unmodified MSCs and their surface markers remained unchanged. Moreover, we evaluated GM-CSF functionality analyzing the ability of conditioned media produced by MSCs expressing GM-CSF to mature dendritic cells in vitro. We observed that the GM-CSF produced by MSC generated matured dendritic cells (68.21 ± 0.24 % CD11c+, CD86+, CMHII+). We conclude that the expression of GM-CSF in MSCs by IVT mRNA is a good and useful strategy for therapeutic purposes.

0269 - SIGNALING PATHWAYS ACTIVATED DURING HEPATIC DIFFERENTIATION AND PROLIFERATION OF AMNIOTIC EPITHELIAL CELLS

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The placenta and fetal membranes have recently been proposed as an important stem cells source for regenerative medicine. Amniotic epithelial cells (hAECs) can be isolated from the amnion of the human placenta at term. They express embryonic stem cells markers and they are pluripotent. These characteristics would make hAECs ideal candidates for regenerative medicine. Hepatic failure is one of the major causes of morbidity and mortality worldwide and the available treatments have several obstacles. Stem cells have been spotlighted as alternative sources of hepatocytes because of their potential for hepatogenic differentiation. The adequate regulation of the signalling pathways activated during a differentiation process is the key for the success of such process. The aim of this work was to study some of the main pathways activated in hAECs during their hepatic differentiation process. Hepatic differentiation (HD) was assayed by specific HD medium (EGF + dexamethasone). Immunofluorescence (IF), Western blot (WB), qRT-PCR, PAS staining and MTT assays were performed. We have found that HD significantly induced an increment in Wnt-1 and B-catenin expression in hAECs, measured by qRT-PCR, WB and IF. Treatment of hAECs with XAV939 (a B-catenin inhibitor) caused the inhibition of HD, as albumin expression and glycogen synthesis were reduced. In addition, we determined that B-catenin pathway inhibition diminished Ki-67 expression and cell viability, during HD. We have also observed that HD promotes phosphorylation of PI3K and Akt, as determined by WB and IF. We observed a significant increment in nuclear localization of P-Akt during hAECs HD. These results suggest that

the activation of the B-catenin and PI3K pathways may be responsible for a successful hepatic differentiation and proliferation of hAECs. Understanding the molecular mechanisms regulating hepatocyte differentiation will significantly facilitate the development of stem cell-based therapy to treat liver diseases.

0287 - NOVEL TREATMENT FOR DERMAL LESIONS USING A SPECIFIC SUBPOPULATION OF MESENCHYMAL STEM CELLS FROM HUMAN UMBILICAL CORDS. IN VIVO ASSAY IN A MURINE MODEL.

Maria Belen PALMA (1) | Guillermo BUERO(2) | Santiago MIRIUKA(3) | Laura ANDRINI(1) | Fernando RICCILO(1) | Ana Maria INDA(1) | Carlos LUZZANI(3) | Edgardo CAROSELLA(4) | Pablo PELINSKI(5) | Marcela GARCIA(1)

CATEDRA DE CITOLOGIA, HISTOLOGIA Y EMBRIOLOGIA A, FAC. CIENCIAS MEDICAS, UNLP (1); INSTITUTO MATER DEI (2); FLENI-CONICET (3); COMMISSARIAT À L'ENERGIE ATOMIQUE ET AUX ENERGIES ALTERNATIVES (CEA), SAINT-LOUIS HOSPITAL (4); HOSPITAL ESPAÑOL, LA PLATA (5)

The chronic venous ulcer (CVU) is described as the spontaneous or accidental tissue absence, in individuals with varicose leg, that can develop for more than fifteen days, with no signs of healing despite receiving conventional treatment. This inadequate and/or incomplete wound healing can lead to significant individual morbidity including a profound reduction in quality of life. In recent years, advances have been made, in particular, the use of stem cell-based therapies has been suggested. Mesenchymal stem cells (MSCs) have been examined in skin repair and regeneration after various acute and chronic skin injuries. All the treatments involve the utilization of autologous cells, which limits their application. The objective of this work was to develop a new treatment for CVU through the allogeneic application of differentiated mesenchymal cells (DMC), obtained from human umbilical cord Wharton's jelly (ucMSC). We characterizated DMC by analyzing surface markers by flow cytometry. DMC possess immunomodulation capacity (by HLA-G expression, among others) allowing its allogeneic use. To check this, we realized an ex vivo assay of inhibition of activated lymphocytes proliferation exposed to DMCs. After that, we performed an in vivo assay, in mice, to evaluate the effect of DMC on the healing on the skin wounds, using DMC derived from murine or human umbilical cords. We have been able to isolate the cell population, the DMC, wich express HLA-G, and we tested its immunosuppressive function. In mice with dermal ulcers, we treated the injury with murine or human DMCs, and we observed that both groups healing more quickly than the control group (untreated), but unexpectedly, we noticed that when using human DMCs there was a 50% reduction in healing time. We developed the basis for a new method for the treatment of CVU through the allogeneic application of DMC from human ucMSC. These results made it possible to register a patent application with the European Patent Office.

0391 - RADIATION MODIFIED BIOMATERIALS FOR APPLICATION IN TISSUE ENGINEERING

Paola BUSTAMANTE | Carolina ANESSI

COMISIÓN NACIONAL DE ENERGÍA ATÓMICA (CNEA)

Tissue engineering applied to bone regeneration aims to develop biological substitutes that allow the restoration or improvement of bone tissue function. The biomaterials used for this purpose must be biocompatible and must also be reabsorbed at a rate similar to that of tissue growth. One method for controlling the degradation rate is the use of ionizing radiation. The goal of this project is to develop a composite biomaterial modified by gamma radiation that can be used for 3D printing of scaffolds used to regenerate trabecular bone tissue. The scaffolding prototypes were made by using a filament, obtained by the extrusion technique, of polylactic acid (PLA) with different concentrations of hydroaxyapatite (HA). They were performed a chemical, physical and morphological characterization. Then, the scaffolding was designed by configuring 3D printer software parameters. Subsequently, the PLA/HA scaffolds were manufactured and were assessed by cytotoxicity test (ISO 10993-5), accelerated hydrolytic degradation test at 19 months at doses of 15 kGy and 25 kGy, and cell adhesion test. The results of the filament characterization allow us to choose the optimal HA concentration that was 3 % w/w since it ensured the correct distribution of the HA in the PLA matrix. The scaffolding prototypes were designed with 60% porosity and pore sizes of 177-331 µm, values similar to those of the trabecular bone. Then, the 3D scaffolding was printed. The result of the cytotoxicity test showed that the printed pieces presented a cell viability percentage greater than 70 % which indicates that they are non-cytotoxic. The adhesion test showed a large number of cells adhered to the surface of the material. While degradation tests showed that the average degradation for both doses was 0.14 %. The results obtained allow us to conclude that the objective was achieved; however, we must continue working on characterization and in the set-up for a future clinical trial.

0394 - IMMUNOMODULATORY OLIGONUCLEOTIDE IMT504 IMPROVES MESENCHYMAL STEM CELLS PROLIFERATION AND MIGRATION IN ADULT NON-OBESE DIABETIC MICE

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ICT MILSTEIN - CONICET (1); IBYME-CONICET (2)

Type 1 diabetes (T1D) is a multifactorial autoimmune disease in which insulin-producing pancreatic β cells are destroyed. No effective clinical interventions for T1D are currently available, and patients are lifelong treat with insulin. There is a consensus that new innovative approaches are urgently needed to predict, treat, and prevent T1D. Different strategies to modulate immunological response and restore β cell mass have been performed, although limited by the availability of transplants and the need for chronic immunosuppression. IMT504 is the prototype of the PyNTTTTGT family of immunomodulatory oligonucleotides (ODNs) known for their regenerative properties and proven to be effective in lowering glycemia in non-obese diabetic (NOD) mice. Here, we investigate its action in bone marrow mesenchymal stem cells (BM-MSCs) and splenocytes in NOD/LtJ. BM-MSCs and splenocytes from pre-diabetic and diabetic NOD mice were treated, in vitro, with different doses of IMT504 (0.5, 1.5, 4, 6.5, 10.5 and 20 µg/ml). No differences were observed in splenocytes activation/proliferation at higher doses. Fibroblasts colony forming units (CFU-F) that originate MSCs, viability, and migration assays were assessed. It was determined that 0.5 µg/ml of IMT504 stimulated MSCs (CFU-F (10^6 seed cells/cm^2): IMT504: 85 vs. Control: 65 $p<0.05$) and migration capacity (migration index: IMT504: 75 vs. Control: 20, $p<0.05$). In conclusion, our results showed that IMT504 exerts an effect on MSCs that might favor the diabetic condition.

0484 - MIR-145, MIR-296 AND MIR-302 FAMILY EXPRESSION FLUCTUATE PERIODICALLY ALONG THE CELL CYCLE OF HUMAN PLURIPOTENT STEM CELLS

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FLENI-CONICET

Human pluripotent stem cells (hPSCs), like embryonic and induced pluripotent stem cells, retain the ability to differentiate into a wide-range of cell types while undergoing self-renewal. They exhibit an unusual mode of cell cycle regulation, reflected by a cell cycle structure where G1 and G2 phases are truncated. hPSCs are primed

to initiate cell fate decisions during their transition through G1. MicroRNAs (miRNAs) are small non-coding RNA molecules involved in the regulation of gene expression. Several miRNAs have been shown to target transcripts that directly or indirectly coordinate the cell cycle of pluripotent cells. miR-145 and miR-296 are induced during differentiation and silence the self-renewal and pluripotency program. miR-302 family is essential for hPSCs stemness and its expression decreases during differentiation. Although, it is clear that miRNAs can regulate components of the cell-cycle machinery, its temporal expression profile along hPSCs cell cycle remains poorly characterized. Thus, we synchronized hPSCs populations using three pharmacological inhibitors: PD0332991, Aphidilcolin and Nocodazole to arrest cells in early G1, G1/S and G2/M phases, respectively. Analysis of cell cycle by DNA content was performed using propidium iodide staining followed by flow cytometry analysis. Then, by RT-qPCR, using specific stem loop primers, we analyzed the expression levels of miR-145, miR-296 and miR-302 family in early G1, G1/S and G2/M arrested hPSCs and determined that all studied miRNAs were periodically expressed. Finally, we performed Aphidilcolin-block and release experiments, and measured the levels of these miRNAs throughout hPSCs cell cycle progression at 0, 4, 8, 12, 14, 16, 20 and 24 hours after release. Importantly, we confirmed the periodic expression of all studied microRNAs and observed that miR-302 family expression is induced at 14-16 h post release, which coincides with G1/S transition, presumably to impede differentiation onset.

0496 - AMNIOTIC MEMBRANE CONDITIONED MEDIUM PROMOTES CELL DEATH IN HEPATOCARCINOMA HEPG2 AND HUH-7 CELLS

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The placenta and fetal membranes have recently been proposed as an important stem cells source for regenerative medicine. Stem cells derived from amniotic membrane offer considerable advantages over other stem cells because of the ease of collection, their low immunogenicity and minimal ethical and legal barriers are associated with their use. Epithelial amniotic cells isolated from the amnion express embryonic stem cells markers and have the ability to differentiate towards all three germ layers. Not only are amnion-derived stem cells applicable in regenerative medicine, but also have antitumoral properties. Hepatic failure is one of the major causes of morbidity and mortality and despite the development in therapies, hepatocarcinoma rates are high worldwide. A few studies have demonstrated the antitumoral effects of the amniotic membrane and their cells but little is known about the molecular and cellular mechanisms involved. The aim of this work was to study some aspects of cell death induced by the amniotic membrane conditioned medium (AM-CM) in hepatocarcinoma cells. Previous results showed that AM-CM inhibits proliferation of HepG2 and HuH-7 cells. We have analyzed the expression of proapoptotic proteins (Caspase-3, PARP-1) by qRT-PCR and Western blot, in HepG2 and HuH-7 cells treated with AM-CM. We have also analyzed p53 expression by immunofluorescence. We found a significant increment in Caspase-3 expression and in cleaved Caspase-3 and PARP-1 - measured by qRT-PCR and Western blot, respectively-, after 24 and 72 h of treatment with AM-CM in hepatocarcinoma cells. We have also observed that AM-CM significant increase p53 nuclear expression, measured by immunofluorescence. Finally, we determined that AM-CM induced DNA fragmentation after 72 h of treatment in HepG2 cells. Our results begin positioning amnion-derived stem cells as emerging candidates in anticancer therapy.

0501 - REGULATION OF APOPTOSIS RELATED PROTEINS IN HUMAN PLURIPOTENT STEM CELLS EXPOSED TO CHEMICAL HYPOXIA

Luciana ISAJA | Sofia MUCCI | Jonathan VERA | Maria Soledad RODRIGUEZ VARELA | Mariela MARAZITA | Gustavo Emilio SEVLEVER | Maria Élida SCASSA | Leonardo ROMORINI

FLENI-CONICET

Human embryonic and induced pluripotent stem cells (hESCs and hiPSCs) are self-renewing pluripotent cells (hPSCs) that can differentiate to a wide range of specialized cells. Notably, hPSCs tend to improve its undifferentiated state and its self-renewal under reduced oxygen conditions (hypoxia). Chemical compounds, like cobalt chloride ($CoCl_2$), can be used in vitro to mimic hypoxic conditions by stabilizing the hypoxia-inducible factor 1a (HIF-1a). We have demonstrated that chemical hypoxia induces apoptosis of hPSCs. Herein, we aim to ascertain the molecular mechanisms underlying these events. First, we screened changes in the expression levels of 43 apoptosis-related proteins using a human apoptosis antibody array at 8 hours post-$CoCl_2$ (250 µM) addition in hPSCs. Our results revealed that the extrinsic and the intrinsic pathway are involved in the apoptotic cascades activated by $CoCl_2$ in hPSCs. Next, we quantified the expression levels of key Bcl-2 family members (not present in the apoptosis array) by Western blot and RT-qPCR in hPSCs after $CoCl_2$ treatment (250 µM for 4, 8 and 24 h). We observed that Bnip-3, Mcl-1 and NOXA protein and mRNA expression levels were significantly upregulated at different time points upon hypoxia induction. In contrast, PUMA expression levels decreased. Importantly, p53 was also upregulated upon $CoCl_2$ treatment (4 h). Additionally, we found that in hPSCs siRNA-mediated downregulation of HIF-1a did not revert the apoptosis rate induced by $CoCl_2$ as judged by PI staining and Trypan blue dye exclusion data. However, the induction of Bnip-3 mRNA and protein expression levels was markedly impaired in HIF-1a-siRNA transfectants. Further supporting these findings, no changes in the percentage of apoptotic cells were observed when using Bnip3 siRNA. Taken together, our results indicate that the apoptosis induced by chemical hypoxia in hPSCs is HIF-1a and Bnip-3 independent and that both extrinsic and intrinsic apoptotic pathways could be involved.

0540 - IDENTIFICATION OF INTERGENIC TRANS-SPLICING IN HUMAN INDUCED PLURIPOTENT STEM CEL

Carolina COLLI | Maria Agustina SCARAFÍA | Alan Miqueas MÖBBS | Natalia Lucía SANTÍN VELAZQUE | Cyntia ABAN | Lucía Natalia MORO | Carlos Daniel LUZZANI | Alejandro Damián LA GRECA | Santiago Gabriel MIRIUKA

FLENI-CONICET

"Non-co-linear" (NCL) events are known RNA sequences, topologically inconsistent with their correspondent DNA sequences in the reference genome. Trans-splicing events are a linear type of NCL transcripts, which are formed post-transcriptionally by junction of separate pre-mRNAs. Trans-splicing has been described for various species, even though, their functions remain unclear. However, in human pluripotent stem cell, one RNA trans-splicing event (tsRMST) was described to contribute to the regulation of early lineage differentiation. We aim to identify intergenic trans-splicing events in induced pluripotent stem cell (iPSCs). First, the RNAseq of FN2.1 (iPSCs line developed in our laboratory) was subjected to NCLscan (a pipeline to discover NCL transcripts in human transcriptome) and we observed 1109 NCL events in iPSCs; of these, only 3 events occur between different genes: TIAM2-SCAFA8-1, TIAM2-SCAFA8-2 and ZRANB1-AL731577.2. Then, based on NCL scan data, we designed primers to amplify the junction for these three intergenic events. For this, total iPSCs RNA was treated with or without RNase R for selectively degrade linear RNA, and cDNA was synthesized using random primers or oligo(dT) primers for identifier events poly(A) and non-poly(A). Negative control was made treating total RNA without MMLV, to discard permanent

fixture of the genome at the DNA level. Trans-splicing intergenic events were validated by RT-qPCR and its subsequent corroboration in agarose gel. Starting from this we could observe that TIAM2-SCAFA8-1 and TIAM2-SCAFA8-2 were detected by RT-qPCR either random primers or oligo(dT) primers but not remained stable after RNase R treatment. ZRANB1-AL731577.2 was only detected by RT-qPCR using random primers and not remained stable after RNase R treatment. We observed that TIAM2-SCAFA8-1 and TIAM2-SCAFA8-2 linear polyadenylated events, while ZRANB1-AL731577.2 is a non-polyadenylated linear event. In summary, we found potentially but scarce relevant transplicing events in PSC.

0583 - ANALYSIS OF CHROMOSOME 19 MICRORNAS CLUSTER (C19MC) IN HIPSC CARDIAC DIFFERENTIATION

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FLENI-CONICET (1); CRG (2)

Human pluripotent stem cells (hPSC) have the capacity to self-renew and differentiate in vitro into any cell type of the three germ layers. Our lab performed a small-RNAseq and described the MIRNOME of hPSC cardiac differentiation (CD). Peculiarly, a 56-microRNA-group was found to be transcribed in pluripotency and its expression decayed over the mesoderm stage. These microRNAs were clustered in a 100 kb-long chromosome 19 sequence, known as C19MC. However, little is known about them in hPSC. Herefore, we decided to study their role in pluripotency and CD. A C19MC deficient human induced PSC line was generated by CRISPR/Cas9 technology. Two sgRNA were designed to flank both 5' and 3' extremes of the C19MC. After clonal selection for the mutant, lack of microRNA expression was confirmed by RT-qPCR. Chromatin immunoprecipitation assay (ChIP) was optimized in order to evaluate whether nearby histones modifications were affected by the big deletion. Eleven loci, ranging from -70 kb to +100 kb from the cluster, were selected: five for H3K27ac, three for H3K9ac, three for H3K4me2, in addition to positive controls, nearby OCT4 and RPL7 genes, and a negative control. ChIP results showed that the selected loci conserved their characteristics in the mutant line. Further, C19MC upstream protein coding gene DRPX and downstream microRNAs mir-371, -372 and -373 expression were evaluated by RT-qPCR, showing no differences. Next, pluripotency genes OCT4, NANOG and SOX2 expression were measured and showed slight upregulation. Although mutant cell line culture was not affected, cardiac differentiation in vitro was dramatically compromised, 74 % of cardiomyocytes (wt) compared to 1 % (mutant) (n= 3). In concordance, MESP1, the master gene for CD, was downregulated. We hypothesize that pluripotency genes balance is altered, and that inhibits OCT4/LEF1 complex formation at MESP1 promoter, important for its normal activation. However, more experiments are necessary to confirm it.

Nanomedicina / Nanomedicine II

Chairs: Mariela Agotegaray | Virginia Aiassa | María José Morilla | Marisa Taverna Porro

0082 - NANOFILMS OF ADSORBED THYMOL FORMED ON TITANIUM SURFACES FOR BIOMEDICAL APPLICATIONS. ANTIMICROBIAL ACTIVITY AND BIOCOMPATIBILITY

Ariel GONZALEZ | Alejandro MIÑAN | Claudia Alejandra GRILLO | Patricia Laura SCHILARDI | Mónica Alicia FERNÁNDEZ LORENZO

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Titanium (Ti) and its alloys are widely used in the construction of permanent orthopedic and cardiovascular implants. However, one of the most frequent causes of failures are bacterial infections by Staphylococcus aureus. This is aggravated by the abusive use of antibiotics that generate microbial resistance to conventional therapies. As a consequence, new antimicrobial nanotechnologies (AMN) emerge as promising alternatives to prevent prosthetic infections. The aim of this work was to evaluate the antimicrobial effect of an innovative AMN: thymol (TOH, phenolic phytocompound) nanofilms adsorbed on Ti (NPTOH-Ti) against S. aureus. The biocompatibility was also determined using preosteoblast cells (MC3T3-E1). To that end, 1 cm diameter grade 2 Ti discs were used and TOH was adsorbed onto their surface by 2h immersion in 0.1 M TOH acid solution. NPTOH-Ti was detected by infrared spectroscopy (FTIR-ATR). The antibiofilm activity of NPTOH-Ti and Ti (control) was determined by immersing the metal discs in a suspension of S. aureus (10^8 bacteria/ml) for 3 h. Subsequently, the number of bacteria adhered on the discs was caunted after sonication by colony forming unit (CFU). In addition, Live/Dead (Invitrogen) staining was used to determine if the adhered bacteria were alive or dead. Finally, biocompatibility of NPTOH-Ti and Ti was assessed by staining the preosteoblast cells with acridine orange. The results showed that NPTOH-Ti has effective anti-biofilm properties. On the one hand, viable bacteria were not observed by the plating count method and Live/Dead staining exhibited only dead (red) bacteria on the surface. On the other hand, control Ti revealed $4 \pm 0.5 \times 10^5$ adhered bacteria that were mostly (95 %) alive (green). In addition, NPTOH-Ti and Ti showed similar cell adhesion and growth (107 ± 12 and 100 ±16 % respectively; $p>0.05$). It was concluded that NPTOH-Ti are biocompatible and have anti-biofilm properties which make them promising to prevent prosthetic infections.

0085 - INFLUENCE OF SURFACE NANOESTRUCTURES OF ANODIZED TITANIUM ALLOY ON THE ADHESION OF FIBROBLASTIC AND OSTEOBLASTIC CELLS

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Ti-6Al-4V is widely used for orthopedic, cardiovascular and dental applications. Different cell/surface interactions take place according to the implantation site. The aim of this work was to analyze, comparatively, the influence of surface features on the adhesion of two cell lines of different characteristics, L929 fibroblasts and MC3T3-E1 pre-osteoblast cells. With this purpose, different topographies were generated by using electrochemical anodization, employing mirror polished Ti-6Al-4V disks (TAD) as working cathodes, graphite rod as anode and HF/H3PO4 solution as electrolyte. The TAD were anodized at 10 V and 30 V for one hour. Cell adhesion was evaluated using Acridine Orange staining and the areas covered by cells (polished TAD as control, 100 %) were obtained from the anodized-TAD. Biological assays were made by triplicate. Microscopic examinations of the surfaces depicted different topographical characteristics and contact angle evaluations showed higher wettability for the anodized-TAD in relation to the smooth metal. Comparative analysis showed that cell adhesion on 10V-anodized-TAD was similar to that of the control for both cells lines (100.05 ± 7.99 for MC3T3-E1 and 92.20 ± 19.93 for L929, smooth control 100% ± 8.84 %, $p>0.05$). Interestingly, when TAD were anodized at 30 V important surface morphological modifications and a marked difference in the adhesion of the two cell lines were noticed ($p<0.001$). A higher agglomeration of cells was also observed on anodized-TAD. A slight decrease of adhesion (82.74 ± 12.89 %) was obtained in case of MC3T3-E1 while an important reduction was noticed for L929

(30.66 ± 17.28 %). It was concluded that L929 cell line is more sensitive to the changes in surface characteristics (wettability and roughness) than MC3T3-E1. Consequently, the appropriate choice of cell the line and surface characteristics of the biometals are key factors to take into account when cell adhesion is evaluated.

0270 - IMMOBILIZATION OF BENEFICIAL VAGINAL LACTOBACILLI IN POLYMERIC NANOFIBERS FOR ITS POTENTIAL INCLUSION IN VAGINAL PROBIOTIC FORMULATIONS

Jessica Alejandra SILVA | Priscilla Romina DE GREGORIO | Guadalupe RIVERO | Gustavo Abel ABRAHAM | **María Elena Fátima NADER**

CONSEJO NACIONAL DE INVESTIGACIONES CIENTÍFICAS Y TÉCNICAS (CONICET)

Lactobacilli are the predominant microorganisms in the vaginal microbiome of healthy women. Probiotic formulations containing lactic acid bacteria (LAB) must include a high number of viable and active bacteria. The aim of this work was to evaluate the compatibility, survival and maintenance of beneficial properties of Lactobacillus gasseri CRL1320 and L. rhamnosus CRL1332 during their immobilization in polymeric nanofibers by electrospinning and after storage. The compatibility of lactobacilli with mucoadhesive polymers [polyvinylalcohol (PVA), polyvinylpyrrolidone (PVP) and chitosan/polyethylene oxide (Quit/PEO)] were evaluated. Lactobacilli were electrospunned with 15 % w/v PVA (12 kV, 0.3 mL/h, 12 cm distance to aluminum collector). The membranes were later stored at room temperature, 4 and -20 °C. Lactobacillus viability, maintenance of beneficial properties (hydrophobicity, self-aggregation and antimicrobial activity against urogenital pathogens) and nanofibers characterization was performed by SEM and FITR. The combination of PVA and PVP does not affect the bacteria viability, while Quit/PEO mixture was non-compatible. Therefore, PVA was selected for LAB immobilization. Electrospinning process was efficient since it allowed the recovery of a high number of lactobacilli (1010 UFC/g nanofiber) without modifying the surface and antimicrobial properties of the two strains. Lactobacillus immobilized in nanofibers were evidenced by SEM and FTIR. A higher survival rate was obtained in L. rhamnosus CRL1332 than in L. gasseri CRL1320 after the immobilization. The highest viable cells were kept in nanofibers stored at -20 °C. However, a decrease of viable cells (lower than 1 x 10^7 CFU/g) was observed in L. gasseri CRL1320 and L. rhamnosus CRL1332 at 28 and 56 days, respectively. The results obtained support the inclusion of lactobacilli into polymeric nanofibers for the design of vaginal formula. However, further studies are being carried out to improve the Lactobacillus survival.

0274 - DERMATAN SULFATE/CHITOSAN NANOMATERIALS LOADED WITH IRW MODULATE HUMAN ENDOTHELIAL STERILE INFLAMMATORY RESPONSE.

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UNIVERSIDAD DE BUENOS AIRES, FACULTAD DE FARMACIA Y BIOQUÍMICA, CÁTEDRA BIOLOGÍA CELULAR Y MOLECULAR (1); UNIVERSIDAD DE BUENOS AIRES, FACULTAD DE FARMACIA Y BIOQUÍMICA, BIOTECNOLOGÍA, NANOBIOTEC-CONICET (2); UNIVERSIDAD DE BUENOS AIRES. FACULTAD DE FARMACIA Y BIOQUÍMICA.TECNOLOGÍA FARMACÉUTICA (3)

The present work describes a novel delivery system for the selective targeting of egg-derived anti-inflammatory tripeptide Ile-Arg-Trp (IRW) to modulate human endothelial cells inflammation, in the context of high levels of oxidized triglyceride-rich lipoproteins (VLDLox). IRW is produced by solid phase peptide synthesis. Dermatan sulfate/Chitosan nanoparticles loaded with IRW (8.00% w/w) (DS/CS-IRW) are prepared by ionotropic gelification method and characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM). VLDLs were isolated from healthy human volunteers by density-gradient ultracentrifugation and oxidized by 5µM CuSO4 for 2 at 37 °C. To analyze the selective binding and uptake, human endothelial cells (EA.hy926) and human macrophages were incubated in the presence of FITC-nanoparticles at the biologically active concentration of DS, 10 µg/mL. Endothelial sterile inflammatory response was evaluated by NFkB subcellular distribution through immunofluorescence analysis and zymography studies. The incorporation of IRW results in a stable nanoparticle dispersion with a single size population of 539 ± 75 nm (n= 6). TEM shows that IRW inclusion resulted in compact spherical-like particles. Co-cultures between endothelial cells and macrophages confirm the selective interaction of fluorescent DS/CS-IRW with EA.hy926. After incubating endothelial cells with 100 µg protein/mL of VLDLox for 24 h, NFkB is localized both at the cytoplasmic and nuclear compartment. Nevertheless, the transcriptional factor is restricted to the cytoplasm in the presence of DS/CS-IRW nanoparticles. NFkB subcellular distribution was correlated with endothelial inflammatory response through the evaluation of the effect of DS/CS-IRW nanoparticles on matrix metalloproteinases activity-9. Zymographic analysis reveal no detectable MMP-9 activity after DS/CS-IRW treatment. We report here on the capability of these IRW-loaded complexes to modulate endothelial inflammatory response by as simple and potentially scalable nanotechnological platform.

0382 - DEVELOPMENT OF VITAMIN COATED TITANIUM DIOXIDE NANOPARTICLES WITH IMPROVED BIOCOMPATIBILITY

Maria Virginia VAUDAGNA (1) | Ariana ZOPPI(2) | Maria Cecilia BECERRA(3) | Virginia AIASSA(2) | Maria Jazmin SILVERO(3)

DPTO. CS. FARMACÉUTICAS, FAC. CS QUÍMICAS, UNIV. NACIONAL DE CÓRDOBA (1); LINBIO - DPTO. CS. FARMACÉUTICAS, FAC. CS QUÍMICAS, UNIV. NACIONAL DE CÓRDOBA - UNITEFA (CONICET) (2); LINBIO - DPTO. CS. FARMACÉUTICAS, FAC. CS QUÍMICAS, UNIV. NACIONAL DE CÓRDOBA - IMBIV (CONICET) (3)

Titanium dioxide (TiO_2) is widely used in sunscreens because it protects against UV radiation. Current ones are micronized or nanoparticle formulations (TiO_2@NP), which blend in with the skin tone and attain better cosmetic effect. Nanosized TiO_2 is approved by the Food and Drug Administration, but its biocompatibility is controversial. Concern about negative effects has lately been raised. In fact, cytotoxicity and oxidative stress produced by TiO_2@NP when exposed to sunlight were demonstrated in some studies. The goal of this work was to coat this kind of nanoparticle to protect skin cells from the damage generated upon the interaction with light. Functionalization of TiO_2@NP was done with antioxidant vitamin B_2 (riboflavin) because it has the potential to bind to the nanoparticle through an amine group. Binding was achieved after few minutes of sonication in aqueous media, followed by characterization. We used a model of prokaryotic cells (methicillin-sensitive Staphylococcus aureus biofilm) exposed to light to study the protective capacity of vitamins@TiO_2NP. Viability was assessed using XTT salt. The absorbance values are proportional to the metabolic activity of the cells and indicate cell survival. The analysis of the supernatant by UV-Vis spectrometry showed that every gram of TiO2@NP is loaded with 0.8 grams of vitamin B_2, after washing them. The IR spectrum of vitaminB2@TiO2NP showed signs of binding between compounds. The OH bending peak (1634 cm^{-1}) corresponding to bare nanoparticle disappeared and the NH_2 bending band characteristic of vitamin B_2 appeared (1650 cm^{-1}). Cell viability percent was higher for prokaryotic cells when they were treated with vitaminB_2@TiO_2NP (153 ± 9 %) compared to the ones treated with

bare TiO_2@NP (82 ± 3 %). Results proved the great potential of vitamin B_2 to coat TiO_2@NP and protect the cells, therefore we continue the study of this and other vitamins@TiO_2NP on eukaryotic cells.

0430 - 2-PHENIL-4-SUBTITUTEDQUINAZOLINES WITH ACTIVITY AGAINST BOVINE VIRAL DIARRHEA VIRUS (BVDV): BIOLOGICAL EVALUATION AND PHYSICOCHEMICAL PROPERTIES.

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Bovine viral diarrhea virus (BVDV) belongs to the Pestivirus genus of the family Flaviviridae. Because BVDV infections lead to considerable financial losses within the livestock production, having effective and selective antivirals could be very useful. Viral RNA-dependent RNA polymerase (NS5B RdRp) is responsible for viral RNA synthesis. In previous studies, after in silico molecular screening with BVDV RdRp and in vitro evaluation, we identified molecules with anti-BVDV activity. Between them, N-(2-morpholinoethyl)-2-phenylquinazolin-4-amine (1) presented an EC_{50} value of 9.68 ± 0.49 µM, and it was chosen for further optimization. Therefore, we synthesized 26 derivates of 1 via nucleophilic aromatic substitution (SNAr) reactions with several amines and anilines. Six derivatives showed improved antiviral activity (EC_{50} from 1.37 ± 0.27 to 1.78 ± 0.37 µM) with better selectivity index (SI) than 1. Derivative 1.9 with the highest selectivity index (17.95) was further studied. Physicochemical properties of 1.9. The solubility of compound 1.9 was tested at 1.2, 6.8, and 7.4 pH values, employing the shake-flask method. As result, 1.9 presented good solubility values within the range generally observed for oral drugs: simulated gastric fluid (pH 1.2): 1,060.8 ± 100.2 µg/mL; simulated intestinal fluid (pH 6.8): 420.4 ± 23.7 µg/mL; and phosphate buffered saline (pH 7.4): 187.2 ± 5.0 µg/mL. Stability studies in different media and the encapsulation of the compounds are currently ongoing in our lab. We hypothesize that encapsulation could improve compound's solubility. We will also test the cytotoxicity of these encapsulated compounds with the aim of obtaining better selectivity index, which can be traduced in more promising candidates as anti-BVDV agents. In summary, we obtained potent anti-BVDV molecules after lead optimization. Biological characterization and in silico studies indicated that these compounds may act as BVDV-RdRp inhibitors.

0446 - IN VITRO PASSIVE PERMEABILITY OF PROPANOLOL ACROSS A CHITOSAN / ALGINATE / MUCIN FREE-STANDING MULTILAYERS FILM

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In this work we reported an in vitro permeability assay by using a biomimetic chitosan (CHI)/alginate (ALG)/mucin (MUC) free-standing multilayers film, fabricated by layer-by-layer (LbL) assembly, to predict the in vivo intestinal passive permeability behavior of propranolol (PRO). The CHI/ALG/MUC free-standing multilayers film was designed by functionally mimicking the gut mucosa because they are natural polymers mimic the extracellular matrix components of tissues, while MUC is the main component of the mucus that covers most of the mucosal epithelia and plays a fundamental role in the permeability profiles of drugs, either favoring or impairing their absorption, as drugs administrated through the mucosa will first have to pass the mucus layer. In order to study the interaction between the mucus layer and PRO, permeability studies were performed by assembled the CHI/ALG free-standing multilayers films with or without MUC adsorbed into the film surface. It was found that MUC had not a significant effect on the PRO permeability behavior (p= 0.974). After the PRO permeability tests, films were analyzed by SEM, circular dichroism (DC) and by measurement their contact angles. SEM images of films without MUC showed the presence of the adsorbed drug on the film surface. On the other hand, it was found that PRO produced a structural conformation change of the MUC adsorbed into films from a globular structure to a flat conformation. These results were confirmed by the DC study, where it was clearly observed the conformational change of the MUC by the PRO. Contact angle measurements revealed a different surface wettability behavior of films, being films CHI-ending hydrophobic. In contrast, this film changed its surface to very hydrophilic after into contact with PRO. Results allow concluding that PRO interacts with MUC, affecting the structural conformation of the glycoprotein, without producing a significant effect on the permeability behavior of PRO.

0449 - ANTIMICROBIAL ACTIVITY AGAINST STAPHYLOCOCCUS AUREUS AND RESPIRATORY BIOCOMPATIBILITY OF RIFAMPICIN NANOPARTICLES

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Infections caused by intracellular bacteria in the lower respiratory tract are among the most important causes of death worldwide. In particular, pulmonary intracellular infections are difficult to eradicate due to various contributing factors which might lead to the emergence of antibiotic-resistance. Nanotechnology is a very promising technological tool to combat health problems associated with the loss of effectiveness of currently used antibiotics. Based on this problem, this work aimed to evaluate polymeric nanoparticles (NPs) as efficient carriers of rifampicin (RIF) for the treatment of intracellular lung infections. To this end RIF loaded NPs were incubated with human lung-derived cell lines and tested for cytotoxicity, uptake capacity and antimicrobial activity against S. aureus. MTT assay was carried out to study NPs cytotoxicity in human fetal lung fibroblast cells (MRC5 cell line) and adenocarcinomic human alveolar basal epithelial cells (A549 cell line). The cellular uptake capacity of RIF NPs was studied by confocal laser scanning microscopy (CLSM) and the drug quantification by high performance liquid chromatography (HPLC). Finally, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in different S. aureus strains were determined and interactions between NPs and bacterial cells were evaluated by CLSM. RIF NPs had less cytotoxicity than free RIF and were internalized by lung cells. Moreover, RIF NPs showed a significant improvement in antimicrobial activity against S. aureus strains. The interfacial assembly of NPs in the bacterial membrane was determined by CLSM, which indicated a strong interaction between bacteria and NPs, this was a key contributor to the biocide activity of proposed nanocarrier. The findings of this work show that RIF polymeric NPs could be an adequate system for the delivery of antibiotics which are known to be losing efficacy due to acquired antimicrobial resistance, with promising prospects regarding effective treatment of pulmonary intracellular infections.

0517 - COMBINED TRAPPING: TOBRAMYCIN + THYMUS VULGARIS ESSENTIAL OIL WITHIN

NEBULIZABLE ULTRA SMALL NANOSTRUCTURED LIPID NANOPARTICLES

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UNIVERSIDAD NACIONAL DE QUILMES

Essential oils (eo) are mixtures of volatile terpenes having pleiotropic activity on multiple therapeutic targets. Their use as antibiotics or antitumorals however, is limited by a high physicochemical unstability and toxicity for eukaryotic tissues. Aiming to solve such challenges, in this work we explore the concept of combined trapping of actives within targeted nanoparticles. Specifically, there were prepared nanostructured lipid nanoparticles (NLN) covered with lipid surfactants (total polar archaeolipids, TPA) extracted from the hyperhalophilic archaebacteria Halorubrum tebenquichense (NLN-TPA). The nanostructure of such nanoparticles resulted efficient to entrap and protect variable amounts of Thymus vulgaris eo (eoT) together with the hydrophilic antibiotic tobramycin (TB) and were named as "NLN-TPA (eoT+TB)". Our preliminary data showed that NLN-TPA (eoT+TB) made of [(2 %) compritol: (0.5 %) miglyol: (1 %) TPA: (3 %) Tween 80: (89 %) H_2O: (5 %) eoT: (0.03 %) TB] were structurally stable along 6 of storage (~ 300 nm Z average, 0,45 pdi, ~ -40 mVζ) and importantly, to the nebulization stress. Remarkably, the presence of TPA was critical to stabilize the combined trapping of the two actives. Besides, the presence of TPA provides a selective targeting to cells expressing SR-AI/II, such as macrophages and dendritic cells, as well as optimizes the interaction with bacterial biofilms in the respiratory tree. It is expected that in NLN-TPA, the combined trapping of eoT and TB would magnify the antibacterial activity of both agents, providing higher therapeutic activity that the achieved by TB alone and at doses small enough of eoT to be innocuous for eukaryotic cells.

0658 - ANALYSIS OF COATED MICROPARTICLES IN A SMALL-SCALE FLUID-BED

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The design of multiparticulate systems is a technological strategy of high impact in the formulation of immunomodulators for the oral route, being the fluid bed coating an attractive obtaining method, due to its versatility and relatively low cost, allowing that substances such as the cell wall of Saccharomyces cerevisiae (EPL), can be transported in low concentrations together with delayed release products. The objective of this work was to coat a solid support (SUGLETS®) with EPL and with an aqueous acrylic enteric system (Acryl-EZE®) to subsequently evaluate the way in which the active component is liberate of the system, through in vitro dissolution tests. METHODS The design and development of the microparticles (MP) was carried out in a Caleva Mini Coater Drier - 2 unit. The influence of the operating and formulation conditions on the quality and performance of the SUGLETS® coated by EPL was determined through a fractionated factorial design. Then, the MP were coated with Acryl-EZE® and in vitro dissolution tests were performed to determine the effectiveness of the film coatings and the way in which the active component is released from the system using a Semi-automatic Dissolution System T XtendTM, Apparatus 1 (basket) and ultraviolet–visible Spectroscopy. The conditions that resulted in the best performance (93 %) and gain in weight (42 %) were from the equipment: fan: 14.1 m/sec, temperature: 26°C, pump: 1.15 rpm, pressure: 20 psi. and from formulation: EPL: 1 g, Methocel: 0.2 g, DS: 1 g, glycerin: 2 g. The conditions in relation to the dissolution test: In the acid phase: 0-120 minutes, not more than 10 % dissolved and buffer phase: 120-210 minutes, not less than 80 % dissolved. By means of the use of a fluidized bed, it was possible to obtain microparticles coated with immunomodulatory compound (EPL) which, after coating with an enteric film coating, it was possible to determine the delayed release by colorimetric reaction.

0669 - STUDY OF ROUTES OF ADMINISTRATION OF ALBUMIN NANOPARTICLES FOR THE TREATMENT OF RETINAL PATHOLOGIES

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Retinal pathologies are treated with therapeutic agents that are administered invasively and periodically. Nanoparticles (NPs) could be a new way of performing therapies directed to the retina. To evaluate different routes of administration of NPs to reach the retina and to analyze the response of the retinal pigment epithelium (RPE) to the presence of NPs. We evaluated NPs of 20 nm and 100 nm in diameter of human albumin associated with a quantum dot. We inoculated the 20 nm NPs through intravitreal (IV), subconjunctival (SCj), and suprachoroidal (SC) injections and the NPs of 100 nm only through SCj injections. The distribution was observed at 3 and 24 hours after the inoculation in wholemounts of retinas and choroids in a fluorescence microscope. In addition, the RPE cell line, ARPE-19, was incubated with NPs for 3 and 24 h, and were observed under fluorescence microscopy. In the IV inoculations, the NPs were detected mainly in the retina and vitreous humor, both at 3 and 24 h. After 24 h, we observed inflammatory cells containing NPs. The SC inoculations showed NPs in the choroid and in the retina mainly in regions associated with blood vessels (after 3 h), persisting in inflammatory cells after 24 h. In SCj inoculations, NPs of both 20 nm and 100 nm were detected in the choroid (after 3 and 24 h) and in the RPE (after 24 h). In the ARPE-19 cells, we observed presence of NPs in the cytoplasm and also in the extracellular matrix, mainly after 24 h of incubation, for both NPs. Conclusions NPs compose of protein showed high biocompatibility, since we did not observed aggregation in none of the tissues analyzed. From the in vitro studies we can infer that the ARPE-19 cells could endocyte the NPs. From the in vivo studies the most promising route of administration would be the SCj, because of the NPs reach the target tissues being less invasive than the others studied.

0712 - NEW ANTIBIOTIC-ANESTHETIC MODIFIED RELEASE FILMS. PRECLINICAL EVALUATION ON DEEP SECOND DEGREE BURNS

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Most important burn treatment challenges are associated with control of infections and pain; and also functional and aesthetic wound healing. The aim of this study was to evaluate the wound healing efficacy in a burn rat model of a treatment with an anesthetic-antibiotic polymeric films (AAF), compared to the reference treatment. A deep second degree burn model was used in male Wistar rats (CICUAL UNLaR protocol nº 5/18). Each anesthetized animal was injured in the dorsal region with a metallic device (1 cm ø, 90 ± 2 ºC, 30 sec). The AAF (Argentine Patent

Application nº 20190102232), control film (CF) or reference cream treatments (RC: silver sulfadiazine 1.0 %, lidocaine 0.67 % and vitamin A 248000 UI) were applied once daily for 21 days (n= 6). Control groups: untreated (UT) and not burned. Photographs and biopsies (H&E) were taken on days 0, 7, 14 and 21. Epidermal continuity and dermal organization were evaluated with scores according to Sanchez et al[1]. Biopsies analysis showed that epidermis closure was reached in the order AAF > CF > UT > RC. Besides, burns treated with AAF presented complete dermis organization at day 21 and histological characters similar to unburned control. These results could be related to the favorable moist environment provided by the components of AAF, that positively impacts on the tissue recovery. In contrast, burns treated with RC did not complete its regeneration at day 21 and even a regression was observed respect to day 14. Most of UT animals presented dense dermis and absence of skin annexes (day 21). These findings suggest that the use of the AAF allowed a more rapid and better quality skin regeneration process with respect to the available reference treatment.

Reference: 1- Sanchez MF et al. Drug Deliv Transl Res. 2018: 8 (5): 1000-13.

0715 - INTERACTIONS BETWEEN HYBRID NANOPARTICLES DESIGNED BY RADIO-SYNTHESIS AND THE HEMATOLOGY SYSTEM

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Nanoparticles (NPs) are extremely promising due to their physicochemical properties and their distinctive features for therapeutic applications. When NPs enter the bloodstream, they immediately interact with plasma and hematology cells (erythrocytes, monocytes and platelets). In the case of blood plasma, the surface of the NPs interacts with several different biomolecules, mostly proteins, forming irreversible layers called the `protein corona´. This adsorption of proteins onto NPs modify the diverse physicochemical properties of NPs such as size, surface charge, surface composition, and functionality, hence giving NPs a new biological identity and different biological responses. The aim of this work is to determine the effect of hybrid NPs on the formation of the protein corona in vitro and erythrocyte interaction and coagulation time. These hybrid NPs are AuNPs coated with human serum albumin (Alb) multilayers by a novel radiation-induced crosslinking process and are called Au/Alb core/shell nanoparticles (Au/Alb NPs). Albumins from human serum were added to the AuNPs suspension, 30% v/v ethanol was added and then these NPs were irradiated at 10 kGy with a gamma source to induce protein crosslinking. The protein corona effect was evaluated in terms of the hydrodynamic diameter (HD) of the NPs, isolation of the corona complex by size exclusion chromatography and polyacrylamide gel electrophoresis. In addition, not changes in the HD were found. In conclusion, our results suggest that Au/Alb NPs show a better hemocompatibility than albumin monolayer and pegilated Au.

0763 - GLUTAMIC ACID AS COATING FOR MAGNETIC NANOPARTICLES: A PLATFORM FOR DIVERSE BIOMEDICAL APPLICATIONS

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Coating of iron oxide magnetic nanoparticles (IOMNPs) with biocompatible molecules is mandatory for biomedical applications. The election of amino acids as coating agents is because their ability in surface stabilization of IOMNPs being also inexpensive and nontoxic. In the present work, glutamic acid (GA) was selected as coating agent aiming to obtain GA-modified IOMNPs with suitable physicochemical properties, including stability, for biomedical applications. The co-precipitation method was applied to obtain IOMNPs functionalized with GA. Two experimental procedures were evaluated varying the order of GA incorporation. In the first procedure (1), an aqueous solution of GA was added under magnetic stirring to a mixture of $FeCl_3.6H_2O$ and $FeSO_4$. In the second case (2), the mixture of iron salts was added to the aqueous solution of GA. Both reactions were conducted under N_2 atmosphere at 70 °C. A solution of NaOH was added dropwise. After 30 min, the supernatants were removed, and the resultant solids were washed and dried at 45 °C. The same procedure was applied to obtain bare IOMNPs. The resultant formulations were studied by FTIR, DLS to determine hydrodynamic diameter and isoelectric point, TEM, atomic absorption spectroscopy (for iron content determination) and DRX. Data analysis revealed that the method 2 allowed the efficient functionalization of IOMNPs with GA rendering a formulation with hydrodynamic diameter of 226.0 nm with spherical shape. The first method renders IOMNPs with similar properties than bare IOMNPs, revealing that functionalization with GA was not successful. The method applied to functionalize IOMNPs with GA was dependent on the order of reactants aggregation. It was possible to obtain IOMNPs coated with GA (IOMNPs@GA) with suitable properties for biomedical applications. The exposure of functional groups such as carboxylate moieties allows to the potential anchoring of diverse molecules (drugs or biomolecules) for the located treatment of multiple diseases.

0790 - F127 (OR P407) POLOXAMER AS A PROMISING GELLING AGENT IN SERTOLI CELL DELIVERY FOR CELL THERAPY OF THE TESTIS

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Sertoli cells (SCs) play an important role in creating an immune-privileged environment and supporting spermatogenesis. Their ability to survive transplantation long term without immunosuppression leads to the notion that SCs may be a tool for cell therapy of many chronic inflammatory diseases. Infertility has become a major health issue in the world, 40% due to male factor. There is no specific treatment to cure infertile patients; overall therapies try to bypass the cause using artificial reproductive technology. We hypothesize SC transplantation would be assessed as a practical approach for recovering spermatogenesis in azoospermic male with chronic inflammation. The trophic potential of SCs has been challenged by transplanting them in the testis of rats devoided of spermatogenesis by chemical treatment. However, experimental protocols employing a high number of SCs which disperse all around the testis had a very limited success. The aim of our work was to improve SC transfer protocols in order to increase cell density in specific areas of testis allowing diffusion of their secreted factors. We use biocompatible linear tri-block copolymer (Pluronic® F127) as cell delivery hydrogel and the SC line TM4. F127 was dispersed in saline to obtain 16-22 % (w/v) dispersions and placed at 34 °C. F127 preparation (22 %) turned into hydrogel in less than 1 min. TM4 cells dispersed in 22% F127 kept viable up to 24 h at 34°C (Trypan blue method). 2-4 x 10^6 TM4 cells labeled with carboxyfluorescein-succinimidyl ester (CFSE) dispersed in 22 % F127 or saline (100 µL) were injected ex-vivo in the testis from adult rats. After 7 min testes were frozen or fixed (PFA) for histology evaluation and fluorescence microscopy. Results showed that F127 increased cell density near injection sites limiting

their dispersion vs cells transferred in saline. We propose that F127 is a useful copolymer matrix for cell therapy as it would allow secreted soluble factors reach therapeutic levels minimizing cell number requirement.

0837 - "LATANOPROST LOADED PHYTANTRIOL CUBOSOMES FOR GLAUCOMA TREATMENT"

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Glaucoma is a degenerative optic neuropathy; it is characterized by increased intraocular pressure (IOP) that results in irreversible damages in all ocular nervous structures and is the main risk factor that can be modified and treated. Liquid crystal systems, such as cubosomes (CUB), emerge as a new way of transporting drugs with properties as biodegradables, bioadhesives and besides show a prolonged release, which makes as potentially useful for administration ocular. Latanoprost (LN) is an esterified prodrug that has a lipophilic nature, it reduces IOP through drainage of the aqueous humor. The principal goal was obtaining nanometric sized CUB through Top-down (TD) method and encapsulating LN for the treatment of glaucoma. TD approach was employed to prepare CUB of phytantriol in water with a solution of Pluronic®F127 as a stabilizer using ultrasonication. CUB were prepared at different concentrations of LN. The average particle size and zeta potential were measured using DLS. The CUB was characterized using small-angle X-ray scattering (SAXS) and isothermal titration calorimetry. The loading capacity was determined by HPLC. The in vitro release of LN from the CUB was studied using a dialysis method in cells coupled with LC/MS technique. To evaluate in vivo efficiency, the formulations were administered subconjuntival manner in normotensive rabbits. IOP and irritation were evaluated. The CUB had an average particle size of 200 nm with negative zeta potential values and it showed a good encapsulation efficiency of LN around 90 %. The SAXS studies revealed a double diamond Pn3m cubic structure for blank and LN-loaded CUB with a constant in the lattice parameter. The ITC assays showing a slight exothermic process of interaction between CUB and the drug. In vitro release essays exhibited a sustained release in the time at each concentration evaluated. In vivo essays showed an important reduction of IOP of about 20 % for 4 days without signal of significant irritation.

0839 - METHOD OF PREPARATION AND CHEMICAL PHYSICAL CHARACTERIZATION OF NP-HSA LOADED WITH MELATONIN WITH POSSIBLE APPLICATION IN NEURODEGENERATIVE EYE DISEASES

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The development of ophthalmic formulations for the treatment of ocular pathologies, such as glaucoma, presents a challenge due to the low absorption and bioavailability of drugs in the ocular pathway. Transport systems based on human serum albumin nanoparticles (NP-HSA) represent an important strategy, since in addition to being an endogenous molecule, significant amounts of active ingredients can be incorporated into the particle. NP-HSA are of our interest to transport melatonin, an insoluble drug that has been described as an effective antioxidant in the retina with direct and indirect free radical scavenging activity. The objective of this work was design, formulate and characterize NP-HSA loaded with melatonin with possible application in eye diseases, such as glaucoma. The obtaining of NP-HSA was carried out by desolvation process. Different crosslinking agents (CA) such as Gantrez, polyethylene glycol 400, Eudragit S100 and hydroxypropylmethylcellulose-phtalate were tested, based on the existence of functional groups capable of interacting with NP-HSA, all of them non-toxic compounds and approved as excipients for pharmaceutical formulations. In addition, it was evaluated if the change in the sequence of CA addition in the formulation, produces changes in obtaining NP-HSA, in order to achieve greater stability, yield and encapsulation efficiency. Colloidal dispersions with a pH close to neutrality were obtained. Regardless of the CA used and the order in which it was added to the system, all NP-HSA presented a unique and uniform population with a particle size between 150-230 nm. The percentages of yield (75,9 %) and encapsulation efficiency (16,8 %), showed that the method of obtaining NP-HSA whose CA is Eudragit S100, was the most efficient, obtaining optimal and stable systems in time. These results will allow us to advance in a greater physicochemical characterization, as well as in in vitro release tests of Melatonin to evaluate its release over time.

0862 - MAGNETIC NANOCLAYS: INCORPORATION AND DIRECTED DELIVERY OF NAPROXEN

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Layered double hydroxides (LDH) nanoclays have many applications as matrices in pharmaceutical fields as support for controlled release systems of drugs, vitamins, biomolecules, with potential applications orally or intravenously of modified release systems. Naproxen (Nap) is a nonsteroidal anti-inflammatory drug (NSAID) used to reduce pain involved in osteoarthritis, rheumatoid arthritis, bursitis, gout. This work reported studies of intercalation drug content and magnetic response. Nap has been incorporated by the direct method (coprecipitation) into nanoclays MgAl LDH-type material at pH 10 with molar ratio equal 2, and them was impregnated with magnetic nanoparticles of Fe_3O_4. By X-ray diffraction its possible observed the drug incorporation into nanoclay and the magnetic response was studied in a vibrating sample magnetometer (VSM) at room temperature, in order to obtain super paramagnetic materials. The amount of intercalated Nap was determined by UV-visible spectroscopy at 271 nm. The basal spacing recorded was 2.3 nm this value suggests the drug has been incorporated in the interlayer of the solids, since the interlayer distance for MgAl LDH is 0.76 nm. The new gallery heights indicate that the drugs have been stacked as monolayer particles perpendicular to the LDH plane. Drug content obtained was 65 %, which indicates that it was partially incorporated into nanoclays. This system presented super paramagnetic behavior at room temperature, a property desired for use in biomedicine. The modified nanoparticles obtained suggest that these materials have potential applications as directed release systems of Nap by magnetic fields use. This work provides significant in-sight into the important area of storage, transport, and delivery of anionic drug using MgAl-LDH as host solid.

0863 - LUMINESCENT SILICA- BASED NANOMATERIALS FOR BIOMEDICINE

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Silicon dioxide (SiO_2), a material known as "silica" presents properties that make it a good candidate as a biomaterial: it has a very labile surface for functionalization, it is easy to synthesize and it is biocompatible. Calcination after synthesis of silica NPs yields

luminescent nanomaterials. Fluorescent NPs themselves would offer enhanced functionality in terms of these properties with respect to conventional organic fluorophores. In this work, the influence of the synthesis method on the hydrodynamic diameter of silica fluorescent NPs was studied in order to obtain an optimal formulation as potential theranostic. Stöber process, involving tetraethylorthosilicate (TEOS) and 3-aminopropyltriethoxysilane (APTES), has been applied to obtain different formulations from increasing APTES initial concentration. After synthesis, the NPs were calcined at 450 °C. Characterization was performed by FTIR, TEM, DRX, fluorescence spectroscopy/microscopy and DLS to determine hydrodynamic diameter (Hd). NPs synthesized without APTES do not present fluorescent properties, while the APTES containing NPs are fluorescent. Increasing concentration of APTES induces larger NPs with low stability in physiological medium. The optimal synthesis condition resulted with a TEOS:APTES ratio of 1:0.1 rendering a Hd of 450 nm with polydispersion index near 0.20. This formulation is suitable for biomedical applications in terms of Hd and stability as potential theranostic agent for multiple pathologies, including Chronic Lymphocytic Leukemia that is of our particular interest. CLL is the most common adult leukemia in Western countries and is defined by the accumulation of mature, CD5+ B lymphocytes in peripheral blood, bone marrow and secondary lymphoid organs. Despite important progress in treatment, relapse occurs and this leukemia remains incurable in many cases. New therapeutic approaches by innovative tools acting in synergism with the last therapies approved in CLL are desirable.

0980 - SOLID DISPERSIONS AS A PHARMACEUTICAL STRATEGY FOR CARRING BENZNIDAZOLE

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Solid dispersions (SD) are considered one of the most successful strategies for improving the dissolution of poorly soluble drugs. Benznidazole (BZL) is an antiparasitic agent with low water solubility, used as a first-line drug for the treatment against Chagas disease. The aim of this work was to evaluate the dissolution properties of BZL from SD based on Gelucire® and poloxamer. SD based on Gelucire® 44/14 (G4414) were prepared by a modification of the fusion method with 20, 40 and 50 % w/w BZL loads (DS G4414-20, DS G4414-40 and DS G4414-50, respectively). A SD using a mixture of G4414 and poloxamer 407 (P407) (1:1) with 40 % w/w BZL load was also prepared. SD morphology was compared with the corresponding physical mixture by scanning electronic microscopy (SEM), and the dissolution profiles were obtained at 37 °C using 0.1 N HCl as dissolution medium. The data were adjusted by the Lumped model, a mathematical model developed and validated by our research group, which allowed to calculate the initial dissolution rate (DRi), the time needed to dissolve 80 % of the drug (t80%) and the dissolution efficiency (DE). SEM revealed that drug crystals were not distinguished in the SD, whereas they were clearly present in the corresponding physical mixtures, confirming that the BZL was dispersed in an amorphous matrix. The data from the dissolution profiles were properly adjusted using the Lumped model (R^2 >0.89). Not only the DRi but also the amount of BZL dissolved were improved when formulated in a SD, and decreased along with an increase in the BZL load. The SD based on the mixture of G4414 and P407 showed the greater DRi (13.70 mg/min), which was 16 times higher than the DRi of the free drug. It can be concluded that the SD developed are promising to formulate an extemporaneous suspension of BZL with adequate biopharmaceutic properties, what would lead to better oral bioavailability in the treatment against Chagas disease.

0981 - ANPHOTERICINE B RELEASE FROM POLYMERIC FILMS. MODELING AND PHARMACEUTICAL PARAMETERS DETERMINATION

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Leishmaniasis is a "neglected" endemic disease and is a priority public health problem for Salta and Argentina. Treatment currently available in our country is very painful and invasive. Particularly for cutaneous leishmaniasis, there is no topical/local treatment that meets the activity, safety and cost requirements. For this reason, polymeric films with an appropriate drug load are proposed as alternative systems. Two polymers were selected, one of natural origin, sodium alginate (SA), and one synthetic, carbomer (CB). Amphotericin B (AnB) was used as a specific drug for the leishmaniasis treatment. The films were prepared with different drug concentrations using only SA or a combination of SA and CB. The cross section of the films was observed by scanning electron microscopy (SEM) and the in-vitro release assays were performed at 37 °C. The release profiles were analyzed and adjusted using the Lumped model developed and validated by our research group. Pharmaceutical interest parameters were calculated: t80% is the time required to reach 80 % dissolution of the total drug available, the dissolution efficiency is defined as the area under the dissolution profile up to a certain time, and the average dissolution time provides information about the ability of the polymer platform to delay the drug release. SEM images showed that films loaded with AnB maintained a dense structure and the drug was evenly distributed throughout the thickness of the film. AnB release profiles revealed that the CB incorporation caused a marked increase in the initial release rate, with a burst drug release in a short period of time, effect that is not suitable for systems that must modulate the drug release. Pharmaceutical relevance parameters were calculated and compared. It was determined that the SA films were able to modulate the drug release rate and presented optimal values of the parameters, in particular the film loaded with the highest percentage of AnB.

Toxicología / Toxicology III

Chairs: Florencia Chiappini | Sandra Ferreira

0139 - IMBALANCE IN HOMEOSTASIS RAT PLACENTA EXPOSED TO CADMIUM (G20). EFFECTS OF SOY PROTEIN AS A PROTEIN SOURCE

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Autophagy and apoptosis are two crucial and interconnected processes in the placenta that are often influenced by oxidative stress. Alteration between the protective and destructive mechanisms of autophagy and apoptosis seems to be associated to pregnancy-related disorders. In addition, Cd can pass through the placenta and accumulates in fetal tissues, so that rat fetal toxicity is caused by placental or maternal Cd-induced dysfunction, not by a Cd direct effect on fetuses. On the other way, soy protein is becoming increasingly important in the human diet. Isoflavones (genistein) could cause hypertrophy in endometrium and alter reproductive function in different species. To evaluate the possible protective role of soy protein consumption compared to the

mechanisms by which Cd exerts its toxicity, 4 lots of female Wistar rats were used: 2 lots received casein (Cas) and 2 lots soybean (Soy) as protein source. Within each group, 1 lot received regular water (Co) and the other, 15 ppm of Cd in the drinking water. We determined TBARS, CAT and GPx activity, and nitrite concentration. RT-PCR was performed using the following primers: MT I; MT II; Nrf-2; NOX-2, SOD-2 and CAT. In Soy-Cd group Nrf-2, SOD and MT I expression increased ($p<0.05$, $p<0.01$, $p<0.001$). While mRNA NOX-2 and MT II expression decreased ($p<0.01$; $p<0.01$; $p<0.00$, respectively). Bcl-2 positive immunostaining increased in both intoxicated groups ($p<0.001$; $p<0.01$), while PCNA increased ($p<0.001$), compared to control group. Caspase-3 immunohistochemical stain decreased in Soy-Cd group ($p<0.001$). Oxidative stress and placentation are closely interrelated. We demonstrated the presence of oxidative and nitrosative stress in placental tissue. This situation may lead to an imbalance in the placental process and result in early pregnancy loss and IUGR and this situation is aggravated with Cd intoxication.

0238 - HYPOMETHYLATION OF LINE-1 RETROTRANSPOSON IN BREAST CANCER CELLS EXPOSED TO PESTICIDES. ROLE OF TGF-ß1 PATHWAY

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Long interspersed nuclear element 1 (LINE-1) is the most common non-LTR retrotransposon in the human genome and comprises 17% of the genome. Reactivation of LINE-1 has been linked to breast cancer progression and metastasis. Strong ligands of aryl hydrocarbon receptor (AhR) activate LINE-1 through the transforming growth factor-ß1 (TGF-ß1) pathway in human liver cancer cells. We have observed that environmental doses of two weak AhR ligands: the organochlorine pesticide hexachlorobenzene (HCB, 0.005 µM) and the organophosphate chlorpyrifos (CPF, 0.5 µM) activate the TGF-ß1 signaling and enhance LINE-1 mRNA expression in the human breast cancer cell line MDA-MB-231. Our aim was to investigate the role of the TGF-ß1 signaling in the mechanism of action and if the pesticides induce epigenetic changes. To study the involvement of the TGF-ß1 signaling on LINE-1 expression, cells were exposed to HCB or CPF in the presence of the TGF-ß1 receptor I inhibitor (SB431542, 2 µM). qPCR results showed that the enhancement exerted by the pesticides was prevented by the treatment with the inhibitor ($p<0.05$). Considering that LINE-1 expression is regulated by methylation of its internal promoter, MDA-MB-231 cells were exposed to HCB or CPF and the methylation status of the LINE-1 5´-UTR was determined. Six sites (+38, +103, +167, +234, +306, +373) were analyzed by a combination of single digestions with methylation-sensitive restriction enzymes and qPCR. We found that the methylation was reduced by HCB at the sites +38, +103 and +167 ($p<0.05$) and by CPF at the sites +38 and +103 ($p<0.05$). In conclusion, HCB and CPF induce the demethylation of LINE-1 and enhance mRNA expression through TGF-ß1 pathway. Our findings support previous investigations showing that methylation of the first sites in 5´-UTR are essential for the LINE-1 transcriptional regulation. Finally, environmental doses of pesticides promote epigenetic changes that could increase human breast cancer risk.

0242 - IN-VITRO EFFECTS OF BISPHENOL A, BENZOPHENONES 2 AND 3 ON CYTOKINE EXPRESSION IN WHOLE HYPOTHALAMI AND IMMORTALIZED GNRH NEURONS

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Bisphenol A (BPA), a monomer of polycarbonate plastics, and Benzophenones (BPs), UV-filters, are endocrine disrupting chemicals (EDC). The hypothesis is that EDC exposure causes brain inflammation and predisposes animals to develop obesity and infertility. Previous results showed that the in-vitro exposure to BPA increases Glial Fibrillary Acidic Protein (GFAP) in hypothalami and IL-18 in mature GnRH neurons. Here, gene expression of cytokines (interleukins 6 and 1ß, IL-6 and IL-1ß) were evaluated in whole hypothalami of adult Balb/c mice and in immortalized GnRH neurons. Hypothalami were incubated in Krebs-Ringer medium in the presence or absence of BPA, BP2 or BP3 ($1x10^{-9}$ M) or medium alone (C) for six hours, and mRNA was extracted for gene expression analysis by qPCR. GnRH neuronal cell lines were cultured in 12-well plates (200,000 cells/well) in DMEM supplemented with 10 % FBS. After 24 hours, media were changed to DMEM without phenol red with 10% charcoal-stripped FBS. Cells were incubated with BPA, BP2, BP3 ($1x10^{-9}$ M) or vehicle (C) for 12 or 24 hours, RNA was extracted and gene expression analyzed by qPCR. In the hypothalami, IL-1ß expression was not changed by EDC exposure (ANOVA ns, n=8). Exposure to BP2 decreased IL-6 compared to C, although not reaching statistical significance (C= 1.1 ± 0.3; BP2= 0.5 ± 0.2; C vs. BP2, p= 0.08, n= 8). Neither BPA nor BP3 changed IL-6 expression. In GT1-7 cells (mature GnRH neurons), 24-hour exposure to BPA decreased IL-6 expression, without reaching statistical significance (C= 1.1 ± 0.2; BPA= 0.7 ± 0.1, C vs. BPA, p= 0.09, n= 4). Neither BP2 nor BP3 changed IL-6 in this cell line. Further experiments are underway to confirm the gene expression changes. The results will help us to further understand how exposure to EDC affects brain inflammation.

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0252 - HEXACHLOROBENZENE AS A CONTRIBUTOR TO TUMOR PROGRESSION: EFFECTS ON A HER2-POSITIVE MODEL, THE LM3 MURINE BREAST CANCER CELL LINE

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Pesticide exposure is proposed as a risk factor for breast cancer, the most frequent tumor in women worldwide. Polychlorinated biphenyls and organochlorine pesticides such as Hexachlorobenzene (HCB), have been identified as endocrine-disrupting-chemicals, compounds that affect the normal mammary gland development. In breast cancer, estrogen receptor ß (ERß) is a favorable prognosis indicator (cell anti-proliferation marker), and is expressed in 60% of triple negative breast cancer cell lines. Vascular endothelial growth factor (VEGF) and cyclooxygenase-2 (COX-2) are associated in tumor angiogenesis. We previously demonstrated that HCB induces migration and invasion in (ERalpha-, PR-, HER2-) MDA-MB-231 human breast cancer cells, as well as tumor growth and metastasis in vivo. In the present study, we examined the action of HCB (0.005, 0.05, 0.5 and 5 µM) in (ERalpha-, PR-, HER2+) LM3 murine breast cancer cells. Our results indicated that HCB increases cell proliferation (clonogenic assay) and migration (wound healing assay) at 0.05 and 5 µM ($p<0.001$).

Besides, HCB enhances cell viability (MTT assay) at 72 h of exposure at all tested doses ($p<0.001$), while decreases at 48 h ($p<0.05$). Regarding the protein levels of pro-angiogenic factors evaluated by Western Blot (WB) at 24 h of exposure, HCB increases the expression of VEGF and COX-2 at 0.05 and 0.5 µM ($p<0.05$; $p<0.001$), exhibiting a similar expression pattern. On the other hand, HCB enhances protein levels (WB) of proliferating cell nuclear antigen (PCNA, cell proliferation marker), at 0.05 and 0.5 µM ($p<0.05$; $p<0.001$), while reduces the expression of ERß at all assayed doses ($p<0.001$). In conclusion, our results show that HCB promotes cell proliferation, migration and expression of proangiogenic factors, while reduces ERß levels in LM3 breast cancer cells. These results suggest that organochlorine pesticides exposure can influence tumor phenotype and contributes to a high degree of malignancy.

0559 - GLYPHOSATE-BASED HERBICIDE DISRUPTS ANGIOGENESIS IN THE RAT UTERUS

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It has been demonstrated that neonatal exposure of rats to glyphosate-based herbicides (GBH) alters the uterine development and fertility. Rats neonatally exposed during postnatal days to GBH shows post-implantation failures associated with diminished decidualized area of implantation sites (IS). The aim of the present work was to investigate the effects of neonatal exposure to GBH on angiogenesis in the rat uterus. Female Wistar pups received saline solution (control, C) or an environmentally relevant dose of GBH (2 mg/kg) by sc injection on postnatal day (PND) 1, 3, 5 and 7. Pups (n= 8/group) were sacrificed on PND8 to evaluate angiogenesis immediately after the end of treatment. On PND90, other group of females were mated with fertile males and pregnant rats were sacrificed on gestation day 9 (GD9) to investigate genes associated with angiogenesis in the IS. The uterine angiogenesis was assessed on PND8 by IHC expression of VEGF. The mRNA expression of iNOS, Notch1, COX2 and angiopoietin-2 (Ang-2) genes were assayed by RT-PCR in uterine samples on PND8 or in IS on GD9. On PND8, angiogenesis measure by VEGF increased in luminal epithelium (2 vs. 26 %) and stromal compartment (9 vs. 17 %) of GBH rats. On PND8, the uterine mRNA expression of both iNOS (83 vs. 54 %) and Notch1 (19 vs. 13 %) diminished in GBH rats. On IS of GBH-exposed group diminished the mRNA expression of Notch1 (21 vs. 12 %), COX2 (19 vs. 5 %), and iNOS (47 vs. 20 %). While, the mRNA expression of Ang-2 increased (28 vs. 62 %) on IS of GBH rats. Present results demonstrate that the neonatal exposure to GBH interferes with the molecular pathway responsible of vascular adaptation during uterine development and decidualization process. Dysregulation of the studied molecules occurs early in GBH exposed rats and remains long even when pregnant rats begins embryo implantation. Altered angiogenesis during embryo implantation might explain reproductive failures found in neonatal GBH-exposed rats.

0627 - ASSESSMENT OF NEUTRALIZING CAPACITY OF ANTIBODIES RAISED IN MICE AGAINST A CROTALUS DURISSUS TERRIFICUS PHOSPHODIESTERASE

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Phosphodiesterases (PDE) belong to a super-family of enzymes that have multiple roles in the metabolism of nucleotides. These enzyme classes have played such a critical role as tools in the field of molecular biology, but their function is little known today. Here, we evaluated the humoral response induced by a PDE from Crotalus durissus terrificus (CDT) venom used as antigen. The PDE of the Crotalus durissus terrificus venom (CDT-PDE) was purified by gel filtration chromatography (Sephadex G-75) and ion exchange chromatography (HiTrap Q-FF). The CDT-PDE activity was tested by chromogenic reaction with sodium salt of bis (p-nitrophenyl) phosphate and the purity was monitored by SDS-PAGE. BALB /c mice (n= 5) were immunized subcutaneously with 0.3, 0.6 and 1.2 µg of enzyme (CDT-PDE) on days 1, 15 and 30, respectively. Freund's complete adjuvant was used in the first immunization and incomplete adjuvant in the booster. On day 50, mice were sacrificed and plasma antibody titers were evaluated by ELISA and cross-reactions with Bothrops alternatus and B. diporus venoms were analyzed by dot blotting. Mice plasma showed a specific response to CDT-PDE and antibodies gave titers around 1/1600. About of dot blotting test, C.d.t venom and it's PDE showed strong spots were noted in each case, but partial and negative reactions were noted on B. diporus and B. alternatus, respectively. The neutralization capacity of PDE- antibodies was 40 and 20 % when different amounts of serum were analyzed (36.66 µl; 18.33 µl). These results demonstrated that CDT-PDE produces a humoral response. PDE-CDT antibodies recognize the crotalic PDE and, to a lesser extent, the PDE present in the venom of B. diporus. These antibodies can be used as tools in the study of the effect of PDE on platelet aggregation, as well as in the purification of PDE by affinity chromatography to improve the performance in the isolation from CDT venom.

0629 - TIME COURSE OF INFLAMMATORY AND OXIDATIVE RESPONSE IN ENVIRONMENTAL PARTICULATE MATTER EXPOSED MACROPHAGES

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Alveolar macrophages are key pulmonary effectors which induce inflammation and oxidative stress after environmental particulate matter (PM) exposure. Therefore, it is of great interest to characterize the oxy-inflammation crosstalk in this scenario. The aim of our work was to study the time course of both oxidative and inflammatory response observed in macrophages after the exposure to Residual Oil Fly Ash (ROFA), a PM surrogate rich in transition metals. The murine cell line RAW 264.7 was exposed to ROFA at 100 µg/mL for 1, 3, 6, and 24 h. Cell viability was not significantly affected under these experimental conditions. Intracytoplasmic complexity was assessed by flow cytometric analysis of light scattering properties (SSC-H) as a cell activation indicator. We found an increase beginning at 1 h time point for the ROFA exposed group (1.00 ± 0.01 vs. 1.30 ± 0.02 % control fold change, $p<0.001$). This result was confirmed by transmission electron microscopy. Regarding inflammatory response, we found increased TNF-α levels in cell culture supernatants beginning at 1h time point for ROFA exposed cells (10 ± 1 vs. 580 ± 60 pg/mL, $p<0.001$), and increased DAF-FM signal, which indicates NO production (1.0 ± 0.1 vs. 1.1 ± 0.2 % control fold change, $p<0.01$), in the same experimental conditions assessed by flow cytometry. However, dichlorofluorescein oxidation assessed by flow cytometry increased at 24 h time point (1.00 ± 0.01 vs. 3.00 ± 0.30 % control fold change), accompanied by a 25% increase in SOD activity (control: 8.00 ± 0.10 USOD/mg protein, $p<0.05$), and a 3-fold increase in H_2O_2 content in cell culture supernatants (control: 0.20 ± 0.02 µM H_2O_2, $p<0.05$), also at 24 h time point. Taken together, these results suggest that an inflammatory response precedes the

observed changes in oxidative metabolism that take place in macrophages exposed to PM.

0634 - NANOVENOMS SYNTHESIS FOR ITS POTENTIAL USE IN ANTISERA PRODUCTION AGAINST RATTLESNAKE VENOM (CROTALUS DURISSUS TERRIFICUS)

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Snake antivenoms (AV) production involves successive inoculations of venom (V) in increasing form together with the addition of adjuvants (ADJ) that improve the immune response of animals destined for immunizations. On the other hand, nanoparticles (NP) are being studied with multiple purposes due to their potential therapeutic and immunomodulatory use, and for their ability to transport antigens and induce a specific response against it. Therefore, it is of interest to associate V to NP in order to replacing conventional ADJ in AV production. In this work, nanovenoms (NV) were synthesized by adsorption of Crotalus durissus terrificus (C.d.t.) V proteins with silica NP (NPsi) of 400 nm size (positive/negative charge, +/-). The NPsi (-) were synthesized according to the Stöber method, while a fraction was modified with APTES (NPsi +), and its load and size was verified by potential Z and DLS (dynamic light scattering) respectively. Then, 10 mg of NPsi +/- were incubated with 1 mg / ml of whole V in PBS for 3 h under stirring to favor proteins adsorption to the NPs surface. NV were photographed under MET and also a FTIR (Fourier Transform Infrared) was performed. Crotoxin presence, the main toxic component of C.d.t. venom, was analyzed by hemolysis radial test both in NV and supernatant. NV proteins desorbed by heat treatment were analyzed by SDS-PAGE and immunoblotting. NV FTIR spectra showed intermediate values between those that exhibited C.d.t. V and the NPsi separately. SDS-PAGE and immunoblotting tests confirmed the presence of proteins in NPsi particles and hemolytic halos demonstrated that NPsi +/- were capable of binding crotoxin molecules on their surface. The results reveal the presence of V in both types of NPsi, preserving its activity and therefore its native structure, evidences that allow progress in an upcoming study such as the evaluation of immunogenic activity in experimental animals.

0662 - SILVER NANOPARTICLES SYNTHESIZED WITH QUERCETIN: SYNTHESIS, CHARACTERIZATION AND EFFECT IN HUMAN TROPHOBLAST EXPOSED TO NEONICOTINOID

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Silver nanoparticles gained popularity due to their applications in medicine. The synthesis of nanoparticles using plant extracts or their antioxidant metabolites as reducing and stabilizing agents is the most adopted method for green production. Previous studies showed toxic effects of the insecticide neonicotinoid (Neo) acetamiprid on human trophoblasts mainly due to oxidative imbalance. The aim of this work was to obtain green silver nanoparticles (AgNPs) able to avoid Neo toxicity. AgNPs were synthesized in the presence of the antioxidant plant metabolite quercetin (Q), a flavonoid isolated from Flaveria bidentis. AgNPs synthesis conditions evaluated: $AgNO_3$ (1-10 mM), Q (0.5-2 mM), use of sodium citrate as stabilizer, stirring time (0-24 h) and pH (7-12). AgNPs were characterized by UV/Vis spectroscopy, TEM and DLS. To test the possible protective effect of AgNPs, the HTR-8/SVneo trophoblast cell line was pre-treated 1 h with AgNPs, and then incubated for 24 h with Neo (100 µM). Controls of synthesis precursors, Neo and AgNPs dilutions (1/5-1/1000) were included. Three AgNPs were obtained, AgQ3NPs (18 nm), AgQC4NPs (50 nm) and AgQC7NPs (33 nm). AgQC7NPs, unlike its precursors, showed no cytotoxic effect on trophoblast, while AgQ3NPs and AgQC4NPs caused a significant decrease in cell viability at 1/5 dilution. Regarding the protective AgNPs effect against Neo toxicity, AgQ3NPs and AgQC7NPs at 1/250, 1/500 and 1/1000 dilutions, avoided the decrease of cell viability (MTT method) and the reactive oxygen species (ROS) production (NBT method) induced by Neo. AgQC4NPs, avoided cell viability loss at 1/500 and 1/1000 dilutions, with a concentration-dependent effect in ROS production reaching values significantly similar to the control at 1/1000. Q synthesized green AgNPs demonstrated a protective effect for Neo induced cytotoxicity and oxidative imbalance in trophoblast. Future studies will be necessary to deepen the study of this protective effect.

0694 - THE PESTICIDE CHLORPYRIFOS INDUCES EPITHELIAL-MESENCHYMAL TRANSITION RELATED WITH BREAST CANCER PROGRESSION AND METASTASES.

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Breast cancer is the malignancy most common diagnosed in women around the world. Endocrine disruptors and some environmental factors may act as breast cancer risk. Chlorpyrifos is widely used for control crops in agriculture in our country. We have previously demonstrated its xenoestrogenic action. Epithelium-mesenchymal transition (EMT) is known to be related to tumor progression and metastasis. In order to determine if CPF may promote EMT and thus became a risk factor promoting breast cancer progression, we exposed MCF-7 and MDA-MB-231 breast cancer cells to CPF (0.00; 0.05 or 50 µM). We evaluated cell morphology after 72 h of exposure by fluorescence and optic microscopy, spheroids formation by hanging drop technique, invasion induced in spheroids laden in collagen gel matrix, proteins involved in EMT activation by WB and immunofluorescence. Mamosphere formation was used as an indicator of an enrichment of cancer stem cells (CSC). Results: CPF 0.05 and 50 µM diminished the number of cell-cell contacts, increased cytoplasmatic projections and changed nucleous-cytomplasm rate in MCF-7 cells. An increment of actin polymerization foci and cytoplasmic projections were observed in MDA-MB-231 cells. Both concentrations of CPF induced an increment of the area of invasion after 7 days of exposure ($p<0.01$). E-cadherin ($p<0.01$) and beta-catenin ($p<0.01$) were downregulated by CPF (0.05 or 50 µM) in an ER-dependent way and both proteins were detected in the perinuclear zone in MCF-7 cells. Vimentin was induced after 72 h of exposure in this cell line. In MDA-MB-213 cells, we detected an increment of vimentin ($p<0.001$) and a decrease of beta-catenin ($p<0.01$) in a c-SRC dependent way. CPF 0.05 µM increased the number and the diameter of MCF-7 mammospheres in a ER dependent way. Our results support evidences that point CPF

regulates EMT markers an invasion and may increase the risk aggravating disease for the patients suffering breast cancer.

0735 - ENVIRONMENTAL EXPOSURE TO CURRENT USE PESTICIDES IN PREGNANT WOMEN

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Among modern non-persistent pesticides, the organophosphates (OPs) are the most commonly used worldwide. In Argentina, pesticides as the OP chlorpyrifos (CP), the fungicide chlorothalonil (CT) and the herbicide trifluralin (TF) are utilized. Previous studies have reported that women and children living close to agricultural areas at the Alto Valle of Río Negro and Neuquén region are at risk of pesticide exposure. The aim of the present work was to determine with an interdisciplinary approach, levels of current use pesticides and carboxylesterase (CES) activity in human placenta. Moreover, the association between pesticide exposure and biomarker responses was also considered. Healthy pregnant women were invited to participate in this study (n= 36). Placenta samples from women residing in Neuquén city (control group-CG, n= 21) and in rural areas (rural group-RG, n= 15), were collected during 2018-2019. Demographic characteristics of the studied groups and morphometric parameters of newborns and placenta were collected. Written informed consent was obtained and the study was approved by the local ethical committee (CAIBSH). The levels of CP, TF, and CT were determined by gas chromatography. The biomarker of OP exposure, CES activity was determined by the Morgan method. Preliminary results indicated non-significant differences in the women socio-demographic characteristics and the morphometric parameters of newborns and placenta among groups. A significant CES activity reduction was observed in RG samples collected during spring-summer respect to CG (29 %, p= 0.022), coincident with the highest pesticide rate for agricultural use. Among pesticides, CP presented the highest concentrations followed in decrease order by CT > TF. These results clearly indicate that women are environmentally in contact with different pesticides. Further work is needed in order to increase the number of participants and the significance of CES activity modifications as well as pesticide levels.

0769 - ALTERNATIVE TARGETS OF PESTICIDE TOXICITY: EFFECT OF CHLORPYRIFOS IN TLR EXPRESSION IN HUMAN TROPHOBLASTS

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Organophosphate pesticides (OPs) have been widely used and are the most commonly used insecticides worldwide. OPs insecticides develop different toxicity mechanisms, classic and non-classic ones. Among these later, it has been proposed that OPs may alter the cellular inflammatory response. Trophoblasts are sensitive to danger signals from infections and tissue damage. Toll-like receptors (TLRs) are essential components of the innate response and are expressed in trophoblasts. The objective of this work was to evaluate the impact of OP in vitro exposure, at environmental relevant concentrations, in TLR2 and TLR4 receptors expression in first trimester trophoblast. The first trimester trophoblast cell line HTR-8/SVneo was incubated with the OP chlorpyrifos –CP- (0-100 µM) for 24 h. LPS condition was used as positive control for TLR4. TLR2 and TLR4 transcript expression levels were studied by qPCR. In addition, protein levels of TLR-4 after exposure were evaluated by western blot in 10% SDS-PAGE. β-actin was used as loading control. Statistical differences were analyzed by Dunnet´s pos hoc of three independent experiments. Preliminary results indicated that CP alters the expression of both TLR-2 and TLR-4. The higher CP concentrations (10 and 100 µM) significantly increased TLR2, 8 fold compared to control treatment. Similarly, CP (10 and 100 µM) augmented TLR4 transcript 3 fold respect to controls. TLR-4 protein levels did not show significant differences compared to control as a result of CP treatment. Interestingly co-exposure of CP-LPS, modulate TLR4 response to LPS. The increment in TLRs transcript indicates the possible impact clorpyriphos in the innate immune response in first trimester trophoblast. Further studies are needed to verify modifications in TLR protein expression at longer incubation than the one analyzed. In order to understand this scenario, cytokine balance as well as other components of cell inflammatory response should be studied.

0811 - NEONATAL EXPOSURE TO A GLYPHOSATE-BASED HERBICIDE ALTERS CELL PROLIFERATION IN THE UTERUS OF EWE LAMBS.

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The increasing use of glyphosate-based herbicides (GBHs) raised concern about its effects on animal and human health. Recently, we reported that postnatal exposure of ewe lambs to a low dose of GBH decreased the uterine cell proliferation, regardless the oral or subcutaneous (sc) administration route. Here, we investigate the molecular pathways related to uterine cell proliferation affected by a postnatal GBH exposure. Frisone ewe lambs were sc exposed from PND1 to PND14 to vehicle (control) or a low dose of a GBH (glyphosate at 2 mg/Kg/day). At postnatal day 45 (PND45), uterine horns were collected for paraffin-embedding or stored at -80°C until mRNA extraction. Expression of Ki67 (as cell proliferation marker), p27 and proteins involved in uterine development (ERa, PR, Wnt5a, Wnt7a, ß-catenin, Hoxa10 and Foxa2) were evaluated by immunohistochemistry. Gene expression of insulin-like growth factors (IGF-1, IGF-2), its receptor (IGF-1R) and the binding protein (IGFBP-3), also related to uterine development, were assessed by RT-PCR. Cell proliferation decreased while p27 expression increased in all uterine compartments: luminal (LE) and glandular (GE) epithelia, subepithelial stroma (SS) and myometrium. The mRNA expression of IGFBP-3 was also increased in GBH-exposed lambs. In addition, lower ERa expression was observed in LE, GE and SS; while PR expression was lower in LE, and higher in GE and SS vs control. Moreover, GBH exposure decreased the expression of Wnt5a in GE and Wnt7a in SS; whereas ß-catenin expression was lower in LE and GE. In GBH-exposed lambs a decreased Hoxa10 and Foxa2 expression in SS and GE, respectively, was also detected. To conclude, postnatal exposure to an environmental relevant dose of GBH decrease the cell proliferation in prepubertal sheep uterus by disrupting the expression of molecules responsible of uterine development. Our results suggest that GBH exposure could compromise reproductive performance in livestock animals.

0813 - METABOLIC STABILITY OF GLIFOSATE IN RUMINAL CONTENT FROM CATTLE: PARTITION BETWEEN PARTICULATE AND FLUID DIGESTIVE MATERIAL

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Glyphosate (GLP) is one of the most commonly pesticides delivered to the environment. Farm animals could be exposed to

both GLP and aminomethylphosphonic acid (AMPA, the major GLP metabolite) present in surface and groundwater, but also in foodstuffs used for preparation of concentrate feeds. For instance, detectable levels of GLP were observed in the urine of dairy cows chronically exposed to the herbicide present in their feed. In cattle, the rumen plays a central role in the pre-systemic metabolism of xenobiotics, thus protecting the organism against potentially harmful chemical compounds. This work evaluated the chemical stability of GLP in the ruminal environment. Ruminal contents from 3 steers were collected in a local abattoir. Samples were roughly filtered, kept at 37°C and transported to the laboratory. Aliquots of both whole ruminal content and fluid phase were incubated (3-6 h) in anaerobiosis with GLP (12.5 µg/mL). Metabolic viability of ruminal contents was assessed by the measurement of the SO-reduction of the anthelmintic albendazole sulfoxide (ABZSO). These assays were carried out in the absence (controls) and in presence of GLP. Incubations of boiled (inactive) ruminal content and fluid were used as controls. Samples were analysed by HLPC with fluorescence detection. Incubated ruminal contents were subsequentially centrifuged for determination of the amounts of the herbicide in the fluid phase and in the particulate material. GLP was not metabolised neither in the ruminal content nor in the fluid phase. The percentage of GLP associated to the particulate material ranged between 5.7 (3 h) and 11.2 (6 h). GLP did not affect the SO-reduction of ABZSO. These results may indicate that the ruminal environment is not involved in the pre-systemic metabolism of GLP. As GLP is a hydrophilic xenobiotic, the highest proportion of the herbicide is solubilised in the fluid phase of the ruminal content which may influence in its rate of absorption after ingestion.

0815 - ROLE OF THYROID HORMONES, TGF BETA; AND ON IN ARTERIAL HYPERTENSION GENERATED BY ENVIRONMENTAL TOXINS

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Hypertension is one of the main pathologies associated with environmental pollution. Among the environmental pollutants is hexachlorobenzene (HCB). We demonstrate that HCB increases blood pressure (BP) and alters morphology and vascular functionality (VF) in vivo. TGF-β1; and RE-alfa; are involved in these effects. Since the effects on the VF were endothelium dependent, in this work we deepen the study of the mechanism of action of HCB on VF in the EA-hy926 endothelial line, grown in DMEM, 10 % FBS, at 37 °C treated with HCB (0.05, 0.05 and 5 µM) at different times (18 and 24 hours). We analyze: a- Molecules involved in contractility and BP, TGF-β and RE-alfa [Western blot (W), RT-PCR]. b- Levels of eNOs and nitrites (W, Greiss). c- Role of TGF-β, in the expression of REalfa; (TGF-R RII inhibitor, SB431542). d- Rol of thyroid hormones (TH) in the expression of TGF-β and RE-alfa [serum depleted TH, exogenous T3 (10^{-7} M)], since HCB is an endocrine disruptor and induces hypothyroxinemia, and that TH regulates the expression of these parameters. a- TGF-β; (protein) increased 24 %, ($p<0.05$), HCB (0.05) and 38, 40 % ($p<0.01$), HCB (0.05 and 5 µM), its mRNA 28 and 36 %, ($p<0.01$), and HCB (0.05 µM). RE-alfa; decreased 28 and 36% ($p<0.01$), HCB (0.05 and 5 µM) (18, 24 h). b- eNOs decreased 25 % ($p<0.05$), HCB (µM), and nitrites 20 and 38% ($p<0.05$, $p<0.01$), HCB (0.05 and 5 µM) (24 h). c- Inhibition of RII TGF-β; increased RE-alfa, HCB (5 µM) (24 h). d- The TGF-β increase in TH depleted cells dose-dependent, contrary to RE-alfa. HCB and T3 administration normalized the effect of HCB on both parameters (24 h). HCB alters hormonal homeostasis, deregulates molecules involved in VF via TGF-β and NO. TGF-β and the decrease in NO decreased, in a paracrine manner, could be responsible for the effects observed in vivo in vascular muscle cells of animals treated with HCB, increasing BP.

0820 - SOY BASED DIET MODIFIES CADMIUM-INDUCED LIPID METABOLISM ALTERATIONS IN RAT CEREBELLUM.

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Cadmium is a toxic agent that is also an environmental contaminant. We studied its effects and the protective role of a vegetarian versus animal protein diet in cerebellum. The lipid profile, lipoperoxidation and the expression of lipid metabolism related enzymes were analized. Four lots of female Wistar rats were used: 2 lots received casein (Cas) and 2 lots soybean (So) as protein source. Within each group, 1 lot received regular water (control-Co) and the other, 15 ppm of Cd in the drinking water for 60 days. The animal weight was weekly measured. Cerebellum homogenates were used for TBA assay, and the levels of lipid peroxidation products - mainly malondialdehyde (MDA) - were determined spectrophotometrically as TBARS. Lipids were extracted and total cholesterol (TC), triglycerides (TG) and phospholipids (PL) were determined. Total RNA was isolated with Trizol and cDNA was obtained. Cytidylyltransferase (CT), hidroxymethylglutaryl CoA reductase (HMGCoAR), fatty acid synthetase (FAS) and acetyl CoA catboxylase (ACC) were determined by PCR. S28 was used as an internal control. Cadmium concentration was determined with ICP-MS. Animals weight showed no differences among the groups. Cadmium concentrations showed an increase in CasCd vs. CasCo ($p<0.001$) with no differences among Soy groups. Total lipids content showed a trend to decrease in Soy groups compared to Casein groups, being only significant in SoCd vs. CasCd ($p<0.01$). TC increased in both intoxicated groups ($p<0.05$) when compared to their controls. TG increased in CasCd vs. CasCo ($p<0.05$) and also augmented in SoCo vs. CasCo ($p<0.05$); without differences between soy groups. FL decreased in SoCd vs. CasCd ($p<0.01$) and increased in CasCd vs. CasCo ($p<0.01$). It also augmented in SoCo vs. CasCo ($p<0.05$). TBARS showed a significant increase ($p<0.01$) in Cd groups, and also augmented in SoCo vs. CasCo ($p<0.05$). CT expression increased in CasCd ($p<0.05$) and in SoCo ($p<0.01$) vs. CasCo; it decreased in SoCd vs. SoCo ($p<0.05$). HMGCoAR mRNA levels augmented in both Cd groups ($p<0.05$). FAS expression diminished in both Cd groups vs. their controls ($p<0.05$) and it augmented in SoCo vs. CasCo ($p<0.05$). ACC showed no differences. This shows that TG and PL are altered by Cd, and Soy might confer protection in cerebellum against the metal.

0822 - SYSTEMIC TOXICITY BY NERIUM OLEANDER IN EXPERIMENTATION ANIMALS

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UNIVERSIDAD NACIONAL DEL NORDESTE

Nerium oleander (NO), belongs to the Apocynacea family, known as adelfa and flower laurel, that is frequently grown in gardens and public areas and as all parts of the plant contain numerous toxic compounds. N. oleander has linear and leathery leaves that come in various colours, from dark green to grey green with distinct light yellowish veins. Its flowers are fragrant, funnel-shaped and arranged in clusters at the tip of twigs, with white to pink to deep red colours. The fruit is a narrow pod containing many silky-haired seeds. This plant is native to Mediterranean regions of Africa and Europe. Its toxicity is attributed to Cardiac glycosides, oleandrin and neriin. The aim of this work was to determine the toxicity of an

alcoholic extract of NO for the tissues. In this context, dried and ground leaves were used. After sieving, it was extracted by refluxing with 50 % methanol with the addition of lead acetate (II). The filtered solution was extracted again with dichloromethane/isopropanol (3:2) and evaporated under reduced pressure. Extracts were kept in a refrigerator in closed containers until use. The detection of cardiotonic glycosides was carried out by TLC with ethyl acetate: methanol: water (100:13.5:10) as eluent and antimony trichloride (20 % in chloroform) as revealing reactive. Mice of BALB/c strain were used, forming 3 study groups. The control groups received balanced food and water ad libitum while the treated groups were administered doses of 15 and 25 mg/kg of the extract orally for 7 days. The animals were sacrificed to obtain blood and tissues that were processed. In TCL fluorescent spots of blue, yellow and orange at 365 nm were observed, corresponding to different groups of cardiotonic glycosides. Histopathological studies have shown renal injury, in those animals that received the lower toxic dose, associated with congestion and widespread tubular epithelial necrosis. The group of animals that received the highest toxic dose, also revealed lesions in the liver with focal necrosis of hepatocytes and vacuolar degeneration. There was also myocardial cell necrosis with interfibrillary edema and severe diffuse pulmonary congestion with large areas of hemorrhage and alveolar wall damage. Blood analysis reflects a significant increase in LDH, GOT and an increase in the number of platelet cells in both groups. Based on the foregoin, we can conclude that there was systemic toxicity in mice attributed to Nerium oleander and the lesions observed are compatible with oleander poisoning in a manner similar to other species.

0826 - PREPARATION OF MIXED GLIAL PRIMARY CULTURES FROM NEWBORN MURINE BRAINS

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UNIVERSIDAD NACIONAL DEL NORDESTE

Primary glial cell culture is the most commonly used in vitro model for neurobiological studies. The aim of this work was to develop a protocol in our lab that allows to obtain glial cells for the toxicological study of North Argentina plants that cause disorders in the central nervous system. The method here described has undergone minor modifications from the protocol published by Saura et al., 2003. Briefly, Poly-d-lisine-coating solution was applied to culture plates for 24 h at 37 °C. Wells were then washed 4 times with PBS before cell seeding. Primary glial cell cultures were prepared from CF-1 mouse (1 to 3 days old). Brains (five) were removed aseptically, blood vessels and meninges were discarded. The brain tissues were mechanically dissociated by pipetting. Subsequently, cells were transferred to a precooled 50 ml sterile tube and centrifuged at 1,000 rpm for 5 min at 4 °C and the resulting pellet triturated before seeded in a micro-full media: DMEM-F12 supplemented with FBS (10 %), MEM NEAA (100X-1 %), L-Glutamine (1 %), Gentamicine (10 µg/ml) and Penicillin–streptomycin (1 %). Culture was incubated at 37°C and 5 % CO_2, medium was replaced every 4–5 days and confluency was achieved after 21 days in vitro (DIV). Cells purity was examined by immunohistochemistry using specific antibodies. Morphological observation evidenced refringent amoeboid microglia and polygonal shape astrocytes. These results were confirmed by immunohistochemistry, using Iba-1 and GFAP, which are well-established markers of microglia and astrocytes, respectively. In conclusion, the Poly-d-lisine-coating used, the maintenance of the culture in a complete medium and only the mechanical dissociation of the tissue resulted in astroglial cultures with higher microglial proportions.

0861 - EARLY POSTNATAL EXPOSURE TO XENOESTROGENS ALTERS THE EXPRESSION OF MOLECULES INVOLVED IN THE POSTNATAL DIFFERENTIATION OF THE OVIDUCT OF THE BROAD-SNOUTED CAIMAN (CAIMAN LATIROSTRIS)

Yamil TAVALIERI | Germán GALOPPO | Sofia Candela MORA | Enrique Hugo LUQUE | Mónica MUÑOZ-DE-TORO

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The differentiation of the oviduct of Caiman latirostris is completed postnatally and is characterized by temporal-spatial patterns of histofunctional changes. We have shown that the exposure to Bisphenol A (BPA) or 17β-Estradiol (E2) at critical stages of embryo development affects oviduct postnatal differentiation, leading to a precocious adenogenesis. Adenogenesis is regulated by the Wnt signaling pathway in many vertebrates. The aim of this study was to determine the molecular mechanism behind altered adenogenesis. We established the ontogeny and evaluated the effects of postnatal exposure to BPA or E2 on the expression of molecules of the Wnt signaling pathway: Wnt-7a, Wnt-5a, β-catenin and FoxA2. Archived paraffin-embedded samples were used to establish ontogenies. To assess the effect of xenoestrogens, early postnatal caimans were treated with E2 (0.014 or 1.4 ppm) or BPA (1.4 or 140 ppm). Protein expression was assessed by IHC. ANOVA or Kruskal-Wallis was performed to obtain the overall significance, followed by Dunnett's or Dunn's test, respectively; $p<0.05$ was accepted as significant. Early postnatal oviducts were highly sensitive to E2 and BPA evidenced not only by increased histofunctional score but by decreased α-SMA/desmin ratio. Expression of Wnt-7a was significantly lower ($p= 0.006$) in the oviduct of caimans treated with E2 0.014 ($p= 0.0127$) and E2 1.4 ($p= 0.0049$). Expression of Wnt-5a was significantly lower in the oviduct of caimans treated with E2 1.4 ($p=0.0013$) and BPA 1.4 ($p= 0.0327$). Expression of β-catenin was significantly higher in the oviduct of caimans treated with BPA 1.4 ($p= 0.0023$). Expression of FoxA2 was significantly lower in the oviduct of caimans treated with E2 1.4 ($p= 0.0093$) and BPA 140 ($p= 0.0063$). These molecules must be expressed at the right time and the right place in order to warrant a correct differentiation process, any alteration in their expression patterns may impair the reproductive health of C. latirostris later in life.

0906 - DEXMEDETOMIDINE ATTENUATES RENAL AND PULMONARY INJURY IN EARLY EXPERIMENTAL SEPSIS.

Julieta RONCHI (1) | Gabriela OLEA(1) | Luis HUESPE(2) | Diego FARIZANO(2) | Eduardo SCHLOVER(3) | María AGUIRRE(1) | Tania STOYANOFF(1)

UNIVERSIDAD NACIONAL DEL NORDESTE. FACULTAD DE MEDICINA.LABORATORIO DE INVESTIGACIONES BIOQUÍMICAS (1); HOSPITAL ESCUELA ´´JOSÉ DE SAN MARTÍN´´. CENTRO DE INVESTIGACIONES CLÍNICAS. (2); UNIVERSIDAD NACIONAL DEL NORDESTE. FACULTAD DE MEDICINA (3)

Sepsis remains the most important cause of acute kidney injury (AKI) and acute lung injury (ALI) in critically ill patients. Therapeutics options for treating this condition are limited. Thus, there is a need for pre-clinical studies to provide new knowledge on the mechanisms involved in sepsis. Dexmedetomidine (DEX) is a common sedative drug used in the intensive care units and several evidences have shown that may induce a variety of citoprotectives effects on early sepsis. In this study, we aimed to evaluate the underlying mechanisms of DEX protection in kidneys and lungs on a murine model of lipopolysaccharide (LPS) induced-endotoxemia. Male inbred Balb/c mice were divided into four experimental groups: I) Control; II) LPS (8 mg/kg, i.p.), III) DEX (50 µg/kg, i.p.) and IV) DEX+LPS. Assessment of renal functional parameters, histological examination, immunohistochemistry and/or Western blottings of Bax, Bcl-xL and vascular endothelial growth factor (VEGF) were performed at 24h post LPS administration. Our data revealed that DEX treatment remarkably improving metabolic

acidosis and hypoglycaemia post endotoxemia. Mice demonstrated AKI by elevation of serum creatinine and renal histologic damage. Similarly, LPS-induced ALI was evident by parenchymal lung histopathologic alterations. DEX attenuates renal dysfunction ($p<0.01$) and ameliorates kidney and lungs histopathologic changes. Additionally, DEX administration caused a decreased in the Bax /Bcl-xL ratio within the 24h post LPS accompanied by a significant increase in VEGF expression. This study demonstrates that DEX exerts protective effects in AKI and ALI in endotoxemic mice through the reduction of Bax/Bcl-xL ratio and the regulation of the VEGF expression.

Farmacología/ Pharmacology IV

Chairs: Andrea Errasti | Alejandro Español

0164 - HIGH-THROUGHPUT SCREENING ASSAY FOR CAMP TRANSPORT INHIBITORS: SEARCHING FOR A NEW DRUG FOR PDAC TREATMENT.

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ININFA, UBA-CONICET

In a previous work, we validated the inhibition of MRP4-dependant cAMP extrusion process as a promising therapeutic strategy for pancreatic ductal adenocarcinoma. MRP4 inhibition abrogated proliferation in vitro by both, augmenting intracellular cAMP and diminishing extracellular cAMP. In this work we sought to study the activity of a large series of compounds as cAMP transport inhibitors. In view of this objective, we first analyzed the available information about the inhibitory effect of different molecules on MRPs and classified them according to their chemical structure and their transporter-substrate specificity. Since the data set of cAMP transport inhibitors is scarce, we developed a microplate high-throughput föster resonance energy transfer (FRET) assay that monitors the real-time kinetics of intracellular cAMP levels in cell monolayers, using the EPAC-SH187 biosensor. We established a HEK293T- EPAC-SH187 clone that stably expresses this sensor, which exhibited a wide dynamic range and high sensitivity (2-fold delta FRET in a concentration range of 0.01–500 µM cAMP). Using this technique, we analyzed 60 compounds, with diverse chemical structures and pharmacological uses, which were previously identified in literature as substrates or inhibitors of MRP4. We identified 25 compounds that functioned as inhibitors of cAMP transport and characterized them by performing concentration-response experiments. These set of compounds include traditional MRP4 inhibitors as probenecid and MK571, channel blockers, protease inhibitors, nucleotide analogs and non-steroidal anti-inflammatory drugs. The emerging results will serve as a basis for the further development of specific inhibitors of MRP4-mediated cAMP transport potentially applicable in the treatment of PDAC.

0310 - AT1 RECEPTORS IN STRIATUM DA-UPTAKE: CRUCIAL ROLE AND RELEVANCE IN AN AMPHETAMINE-SENSITIZATION MODEL OF SCHIZOPHRENIA

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Limbic dopamine (DA) hyperactivity, a hallmark of amphetamine (AMPH) exposure, it is considered as a neurochemical feature involved in the expression of schizophrenic positive symptoms. DA neurotransmission dynamics is regulated by the uptake of extracellular transmitter at presynaptic neurons through specific transporter. Angiotensin II, through AT1 receptors (AT1-R), modulates DA neurotransmission at limbic areas. Herein, we studied AT1-R involvement after AMPH exposure on: a) development and expression of behavioral sensitization, b) in vitro striatum DA uptake. To these purposes male Wistar rats (250-300 g) were daily administered with d-AMPH (2.5 mg/kg i.p.) for 5 days. After 3 weeks of withdrawal, the behavioral sensitization was evaluated measuring locomotor activity. The AT1-R blocker, Candesartan (CV 3 mg/kg p.o.), was administered daily for 10 days, starting 5 days prior to the first AMPH injection in the prevention sensitization protocol. In the reversion protocol, either, CV or aripiprazole (antipsychotic drug widely use partial agonist of D2 receptors), were administered for 5 days starting 2 weeks after the last AMPH injection. DA uptake was measured in homogenized striatum using an electrochemical sensor, based on glassy carbon electrode modified with carbon nanotubes and polyethylenimine by amperometry. The results were analyzed by 2-way ANOVA followed by Bonferroni test or t-test. We found that behavioral sensitization was prevented and reversed by AT1-R blockade more efficiently than aripiprazole. Moreover, 5 days of CV administration increased DA uptake fact that could account for the behavioral results. We conclude that the lesser DA signaling, as a result of the increasing of its uptake, would explain the beneficial effects of AT1-R blockade in the behavioral neuroadaptations induced by AMPH.

0328 - POTENTIALLY INAPPROPIATE MEDICATION USE IN OLDER ADULT AFFILIATES OF A SOCIAL SECURITY INSTITUTE IN CORRIENTES CITY. 2018

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To identify potentially inappropiate medication in older adults' affiliates of a Social Security Institute in Corrientes city. Observational descriptive transversal study, also classified as a prescription-indication medications usage study. MPI prescriptions classified as "non clinical evidence for indication" were considered. Based on STOPP-START criteria, 60 years and older adults with polymedication (more than four drugs) belonging to the Social Security Institute of Corrientes city during 2018 were included in the present study. Variables analyzed: sex, age, drug prescription and diagnosis. Were included 192 prescription; with an average of seven drugs per patient (4-21 drugs range), age average 68 years old (60-91 age range), 61% male (n=118). More frequent diagnosis: hypertension, dyslipidemia, diabetes mellitus, hypertensive cardiopathy, gastropathy and ocular diseases. Were identify 28 MPI: multivitamin B complexes (8) associated to paresthesia, muscle cramps, hypopotassemia, and others with non-precise indication; beta-escin (3) for varicose veins; unproteneized calf blood extract (3) for diabetes retinopathy and corneal dystrophy; ascorbic acid (2) with no diagnosis association; silymarin (1) for diabetes; vitamin E (1) for irritable bowel; purified flavonoids (1) for irritable bowel; and others with no precise diagnosed indication (6). In 10% patients (n=20) MPI was detected, drugs with no scientific evidence or with no clinical relation to the patient diagnosis. MPI in Elder adults, who are habitually polymedicated due to their multiple pathologies, increases the risk to adverse effects or harmful drugs interactions and expose patients to a higher morbidity and mortality, also increasing health care expenses.

0365 - CHARACTERIZATION OF EXOSOME-LIKE VESICLES DURING DRUG TREATMENT OF CYST ECHINOCOCCOSIS

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Cystic echinococcosis (CE) is a chronic zoonotic disease which shows a high prevalence in Argentina (Patagonia, Catamarca, Santiago del Estero, Salta and Entre Ríos) caused by Echinococcus granulosus larval cystic stage. Humans acquire the disease by ingestion of cestode eggs from contaminated soil or food. As other parasite helminths, E. granulosus adopt complex strategies of communication with their hosts, driving a physiological and immunological homeostasis to achieve long-term parasitism. Our attention has been focused in the exosome-like vesicles (EVs) and their importance in cellular crosstalk under drug treatment pressure. Previously, we characterized EVs from protoscoleces and metacestodes, and demonstrated for the first time that they interact with host dendritic cells inducing an unconventional activation profile with MHCII decrease. EVs were characterized by dynamic light scattering and proteomic analysis from control, metformin- and albendazole-treated parasite cultures. This allowed us to identify at least twenty small molecular weight antigenic proteins, which showed constant proportion between samples with and without pharmacological treatment. Amongst them, two unknown antigens (W6UFE0 and U6IZE6) were identified. Interestingly, drug treatment led to a decrease in tolerogenic proteins and certain extracellular matrix-interacting and epithelial-mesenchymal transition inducing proteins. These findings could be correlated to the effectiveness of the pharmacological treatment described in murine models and patients for metformin and albendazol, respectively. More precisely, it is known that albendazole-treatment increase the Th1 cytokine profile, indicating that Th1 responses play a role in the process of cyst degeneration. Here, we provide valuable data on the occurrence of cargo proteins that can promote proliferation, induce dissemination and evade immune response in the parasite-host interface. Currently we are engaged in selecting proteins with potential applications as immunosuppressive drugs. *Equal contribution

0422 - IN VITRO STUDY OF THE PARASITICIDAL ACTIVITY OF NOVEL MEBENDAZOLE FORMULATIONS ON FEMALE ADULT FORMS OF TRICHINELLA SPIRALIS

Ana Victoria CODINA (1) | Josefina PRIOTTI(2) | Valeria MARIZZA(1) | Ariana ROSALES(3) | María Delia VASCONI(1) | Darío LEONARDI(2) | María Celina LAMAS(2) | Lucila I. HINRICHSEN(1)

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Oral administration of medications has advantages over other routes. Mebendazole (MBZ) is a benzimidazole widely used in oral chemotherapy against intestinal parasites, due to its broad-spectrum activity and low cost. Its primary disadvantage is poor water solubility and low bioavailability: only 20% reaches systemic circulation due to incomplete absorption and extensive first-pass metabolism. A major challenge for the pharmaceutical industry is to resolve the poor solubility of most drugs, which generates delivery problems as well as low bioavailability. Two novel MBZ formulations were produced to optimize oral absorption of this drug: a nanoparticulate system (Np), obtained through spray-drying techniques, and an inclusion complex with ß -cyclodextrin citrate (Comp). This study aimed to evaluate the in vitro parasiticidal activity of these systems, compared with pure MBZ. Their effect on the in vitro ability of MBZ to kill T. spiralis worms was assessed with the survival curves of the female parasites cultured for 48 h in RPMI 1640 medium containing the MBZ systems. The worms were observed with an inverted microscope at 2, 4, 7, 24, 29 and 48 h, and viability was estimated analyzing parasite motility and morphology. The number and motility of newborn larvae were also examined. Both MBZ systems improved the parasiticidal activity of the pure drug (p= 0.0232). The Np formulation was significantly better than pure MBZ (p= 0.0093), with a median survival of 30 h and a proportion of live worms at 30 h of 47.6 vs. 79.3 % for MBZ. Newborn larvae (NBL) in contact with the systems retained their motility during the period analyzed; however, the NBL number was lower in the nanoparticulate system compared to MBZ. The effect of the formulations on the survival of adult T. spiralis indicates that the methodology to obtain them improves the parasiticidal activity of MBZ. The nanoparticulate system was the most effective and is of choice to test its therapeutic efficacy in vivo.

0469 - ANTIBIOTIC-INDUCED QT INTERVAL PROLONGATION: DRUGS IMPLICATED AND SAFETY MEASURES FOR RISK MINIMIZATION.

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QT interval prolongation (QTP) and arrhythmias (Torsades de Pointes, TdP), a serious adverse drug reaction, have been associated with many commonly used antibiotics in recent times. The comparative risk of different drugs has not been explored, as well as the strategies for their early risk detection and comparative evaluation. The objective of this study was to explore and evaluate the association between TdP/QTP and many available antibiotics using the FDA adverse event report system (FAERS). FAERS reports from January 1, 2004 to June 30, 2019 were analyzed. The Medical Dictionary for Regulatory Activities (MedDRA) was used to identify TdP/QTP cases. We calculated the reporting odds ratios (RORs), proportional ADR reporting ratio (PPR), and Yule's Q, Chi Square with Yate's correction for the association between each antibiotic and 4 different categories (Group 1: standarized medical querry in MedDRA for QT Prolongation, Group 2: preferred MedDRA terms for QT interval prolongation, GROUP 3: unspecific symptoms related with arrhythmia, Group 4: MedDRA terms linked to ventricular arrhythmia). An association was considered to be statistically significant when the lower limit of the 95 % CI was greater than 1.0 (ROR or PPR), greater than 0 (Yule's Q) or the P value was less than 0.05 (Chi Square). Results: A total of 371,002,880 reports (including 7,889,404 TdP/QTP reports) were considered, after inclusion criteria were applied. The most frequently used antibiotics in the hospital setting were linked to prolongation of the QT interval. They were in decreasing order of risk: Ticarcillin + clavulanic acid, Piperacillin + Tazobactam, Ceftriaxone, Ciprofloxacin, Ampicillin + Sulbactam, Moxifloxacin, ampicillin, amoxicillin + Clavulanic acid, Metronidazole, amoxicillin, levofloxacin. The aforementioned antibiotics presented increased risk in the four categories (standardized search strategy, risk of arrhythmias, risk of nonspecific symptoms, and asymptomatic alterations of the QT interval). Conclusion: The prolongation of the QT interval should be taken into account as a frequent and potentially risky adverse reaction in all antibiotics for hospital use. They are associated with a multiplicity of warning symptoms prior to the presence of fatal arrhythmias. The need for evolutionary clinical control for the detection of nonspecific symptoms and electrocardiographic evaluation for early prolongations of the QT interval are clear. This study confirms prior evidence for TdP/QTP associations with antibiotics and proposes safety controls for early detection and risk minimization.

0470 - PHARMACOLOGICAL TREATMENT OF HYPERTENSION: THERAPEUTIC RESPONSE AND

FACTORS INVOLVED IN THE LACK OF EFFECTIVENESS

Guillermo Alberto KELLER(1) | **Marta Graciela FAVRETO** (2) | Diego Fernando SANCHEZ GELÓS(2) | Rocío MARTINEZ(2) | Carlos Federico GAUNA COLÁS(3) | Maria Laura FERREIRÓS GAGO(2) | Milagros GOTELLI(2) | Hector E. DI SALVO(3) | Roberto Alejandro DIEZ(1) | Guillermo DI GIROLAMO(2)

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Hypertension is a highly prevalent disease and causes cardiovascular morbidity and mortality. Despite the great advances in pathophysiological research and available drugs, its control has not been as expected. To investigate the characteristics that determine the therapeutic response in hypertensive patients who undergo ambulatory blood pressure monitoring (ABPM). All patients who underwent ABPM were consulted about their participation questionaire. ABPM results were also recorded and correlated with epidemiological data. Results: 119 patients (59 males, 49.58%) were included. The mean ± SD (min-Max) characteristics were: age 57 ± 14.5 (19-86) years, weight 81 ± 19.4 (43-147) kilograms, height 167 ± 11.5 (137-195) cm, BMI 29 ± 6.5 (16-53) Kg/m^2 and abdominal circumference 104 ± 60 (60-210). Most of the patients had full university studies (52.94%), followed by complete secondary studies (36.97%). The presurometry was requested by recent diagnosis of hypertension (26.89%) or by prior known hypertension follow-up (73.11 %). The majority never smoked (63.87 %), while the rest currently smokes (15.97 %) or quit in recent years (20.17%). They reported sleeping on average 07:12 ± 01:26 h (01:30 to 10:45 h). Only 11.76 % said they were complying with a hyposodic diet, while 39.5 % mentioned it as nutritionally adequate, and 48.74 % said they did not control their diet. Alarmingly, a large percentage do not perform physical activity (47.90 %), or perform it sparingly (18.49 %), while only 33.61 % perform it properly. A striking number recognized risky behaviors such as: forgetting to take the medication (18 %), being irregular in its intake (24 %), not taking it when it feels good (13 %) or stop taking it if they think feeling bad (22 %). Symptoms commonly associated with hypertension records were of very low prevalence: headache (19.33 %), eye pain (5.88 %), dizziness (9.24 %), weakness (5.88 %), nausea (4, 20 %), abdominal pain (7.56 %), dyspnea (8.40 %), neck pain (12.61 %) and fatigue (8.40 %). The pharmacological treatment was correctly indicated in the majority. The pharmacological treatment was correctly indicated in the majority and it was consistent with the recommendations of therapeutic guidelines. Irrational prescription patterns were not detected. Regarding ABPM, 83 % of patients had uncontrolled systolic and diastolic arterial hypertension. The majority (67 %) with an altered circadian pattern. The factors that significantly correlated with the lack of control were: lack of adequate nutritional regimen, obesity, risky pharmacotherapeutic behaviors (no medication), insufficient hours dedicated to sleep. Despite the great pathophysiological and pharmacological knowledge, the response to antihypertensive treatment is still conditioned by sociocultural factors that must be approached in an interdisciplinary way to achieve therapeutic goals.

0576 - SEARCHING FOR NEW PROTEINS OF FUNGAL ORIGIN WITH POTENTIAL MEDICAL APPLICATION

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Currently, interest in obtaining fungal bioactive compounds with medical applications has been renewed due to their great diversity and unique bioactivity compared to other natural sources. Bioactive proteins constitute an important part of the functional components in fungi, as they can have antioxidant, antitumor and immunomodulatory activity. In particular, the antioxidant activity is of great interest since the uncontrolled production of free radicals has been related to several diseases, including different types of cancer. The aim of this work was to obtain extracellular proteins and enriched protein extracts from Pleurotus ostreatus fruiting bodies and mycelium in order to evaluate their antioxidant potential. In order to achieve this goal, P. ostreatus mycelium was grown on potato dextrose agar for 7 days and a plug of culture was inoculated on potato dextrose broth. After 10 days, mycelium was harvested and liquid media was collected. Mycelium and fruiting bodies obtained from the local market were lyophilized and homogenized with Tris-Glycine buffer pH 8.4. The homogenates were centrifuged and the supernatants and collected liquid media were treated with ice cold ethanol to precipitate soluble proteins. The precipitates were resuspended in buffer. Protein concentration was measured by Bradford method and a SDS-PAGE was performed. Antioxidant activity of homogenates and extracts was evaluated using 2,2-azino-bis-3- ethylbenzothiazoline-6-sulfonic acid (ABTS). ABTS radicals were generated by the reaction of ABTS 7 mM and potassium persulfate 2.45 mM overnight at room temperature in the dark. For the assay, homogenates and extracts were incubated with the radical ABTS for 30 min in the dark and the absorbance was measured at 734 nm. The results showed that the homogenates have a great antioxidant capacity, being higher in that obtained from fruiting bodies. As for protein-enriched extracts, although the antioxidant activity of those corresponding to extracellular proteins and mycelium was lower, the one obtained from fruiting bodies showed similar values to mycelium homogenate. Therefore, these results are promising to continue the search for new proteins with application in medicine.

0647 - BIOLOGICAL EVALUATION OF QUINOXALINE COMPOUNDS AS ANTI-HIV AGENTS TARGETING REVERSE TRANSCRIPTASE ENZYME.

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Infections by human immunodeficiency virus (HIV) still represent a serious concern and a global threat to human health. Due to appearance of multi-resistant virus strains, the poor compliance of treatments and the adverse side effects of the antiretroviral therapy, the development of new treatment agents, more active, less toxic and with increased tolerability to mutations is still required. Quinoxaline derivatives are an emergent class of heterocyclic compounds with a wide spectrum of biological activities and therapeutic applications. These types of compounds have also shown high potency in the inhibition of HIV reverse transcriptase (RT) and HIV replication in cell culture. In this context, the aim of this work was the discovery of novel RT inhibitors containing a quinoxaline scaffold. For this purpose, a chemical library of these type of compounds with potential RT inhibitory activity was previously constructed and then screened, using docking and a 3D-QSAR model. This screening led to the synthesis of twenty-five quinoxaline compounds. Here we present the results of the biological activity evaluation of these derivatives formerly synthesized. First, all of the compounds were assayed as inhibitors of the recombinant wild-type RT enzyme. From this evaluation, six compounds showed interesting inhibitory capabilities. The most promising, compound 3, showed an IC50 of 0.33 ± 0.12 µM, slightly higher than nevirapine's (IC50= 0.10 ± 0.02 µM). Then, the anti-HIV

activities on MT4 infected cells and the cytotoxicity of the quinoxaline derivatives with the highest RT inhibitory capabilities were evaluated. Compound 3 showed excellent anti-HIV properties with an EC50 of 0.030 ± 0.001 µM, smaller than nevirapine's (EC50= 0.060 ± 0.001 µM). Also, another compound showed interesting anti-HIV capabilities with an EC50 of 1.6 ± 0.4 µM. In conclusion, two quinoxaline derivatives, 12 and 3, showed promising inhibitory activity profiles and could be defined as hit and lead compounds, respectively. The further development of these will likely provide novel and more potent RT inhibitors for HIV treatment.

0889 - A SUNFLOWER ANTIFUNGAL LECTIN DISPLAYS SYNERGISTIC EFFECT WITH FLUCONAZOLE ON CANDIDA ALBICANS

Marianela Victoria DEL RIO (1) | Érica MELLO(2) | Susana RIBEIRO(2) | Gabriel TAVEIRA(2) | Laura DE LA CANAL(1) | Marcela PINEDO(1) | André CARVALHO(2) | Valdirene GOMES MOREIRA(2) | Mariana REGENTE(1)

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The resistance to antifungal drugs has become an emerging problem in the treatment of Candida infections worldwide. Natural compounds recently appeared in combined alternative therapy to reduce the doses of conventional drugs, providing the additional benefit of decreased toxicity effects on health. Fluconazole (FLCZ), an antifungal agent of the azole group, inhibits the biosynthesis of ergosterol disrupting plasma membrane integrity of fungal cells. We have previously isolated a sunflower mannose-binding lectin (Helja) with antifungal activity on C. albicans. Helja interacts with yeast cell wall and triggers the production of hydrogen peroxide and the loss of cell integrity and viability. The aim of this work was to evaluate the effect of Helja in combination with FLCZ on the growth of C. albicans. Yeast cells were incubated in the presence of Helja (100 µg/ml), FLCZ (1 µg/ml) or combination of Helja-FLCZ to follow optical reading at 630 nm and assess fungal growth. The inhibition of fungal growth in the presence of Helja or FLCZ was 30.91 ± 5.27 and 28.12 ± 11.5% respectively, while the combination Helja-FLCZ exhibited an inhibitory effect of 91 ± 3.5, greater than the sum of both. In addition, cells treated with Helja-FLCZ showed differentials morphological alterations compared to treatment with Helja or FLCZ alone, such as loss of the typical oval form of yeast and the formation of clusters with agglutinated cells. Thus, combination Helja-FLCZ leads to the collapse of cells and their inability to reproduce. The synergistic action of Helja in combination with FLCZ might be helpful in preventing the development of drug resistance and decreasing toxicity, thus it constitutes a promising therapeutic strategy in antifungal treatments.

0894 - HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY (HILIC) – MS/MS APPLIED IN THE IDENTIFICATION OF DRUG METABOLITES IN PATIENTS TREATED FOR CHAGAS DISEASE

Carlos Alberto PÉREZ MONTILLA (1) | Samanta MORONI(1) | Nicolás GONZÁLEZ(2) | Guillermo MOSCATELLI(1) | Jaime ALTCHEH(1) | Facundo GARCÍA BOURNISSEN(1)

INSTITUTO MULTIDISCIPLINARIO DE INVESTIGACIONES EN PATOLOGÍAS PEDIÁTRICAS (1); HOSPITAL DE NIÑOS "RICARDO GUTIERREZ" (2)

Hydrophilic Interaction Liquid Chromatography (HILIC) is done using a HPLC column that retains polar species and can reveal metabolites previously undetectable by the more traditional reverse phase chromatography. Most metabolites of benznidazole (BNZ) and nifurtimox (NFX), the drugs used to treat Chagas disease, have been described in our laboratory using reverse phase chromatography. This study aims to identify and characterize new, more hydrophilic, BNZ and NFX metabolites by HILIC-UHPLC-MS/MS to broaden our knowledge of BNZ and NFX metabolic profiles. Methods: 24-hour urine was collected from healthy controls and from patients treated with BNZ or NFX for Chagas disease (written consent was obtained from all patients to use those samples for research). After sample cleaning by precipitation with ACN/MeOH 1:1 and cold centrifugation, supernatants were injected into a Restek Raptor HILIC-Si column (100 x 2.1 mm, 2.7 µm particles) in a Shimadzu Nexera X2 UHPLC system coupled to an AB-Sciex QTRAP 6500 mass spectrometer by a Turbo-IonSpray ionization source. The chromatography was performed in 50/50 (A) and 95/5 (B) gradients with ACN/Buffer acetic-ammonium acetate 10 mM, pH 4.75. Mass analysis was performed with Enhanced Mass Scan (EMS) and Enhanced Product Ion (EPI) experiments under a Data-Independent Acquisition (IDA) criterion, alternating +/- polarities in a single run. Data were analyzed with ABSciex LightSight software. Comparison of patient urine and controls revealed new phase I and II metabolites for both drugs, including new amino-BNZ derivatives, such as acyl-glucuronide, or denitrated NFX GSH-conjugates (species potentially associated with toxicity). The HILIC-UHPLC-MS/MS methodology allowed discovery of new BNZ and NFX metabolites. The role of these metabolites in the activity and toxicity of these drugs will need to be explored in PK/PD and toxicological studies in Chagas disease, with special attention to the pediatric population.

Reproducción / Reproduction VI

Chairs: Silvina Meroni | Maria Laura Ribeiro

0131 - OOCYTE RETENTION IN ACCESSORY CORPORA LUTEA AND ANOVULATION IN THE SOUTH AMERICAN PLAINS VIZCACHA (LAGOSTOMUS MAXIMUS).

Romina CAPRINO MERCADO | Facundo DUCHONY | María Sol VAVRA | Santiago Andrés CORTASA | Sofia PROIETTO | María Clara CORSO | Ruth CWIRENBAUM | Kevin FEEHAN | Julia HALPERIN | Alfredo VITULLO | **Verónica Berta DORFMAN**

CENTRO DE ESTUDIOS BIOMÉDICOS, BIOTECNOLÓGICOS, AMBIENTALES Y DE DIAGNÓSTICO-UNIVERSIDAD MAIMÓNIDES

Vizcacha shows unusual reproductive features like inhibition of follicular atresia, natural massive poliovulation and reactivation of reproductive axis during pregnancy with follicular recruitment and formation of accessory corpora lutea (aCL) with oocyte retention. Oocyte retention is also observed in ovary follicular cysts of women with polycystic ovary syndrome (POS) whose exact causes are unknown. In addition, POS is characterized by alteration in the hormonal environment and anovulation. Here we evaluated possible similar etiology for aCL and follicular cysts in POS. Progesterone (P4), estradiol (E2), estrone (E1), androstenedione (A4), and testosterone (TT) serum levels were studied by ELISA and luteinizing hormone (LH) by RIA. Also, expression of aromatase (ARO), 17beta-HSD enzyme, LH receptor (LHR), and FSH receptor (FSHR) was determined. Non-pregnant non-ovulating (NPNO), non-pregnant ovulating (NPO) and mid-pregnant (MP) vizcachas were used (n= 5/group). Ovary dynamic was evaluated by H&E staining. NPO showed significant increment of P4 vs. NPNO whereas, MP showed the highest values ($p<0.01$). Similar variations were determined for E2 ($p<0.05$), however E1 values depicted significant decrease in MP ($p<0.05$). A4 and TT did not exhibit variations. LH was significantly increased in NPO ($p<0.01$). ARO, 17beta-HSD, LHR and FSHR showed expression at follicles and CL. Strikingly, ovary dynamics showed similar amount of mature follicles among evaluated groups whereas, significant increment of CL was determined in both ovulating groups related to NPNO ($p<0.01$) with 2.5 % of aCL in NPO and 18.6 % in MP. These results suggest a key role for aCL during the pregnancy of the vizcacha contributing with the hormonal boost. These aCL seem to originate from non-mature

follicles. The hypothesis of the possible similar etiology for aCL and follicular cysts in POS needs more evidence to be confirmed.
Supported by Fundación Científica Felipe Fiorellino, CONICET-PIP110/14, MICyT-PICT1281/2014 and Escuela Técnica ORT grants.

0152 - INHIBITION OF ANDROGEN RECEPTOR AND UTERINE HISTOARCHITECTURE IN A RAT PCOS MODEL

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INSTITUTO DE SALUD Y AMBIENTE DEL LITORAL (ISAL, UNL-CONICET),

Polycystic ovary syndrome (PCOS) is associated with hyperandrogenism and uterine histoarchitecture modifications. The aim of this study was to investigate the uterine effects mediated by androgen receptor (AR) in a rat PCOS model. Beginning at weaning, female rats were injected sc with dehydroepiandrosterone (DHEA) 6mg/100g bw (PCOS) or DHEA 6mg/100g + flutamide (AR antagonist) 2mg/100g bw (PCOS+F) for 20 consecutive days. At postnatal day 41, serum and uterine tissues samples were collected. Similar testosterone, estradiol and progesterone serum levels and no estrous cyclicity were observed in both groups. The uterine water content was not modified between groups, although a decreased in AQP7-8 protein expression was found in PCOS+F rats. No differences in epithelial height, glandular density and subepithelial stroma thickness and nuclei density was observed between groups. However, the myometrium thickness was decreased in PCOS+F rats without changing the nuclei density. Also, in these animals an increase in the uterine collagen remodeling was observed. Cell apoptosis in the luminal epithelium was increased in PCOS+F rats, whereas cell proliferation in the subepithelial stroma and myometrium was decreased. Besides in this group, insulin-like growth factor-I (IGF-1) mRNA expression was increased, whereas phosphatase and tensin homolog (PTEN) expression was no modified in the stroma. Progesterone receptor expression was similar between groups, estrogen receptor 1 (ESR1) expression was reduced in the subepithelial stroma and myometrium, whereas AR expression was decreased in the subepithelial stroma of PCOS+F animals. The results demonstrated that uterine cell turnover and collagen remodeling found in the PCOS rats is regulated by AR, directly or indirectly through changes in ESR1 expression.

0215 - ORAL DAILY MELATONIN SUPLEMENTATION ATTENUATES OXIDATIVE STRESS IN TESTES OF MEN WITH IDIOPATHIC INFERTILITY

Eugenia RIVIERE (1) | Soledad Paola ROSSI(2) | Roberto PONZIO(3) | Elisa PUIGDOMENECH(4) | Oscar LEVALLE(5) | Gustavo MARTINEZ(6) | Claudio TERRADAS(7) | Ricardo Saúl CALANDRA(8) | María Eugenia MATZKIN(2) | Mónica Beatriz FRUNGIERI(9)

IBYME-CONICET; CÁTEDRA DE QUÍMICA, CICLO BÁSICO COMÚN, UBA (1); IBYME-CONICET; DEPARTAMENTO DE BIOQUÍMICA HUMANA, FACULTAD DE MEDICINA, UBA (2); INSTITUTO DE INVESTIGACIONES EN REPRODUCCIÓN, FACULTAD DE MEDICINA, UBA (3); INSTITUTO MÉDICO PREFER (4); DIVISIÓN ENDOCRINOLOGÍA, HOSPITAL DURAND, FACULTAD DE MEDICINA, UBA (5); FERTILIDAD SAN ISIDRO, INSTITUTO MÉDICO DE ALTA COMPLEJIDAD (6); PREFER; HOSPITAL DURAND, FACULTAD DE MEDICINA, UBA; FERTILIDAD SAN ISIDRO (7); IBYME-CONICET (8); IBYME-CONICET; CÁTEDRA DE QUÍMICA, CICLO BÁSICO COMÚN, UBA (9)

Melatonin (mel) modulates testicular function acting indirectly through the hypothalamus/pituitary and directly on different gonadal cells. We previously demonstrated that testicular expression of several inflammatory markers was increased in biopsies of infertile patients compared to biopsies of men with normal spermatogenesis. Oral mel intake attenuated testicular inflammation in infertile patients. Because inflammation and oxidative stress are co-dependent pathophysiological processes, and mel is known as a potent antioxidant agent, in this study we evaluated the impact of oral mel supplementation on testicular oxidative state in infertile patients. Testicular biopsies of patients with idiopathic infertility (n= 35) and normal histology (n= 6) were used. A sub-group of infertile patients (n= 11) were under mel supplementation (3 mg single oral daily dose) to treat sleep disorders. Mel concentration was lower in testes of infertile patients compared to gonads of men with normal morphology. Oral daily mel intake in infertile patients restored testicular mel concentration close to control levels ($p<0.05$). Lipid peroxidation (TBARs assay) as well as the protein expression levels (Western blot) of the antioxidant enzymes superoxide dismutase 1 (SOD1), catalase and peroxiredoxin 1 (Pxr1) were statistically increased in testicular biopsies of infertile patients compared to gonads with normal histology ($p<0.05$) suggesting that, in pathological conditions, testicular oxidative stress activates the enzymatic defense mechanisms to prevent further injury. An oral daily dose of mel, decreased testicular TBARs generation as well as SOD1 and catalase expression to control levels ($p<0.05$). No significant differences were found in Pxr1 and glutathione peroxidase 1-2 expression levels between biopsies of infertile patients supplemented or not with mel. In conclusion, these results reveal that oral daily mel intake reduces oxidative stress in testes of patients with idiopathic infertility.

0251 - SERUM ESTRADIOL LEVELS, BUT NOT PROGESTERONE LEVELS, ARE ASSOCIATED TO CHANGES IN ENVIRONMENTAL FACTORS IN THE SOUTH AMERICAN PLAINS VIZCACHA (LAGOSTOMUS MAXIMUS).

Kevin FEEHAN | Santiago Andrés CORTASA | Alejandro Raúl SCHMIDT | María Clara CORSO | Sofia PROIETTO | Victoria FIDEL | Pablo Ignacio Felipe INSERRA | Noelia LEOPARDO | Alfredo VITULLO | Julia HALPERIN | Verónica Berta DORFMAN

CEBBAD, UNIVERSIDAD MAIMÓNIDES

Lagostomus maximus is a seasonal breeder with two reproductive cycles per year. The cycle ranging from April to August (Cycle 1) usually registers a higher number of gestations than that from October to February (Cycle 2). Our laboratory has previously established that during pregnancy, the plains vizcacha reactivates the reproductive axis allowing follicular maturation, corpora lutea formation and increased circulating steroids. The aim of this work was to evaluate possible effects of environmental factors on hormonal levels during pregnancy of plains vizcacha. We determined by ELISA the serum levels of estradiol (E2) and progesterone (P4) in pregnant animals of Cycles 1 and 2 (n= 230) for 13 consecutive years (2006-2018). The annual environmental factors considered included rainfall, humidity, temperature, wind and light cycle. Both in Cycle 1 and 2, E2 and P4 serum patterns showed a similar behavior throughout pregnancy, with two peaks at 50-70 and 90-118 gestation days. The average level of serum E2 significantly changed over the years in relation to changes in environmental factors. This allowed distinguishing two groups with a different annual reproductive strategy; i.e, years with high E2 and years with low E2 serum levels. On the other hand, no variation in average of P4 serum level was found over the years irrespective of changes in environmental factors. Nevertheless, P4 levels were always significantly higher in Cycle 1 vs. Cycle 2 over the years. Variation of E2 level suggests different hormonal strategies according to changes of environmental factors which could be involved in the determination of seasonal reproduction observed in this rodent.
Supported by Fundación Científica Felipe Fiorellino, CONICET-PIP110/14 and MICyT-PICT1281/2014 grants.

0265 - PLACENTAL APOPTOSIS ENHANCED BY HIF1 ALPHA STABILIZATION IS COUNTERACTED BY LEPTIN

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INSTITUTO DE QUÍMICA BIOLÓGICA DE LA FACULTAD DE CIENCIAS EXACTAS Y NATURALES (IQUIBICEN) (1); HOSPITAL NACIONAL "PROF. DR. ALEJANDRO POSADAS" (2)

Leptin is a pleiotropic hormone produced by the placenta where it plays important regulatory functions. We have previously demonstrated that leptin promotes proliferation and survival of trophoblastic cells. Moreover, leptin prevents cellular stress under hypoxic condition in trophoblastic cells. In this work we aimed to study the mechanisms that mediate the effect of leptin in placental apoptosis induced by cobalt chloride ($CoCl_2$), a hypoxia mimicking agent that stabilizes HIF-1 alpha transcription factor expression. For this study we use Swan-71 cells, a first trimester trophoblastic human cell line, cultured under standard conditions, as well as human term placental explants. Swan-71 cells and placental explants were treated with $CoCl_2$ (50 or 100 µM). The expression of HIF-1 alpha, p53, Caspase-3 and cPARP was determined by Western blot or Immunofluorescence (IF). Apoptosis was determined by DNA ladder assay in placental explants. All procedures were approved by ethical review committee at the Alejandro Posadas National Hospital. We observed that HIF-1 alpha stabilization increased DNA fragmentation in placental explants (*p<0.05). Leptin treatment blocked this effect (#p<0.05 relativized to control treated with $CoCl_2$). On the other hand, treatment with $CoCl_2$ increases clivated PARP and Caspase-3 levels in a dose-dependent manner indicating that apoptosis was induced (**p<0.01). Moreover, p53 protein expression, a key regulator of apoptosis pathway, was enhanced by hypoxic condition (**p<0.01). We also observed that HIF-1 alpha stabilization increased nuclear p53 localization. All these results suggest that HIF-1alpha stabilization enhances placental apoptosis and leptin is capable to protect these cells under hypoxia conditions.

0374 - PREVALENCE OF ESCHERICHIA COLI AND ANALYSIS OF VIRULENCE FACTORS IN ENDOCERVICAL CULTURES FROM PREGNANT WOMEN.

Maria Lujan SCALISE (1) | Patricia LEONINO(2) | Adriana PEREYRA(2) | Roberto CASALE(2) | José A. FERREIROS(2) | Flavia SACERDOTI(1) | Cristina IBARRA(1)

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Presence of E. coli in the endocervical microbiome has been associated to pregnancy complications. We have previously reported that Shiga toxin (Stx) producing E. coli (STEC) infections during pregnancy may cause maternal or fetal damage mediated by Stx2 in rats in early stage of gestation. Moreover, Stx2 inhibits migration, invasion and cell viability in extravillous trophoblast human cells of first trimester. Therefore, we propose to study the presence of STEC in female genital tract in the pregnant women since might be risk factor during gestation. Our objective was to identify different virulence factors of STEC cultures of endocervix of pregnant women. Endocervical swabs from 103 asymptomatic pregnant women with gestational age of 14 to 30 weeks from the National Hospital Posadas were enrolled. Samples were enriched in Tryptic Soy Broth and sub-cultivated on sorbitol-MacConkey (SMAC) agar in order to detect no sorbitol fermenting colonies, characteristic of STEC. Genomic DNA was purified from colonies and the presence of the uidA gene, exclusive for E. coli was analyzed by polymerase chain reaction (PCR). Positive colonies for uidA were checked for rfbO157, lpfAO113, hcp, eae, stx1, stx2-2a genes. STEC strains positive for stx2 genes were also cultured in the presence of mitomycin C (2 µg/ml) to evaluate expression of Stx2 by viability assays on Vero cells. The PCR results showed that 16/103 samples were positive for SMAC agar and 15/103 were positive for the uidA gene. Furthermore, 6/15 E. coli expressed lpfAO113 and hcp, and 9/15 E. coli expressed stx2 being only one sample positive for stx2a variant. All of them were negative for rfbO157, eae and stx1 genes. One STEC strain positive for stx2 gene showed cytotoxic effects even in absence of mitomycin C. These results suggest that STEC strains could colonize the endocervix of pregnant women

0405 - VASOACTIVE INTESTINAL PEPTIDE (VIP) AS AN OVARIAN PROTECTOR: PREVENTION AGAINST PREMATURE OVARIAN FAILURE DURING CHEMOTHERAPY

Yamila HERRERO (1) | Leopoldina SCOTTI(1) | Gonzalo OUBIÑA(1) | Natalia PASCUALI(1) | Rossana RAMHORST(2) | Claudia PÉREZ LEIROS(2) | Dalhia ABRAMOVICH(1) | Fernanda PARBORELL(1)

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The ovary, in addition to its endocrine and intraovarian control, is regulated by direct neural inputs of peptidergic nature. Vasoactive intestinal peptide (VIP) was originally isolated from the small intestine and lung tissues and plays an important role in ovarian function. Previous studies have found VIP immunoreactivity in ovarian follicles. The objective of this study was to determine the effect of VIP on ovarian function in a doxorubicin (DX) induced-premature ovarian failure (POF) murine model. To induce POF, DX 10 mg/kg, i.p. was applied in F1 mice (C57XBalbC 8 weeks old) on day 1. Control and DX mice underwent sham surgery and received an intrabursal injection of saline solution on both ovaries, while DX + VIP groups received either 1 µl or 10 µl VIP 1 µM 1 h prior to DX administration. Sacrifices were made at day 15. The ovaries were isolated for histological analysis and protein extraction for Western Blot assays. For all data analysis ANOVA followed by Tukey test were performed. An ovarian morphological analysis showed that DX decreased the % of primary (PriF), preantral (PF) and early antral follicles (EAF), and increased the % of atretic follicles (AtrF) ($p<0.05$). VIP (1 µl) increased the % of EAF and decreased the % AtrF. However, the highest dose of VIP (10 µl) increased the % of PriF, PF and EAF, and decreased the % of AtrF compared to DX ($p<0.05$). These results were corroborated by IHC for Anti-Müllerian Hormone (AMH), where it was found that DX reduced the % of follicles expressing AMH, while VIP (both doses) increased it ($p<0.001$). DX increased the apoptotic index (cleaved caspase-3-positive follicles/total follicles) in follicles, compared to control ($P<0.01$). VIP (both doses) protected follicles from this increment. In conclusion, VIP might be a promising strategy to protect female fertility in cancer patients. Further studies on VIP effects on female reproduction in chemotherapy-induced POF and on the safety of use of this peptide are required.

0443 - CANNABINOID RECEPTOR 1 (CB1) IS INVOLVED IN PRETERM BIRTH INDUCED BY LPS

Carolina MARVALDI | Julieta SCHANDER | Julieta AISEMBERG | Fernanda DE LA CRUZ | Ana María FRANCHI | Manuel Luis WOLFSON

CEFYBO, UBA

Endocannabinoid system (ECs) is one of several signaling pathways implicated in maternal-fetal interface, and endocannabinoids are implicated in different aspects of physiopathology of reproduction. Preterm birth (PTB) is the leading cause of mortality and morbidity in neonates. It is well known that premature deliveries are mainly associated with infectious process. In mice, it has been shown that one of the major causes of PTB is premature decidual senescence,

which becomes more aggravated by an inflammatory stimulus. Our group developed a murine model of preterm labor, consisting of two injections of bacterial lipopolysaccharide (LPS) that produces an 85% of PTB in BALB/c mice. The aim of the present work was to evaluate if the ECs participates in LPS-induced preterm labor. For this purpose, we administrated two doses of bacterial lipopolysaccharide (LPS, 10 µg/g of weight and 3 h later 20 µg/g of weigh respectively) on day 15 of pregnancy to CD1-wild type mice (CB1-WT) and CD1-knock out mice for the cannabinoid receptor type one (CB1-KO). We found that CB1-KO mice show lower PTB percentage than CB1-WT mice (60 CB1-KO vs. 81 % CB1-WT). We studied different inflammatory mediators in decidua 5h after the second dose of LPS and observed that protein levels of TLR-4 were decreased in LPS treated mice ($p<0.05$) while CD14 and COX-2 protein levels were augmented ($p<0.05$). The same response pattern was observed both in CB1-WT and CB1-KO mice. It has been reported that disruption of autophagy balance (either increase or decrease) can lead to PTB. We evaluated decidual protein expression of LC3b II, a marker of autophagy, and observed that CB1-KO mice presented lower decidual protein levels of LC3b II when compared to CB1-WT ($p<0.05$). Considering the cross-talk between autophagy and senescence, we evaluated the protein expression of H2AX, an indicator of DNA damage, and did not observe differences between genotypes. In summary, our results indicate that cannabinoid receptor type one is involved in the triggering of LPS-induced preterm birth.

0776 - PRO-INFLAMMATORY AGENTS NITRIC OXIDE AND TNF ALPHA ARREST GC-1 SPERMATOGONIA CELL CYCLE THROUGH DIFFERENT MECHANISM

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INBIOMED-UBA-CONICET (1); CEBBAD, UNIVERSIDAD MAIMÓNIDES (2); CEFYBO, UBA-CONICET (3)

Nitric oxide (NO) and tumor necrosis factor alpha (TNF alpha) are pro-inflammatory agents able to interfere with cell cycle. Experimental autoimmune orchitis (EAO) is a model of chronic inflammation associated to infertility. In EAO high levels of NO and TNF alpha are produced by testicular macrophages and pre-meiotic germ cells (spermatogonia and pre-leptotene spermatocytes) proliferation is reduced. We propose that NO and TNF alpha arrest spermatogonial cell cycle in EAO. To evaluate this hypothesis, we explored the effect of DETA-NO (a NO donor) and TNF alpha on cell cycle and death on GC-1 spermatogonia cell line by flow cytometry. Both TNF alpha (50 ng/ml) and DETA-NO (2.0 mM), significantly increased the percentage of GC-1 cells in the S-phase and significantly reduced the percentage in the G1-phase of the cell cycle (propidium iodide incorporation, IP) also inducing cell apoptosis (Annexin V-FITC-IP assay) after 24 and 18 h of incubation respectively. Pre-incubation of GC-1 cells with a general antioxidant, N-acetyl-L-cysteine (NAC, 2.5 and 5.0 mM) significantly reduced DETA-NO effect on cell cycle arrest and apoptosis while NAC did not modify TNF alpha action. DETA-NO induced GC-1 cell cycle arrest and apoptosis was reverted after DETA-NO withdrawal unlike TNF alpha.

1085 - THE RESPONSE TO ENVIRONMENTAL THERMAL STRESS IS NOT SEXUALLY DIMORPHIC AND DEPENDS ON ANDROGENS IN THE INDUCTION OF SEX REVERSAL OF MEDAKA FISH

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LABORATORIO DE BIOLOGÍA DEL DESARROLLO - INSTITUTO TECNOLÓGICO DE CHASCOMÚS. INTECH (CONICET-UNSAM) (1); INSTITUT NATIONAL DE LA RECHERCHE SCIENTIFIQUE (INRS) (2)

In many fish species, environmental stressors (ES), like high temperatures (HT), induce sex reversal of genotypic females (GF) to phenotypic male. In a previous study, we elucidated that all begins in the brain through an early increase of crhb, with the concomitant increase of whole body cortisol level. The synthesis of androgen as a by-product of cortisol inactivation has been proposed in a fish with environmental sex determination, the pejerrey. Nevertheless, the participation of androgen in the sex reversal induced by an ES during the gonadal sex determination period (GSDP) has not been corroborated. First, we performed a microarray after incubate embryos to HT, and control temperature (CT), from fertilization to GSDP. Our results showed a whole stress effects, inducing the differential expression of 2,517 of 11,600 genes. Many of them related to glucocorticoid, *e.i*. crhb, gr2, and thyroid axis, *e.i*. tsh, tg, and iyd; both axes has been related with sex reversal. Moreover, the pathway of sex steroid was up-regulated in HT treated embryos, especially to androgen synthesis, *e.i*. hsd3b, cyo11b, hsd11b2 and hsd11b3. On the other hand, the estrogen pathway was down-regulated, e.i. cyp19a1a. Moreover, it is important to highlight those DEGs analysis between sexes at the same treatment did not display differences, assuming of both genotypes sexes response similarly to an ES. Finally, the participation of androgens in the sex reversal was analyzed with Flutamide (Flu), an androgen receptor antagonist. XX larvae incubated at CT showed only ovarian development, but XX individuals incubated at HT until hatching presented an increased sex reversal towards male; however, in the case of Flu treatment of embryos incubated at HT, the sex reversal percentage decreases in a dose-dependent manner. Therefore, our results are consistent with an androgen synthesis response to an environmental stress, with the concomitant testis development bias.

AACyTAL II

Chairs: Marco Brocca | Gabriel Pinto | Marina Snitcofsky

0034 - BOTRYOMYCOSIS IN NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ MICE: A FOCAL OUTBREAK IN AN EXPERIMENTAL COLONY

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NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ mice were purchased from Jackson Laboratory and maintained under conventional closed barriers in individually ventilated cages (Tecniplast®) with controlled temperature (20-22°) and relative humidity (50–70%) in a 12:12 h light: dark cycle. The animals were fed with autoclaved standard diet and carbon activated filtered water ad libitum. Cages were filled with sterile wooden chips and corn. The experimental protocols were approved by the IACUC. Mice (n=9; 6-12 months-old) included in patient-derived xenografts (PDX) studies exhibited solitary or multiple fibrose nodules of 0.50 cm, located near to the mouth and nose. The cases appeared randomly in cages for over five months. After clinical examination, differential diagnoses included bacterial or fungal infections, foreign body granuloma, sterile pyogranuloma or neoplasia. Mice exhibited poor body condition, weight loss and ruffed coat. Animals were submitted to necropsy. The nodules presented fibroelastic consistency at the cutting. Macroscopic description included hepatosplenomegaly, pale diffuse liver and uterus, ovaries and gastrointestinal tract inflammation, with coagulated blood content in stomach. At histological examination, there was a multifocal dermatitis, with

diffuse bacteria colonies accompanied by fibrillar acidophilic material compatible with a Splendore-Hoeppli reaction, surrounded by neutrophils and pyocytes with moderate presence of epithelioid macrophages. The final diagnostic was botryomycosis, probably due to Staphylococcus spp. This is the first report of botryomycosis in NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ mice. Regarding their combined immunodeficiency condition, skin nodules should be early diagnosed, taking account botryomycosis as a differential diagnosis. Strict housing barriers and procedures have to be taken to the extreme in order to avoid or reduce the presentation and severity of this concomitant pathology.

0071 - REFINING STRATEGIES FOR PHARMACOKINETIC STUDIES: APPLICATION IN A MOUSE MODEL OF TRYPANOSOMA CRUZI ACUTE INFECTION

Daniela ROCCO | **Ernesto GULIN** | Jaime ALTCHEH | Facundo GARCÍA BOURNISSEN

SERVICIO DE PARASITOLOGÍA Y CHAGAS - HOSPITAL DE NIÑOS "RICARDO GUTIÉRREZ"

Benznidazole (BZ) is one of the two available nitroheterocyclic drugs for Chagas disease treatment. The lack of pharmacokinetic (PK) information in human and animal models hampers the development of suitable treatment strategies. Classical PK studies require high volumes of blood and a group of mice for each PK time point. The objective of this work was to develop a BZ PK model in infected mice with T. cruzi, applying population PK strategies to reduce the number of animals and refine the procedures. Twenty-eight 2-months old male BALB/cJ mice were treated with a single 100 mg/kg dose of BZ by gavage; blood was sampled at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6 and 8 h. Mice (n= 12) were infected by i.p. route with 500 trypomastigotes of T. cruzi VD strain and treated with BZ at parasitemia onset. The protocol was approved by the local IACUC (#2017-10). Blood was obtained by sub-mandibular puncture, which allowed obtaining enough volume for BZ analysis but also permitted to take multiple samples from each mouse. Blood samples were centrifuged, serum was precipitated with acetonitrile (1:1) and conserved at -70ºC until BZ measurement by UHPLC-MS/MS. A population PK model was developed using Monolix software (Lixsoft). BZ population PK followed a one compartment model with first order oral absortion; the maximum observed concentration (Cmax) was 67.7 µg/ml at 0.5 h from drug administration. Absorption was fast (ka $4.3h^{-1}$), the distribution volume was 30.3 ml and clearance 15.8 ml/h (estimated half-life: 1.3 h). Significant differences between infected and control mice were only observed in the distribution volume (non-linear mixed effects regression model, $p<0.05$). BZ PK profile in infected animals was conserved when compared to healthy mice, suggesting that drug concentrations reflect those required for drug effectiveness. Moreover, the design of PK studies should address 3Rs principles, as they do not interfere with scientific purposes while providing robust and repeatable results.

0091 - ZEBRAFISH (DANIO RERIO) HEALTH MONITORING IN ARGENTINIAN COLONIES.

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Zebrafish, Danio rerio currently is used as an animal model in embryonic development assays, genetic function analysis and mutagenesis. Its characteristics such as high fertility, external fertilization and molecular mechanisms of embryo development similar to all vertebrates, allowed the zebrafish to gradually become the model of choice among inferior vertebrates. Many of the infections that affect zebrafish are primary or opportunistic pathogens that can alter research results and cause subclinical infections, therefore, individuals under stress conditions, poor management techniques and poor water quality, may end in an illness. Signs of infections include a slight decrease in reproductive efficiency, anorexia and an increase of mortality in the colonies. Mycobacterium spp species are potential zoonotic pathogens and Mycobacterium marinum is the specie with the highest prevalence in zoonotic human infections. The objective of this work was to perform a health monitoring program in Argentine colonies by using molecular techniques (PCR) and bacteriological culture in compliance with the list of the most prevalent zebrafish pathogens. The results found through the PCR techniques, showed fish colonies contaminated with Mycobacterium spp, Aeromonas hydrophila, Ichthyopthirius multifiliis, Flavobacterium columnare and by using bacteriological culture it was isolated Pseudomonas fluorescens. In conclusion, we strongly recommend the implementation of a health monitoring program in zebrafish facilities in order to reduce risks, avoid variables due to infectious agents and ensure reliable research results.

0122 - BALB/CANNLAE MICE BEHAVIOUR: OPEN FIEL ETHOGRAM AND THE IMPORTANCE OF ENVIRONMENTAL ENRICHMENT

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The strain BALB/cAnNLAE is one of the most used mice model in our country in areas such as immunology, oncology, virology and breeding. Know its behavior allows you to understand normal patterns of behaviour, communicate them to the scientific community, establish its relevance to a specific type of study and understand if their behavior under experience corresponds with expectations and the results are more accurate. The open field test is a methodology to evaluate behavior. The animal maintenance way with environment enrichment has allowed to enhance their well-being and enabled that individuals have larger strategies to make their specific behavior. The objective of this study was to analyze the ethogram and frequency of use of behavioural patterns of this strain in the open field test, analyze the variance of the results obtained in animals housed with and without environmental enrichment. 36 mice, 9-week-old were used. The open field trial was conducted and the ethogram for groups and the frequencies in which occurred the behaviour patterns were compared. The variance of data was analyzed by ANOVA for data of normal distribution and Kruskal Wallis data for those who had no such distribution for $p<0.05$. It was concluded that the variances of groups with enrichement was less that of control groups and groups with toy enrichment is higher than the shelter enrichment. In enrichement groups manifestation of behavior occurs in lower frequency compared to groups control resulting in a better adaptation to the new situation. Toy enrichment exacerbates most active behaviors when compared with the shelter enrichment groups. In active behavior, shelter enrichment is more important than toy enrichment and raises an action on some behaviors that occur on the edge of the open field at low frequencies, suggesting a fearful behavior.

This work has been evaluated by the institutional committee for care and use of the laboratory animals of the FCV - UNLP code 02-09-10T.

0124 - NLAE:NIH (SWISS) MICE BEHAVIOUR: OPEN FIELD ETHOGRAM AND THE IMPORTANCE OF ENVIRONMENTAL ENRICHMENT

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CÁTEDRA DE ETOLOGÍA, FACULTAD DE CS. NATURALES Y MUSEO, UNLP (2)

The laboratory mouse is the major non-human model used for research. Inbreed strains are used for specific fields and the outbreed stocks are mainly used in toxicology, as genetic variability models. Although their use is smaller, they continue to be used in significant quantities. The characterization of the strains allows to understand the animal model, and in the most strains used, this is considerably developed. However, this is not the case in outbreed mice, given its high variability and lower proportion of use. Studying their behavior enables their definition as a model and knowledge of their behavior. The maintenance of these laboratory animals has changed in recent years, considering environmental enrichment as a key element for their welfare and specific behavior. In this study, 36 outbreed 9-week-old mice were used with two types of enrichment, toy and shelter. The ethogram for the different groups was elaborated and the open field test was carried out, comparing the frequencies in which the patterns were followed using an ANOVA test for normal distribution data and Kruskal Wallis for non-normal data, for $p<0.05$. Variances of the enriched mice were lower than those of the controls and that of the groups with toy enrichment were higher than those of the refugee. In the enriched animals there is less frequency of behaviors. Toy enrichment increases most active behaviors. In the latter, the refuge is more transcendent than the toy, and poses an action on some behaviors that occur throughout the open field at high frequencies. Passive behaviors such as frezzing are practically absent. This work has been evaluated and approved by the Institutional Animal Care and Use of the FCV - UNLP, under the code 02-09-10T.

0440 - EXPERIMENTATION WITH ANIMALS: A KEY ASPECT OF THE 3RS: THE GENETIC QUALITY

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The genetic quality of laboratory animals is essential for reproducibility of scientific research. Working with animals of certified genetic quality is still a pending issue in Argentina due to the lack of routine genetic controls, of information on the genetic background of animals and of proper training. Apart from being concerned with having their results published and getting funding for research, scientists should know the genetic origin of laboratory animals. Consequently, they should perform genetic controls to verify whether animal integrity has been compromised by accidental genetic contamination or genetic drift. The aim of this work was to evaluate the genetic purity of the inbred C57BL/6J mouse strain from three animal facilities belonging to the Buenos Aires University School of Medicine network by analyzing a panel of microsatellite markers. Female mice tail samples (3-5 mm) were taken and genomic DNA was obtained by organic extraction. The genetic profile of each animal was determined by PCR-fragment analysis, using microsatellites D1Mit155, D2Mit493, D3Mit49, D13Mit13, D6Mit8 and D12Mit12, located on six different autosomal chromosomes and selected from the Mouse Genome Informatics database (www.informatics.jax.org/searches). The results provided key data on the genetic quality of the three inbred animal colonies studied. They were also served as an example for other laboratory animal facilities in Argentina and as a starting point to modify the conditions and management of laboratory animal colonies.We determined the genetic purity of the inbred C57BL/6J mouse strain in all animal facilities evaluated. All six loci analysed were homozygous, certifying their isogenicity and phenotipic uniformity. These results are promising for animal facilities mainly performing biomedical research. They also show a positive evolution in handling animal colonies and using of the 3Rs, and researchers commitment with animal science, since they promote the supply of genetically quality-controlled animals. The positive impact of these results should encourage other researchers using this inbred strain to perform periodic genetic monitoring, there by consolidating the supply of quality-controlled mice. This pioneering study carried out in IGEVET (CONICET-UNLP), should consolidate the genetic monitoring of inbred strains throughout the country

0592 - DETECTION OF NON WANTED PHENOTYPE AND REPLACEMENT OF COLONY BALB/c IN VIVARIUM OF PRODUCTION AND RESEARH IN BASIC ONCOLOGY

Noelia Paola Natalia CARDOZO | **Mónica Viviana CHAMORRO** | Romina Alejandra KARAS | Marianela ABRIGO | Miriam Judith DIAMENT

INSTITUTO DE ONCOLOGÍA ANGEL H. ROFFO

The AH Roffo Institute of Oncology has a vivariumof murine production and basic research. The appearance of body alopecia was observed in different individuals of the BALB/c population, before reaching 3 months of age. To determine the origin of this pathology, microbiological and parasitological analyzes were performed, with negative results. Subsequently, an analysis of the family tree was performed to determine if the abnormal phenotype was a genetically determined trait. It was established that the main ancestor line presented the modification of the phenotype, confirming that the observed alopecia could be hereditary. Suspecting that the animals did not have the required genetic quality, so it was decided to establish a new colony. Because the genetic composition of the new colony was different from that housed in the vivarium, it was necessary to check whether tumors that arose spontaneously and maintained in the IOAHR were developed in the same way. Murine tumors M2 (undifferentiated fusocellular breast carcinoma), M3 (semi-differentiated breast adenocarcinoma) and P07 (semi-differentiated lung adenocarcinoma) were transplanted subcutaneously with trocar. Tumor progression, spontaneous metastasis and the appearance of paraneoplastic syndromes were evaluated in both colonies. Each tumor was thawed and transplanted to 5 animals from the pre-existing colony, in order to stabilize them. Then, a tumor bearing mice from each tumor line was selected to perform a second transplant to 5 BALB /c females of each colony. Finally, a third transplant was performed only to females of the new colony. Tumor and metastatic development (latency, incidence, growth and evolution time) were evaluated. As In both colonies was observed similar characteristic behavior of the M2, M3 and P07 tumors, was decided the elimination of the former colony. We were able to conclude that the new BALB/c colony fulfilled the characteristics of the line necessary for basic research in oncology.

0746 - ARE THE OUTBRED MICE STOCKS FROM ARGENTINA REALLY OUTBRED?

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The laboratory mouse is the most commonly used experimental non-human subject in biomedical research. Furthermore, the inbred strains are commonly preferred over outbred stock by their genetic pureness and phenotypic stability. However, recent work showed that the outbred stocks are comparable to the inbred strains for its use in biomedical research. The data that argued that the outbred stocks are comparable to inbred strains used the data coming from companies that sell mice in the USA and Europe. Its company possesses a well-documented pipeline of maintained of the genetic variability into their outbred strains. Conversely, in Argentina generally due to colony age old, the outbred stocks of mice primarily need to be tested to determine their level of endogamy and to establish if they are really outbreed stock. The

objective of this work is to study the genetic variability in different outbred stocks from Argentinean breeders. Different breeders from Buenos Aires sent samples of tissue from CF-1, CD-1, and Swiss stocks. The samples were proceeding by the Genetic Monitoring Service of INTA using the analysis of the microsatellite. Our results show that the analyzed outbred stocks possess a high level of homozygous (>80%) across the full genome, all 19 autosomal chromosomes tested. The homozygous into an outbred stock should be highest than 75% in all genetic markers. However, it is a challenge for the breeders because the mating system into a closed population tends to increase the inbreeding. Our results show that the analyzed outbred mice stock possesses a very low heterozygosis and a high inbreeding. Probably, even when the mating systems are well used, the condition to maintain the genetic variability higher, as the incorporation of new parental to the stock is frequently not fulfilled. Then, even when the outbred stocks theoretically possess a heterogeneous background, in countries where the stocks don't show the expected variability the introduction of a genetic monitory could help to select the best animals for the mating system.
*These two authors contributed equally.

0959 - USE OF AN ARTIFICIAL FEEDER TO REPLACE BIRDS TO FEED HEMATOPHAGOUS INSECTS: POST-INTAKE EFFECT.

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UNIDEF-CITEDEF-CONICET-CIPEIN (1); UNIDEF-CITEDEF-CONICET-CIPEIN; 3IA UNSAM (2)

The Research Center for the Investigation on Plagues and Insecticides (CIPEIN) aims to develop pest insect control tools, especially vectors of arboviral diseases, with high safety for human health and low environmental impact. In order to study these insects, it is essential to keep breed them. In order to minimize the use of birds for feeding these hematophagous insects, we have acquired and implemented the use of artificial feeders (Hemotec®). First, the optimal temperatures at which each insect species is attracted to feed using physiological solution were studied. The first results obtained indicated that at 40 °C V stage nymphs and adults of kissing bugs (Triatoma infestans) respond quickly trying to bite in 79 sec; at 35 °C in just about five minutes (327 sec) and at 30 °C they approach to the membrane but they don't try to bite. For bed bugs (Cimex lectularius) a similar trend is observed but they respond faster, in less than a minute (50 sec) at 40 °C, in 140 sec at 35 °C and in 160 sec at 30 °C. When Aedes aegypti mosquito females were used it was observed that they practically did not respond at 30 °C and for temperatures of 35 and 40 °C they presented responses as different as trying to bite in 20 sec or take more than 7 minutes. This would indicate that for species that are not obligate hematophagous and that do not live in direct contact with their host (such as mosquitoes) there would be other cues that would influence the behavior of blood search in addition to heat, such as olfactory cues for example carbon dioxide emissions. As a second part of this work, the physiological solution was replaced by guinea pig or chickens blood using the optimum temperatures already evaluated. The effect on the survival rate of bed bugs and mosquito females was evaluated, demonstrating great variability within and between both species evaluated. The results show that the hematophagous insects studied respond to the use of the artificial feeder, and that it is then possible to use the Hemotec® to carry out their breeding according to the international standards and ethics committees required by the scientific journals.

Oncología/ Oncology VII

Chairs: María Fátima Ladelfa | María Fernanda Troncoso

0042 - THE ALOYSIA POLYSTACHYA EXTRACT REDUCES MALIGNANT TUMOR GROWTH

Mileni SOARES MACHADO (1) | Alejandra PALMA(1) | Maria Cecilia LIRA(1) | Francisco Damián ROSA(1) | Maria Fernanda RUBIO(1) | Leonardo PAZ(1) | Guido LENZ(2) | Alejandro URTREGER(3) | Mónica COSTAS(1)

INSTITUTO DE INVESTIGACIONES MÉDICAS ALFREDO LANARI - UNIVERSIDAD DE BUENOS AIRES (1); UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL (2); INSTITUTO DE ONCOLOGÍA ANGEL H. ROFFO (3)

We have previously demonstrated Aloysia polystachya (AP) extract induces apoptosis and also the sensitization of human and mouse colorectal cancer cell lines to chemotherapeutic drugs in vitro. Moreover, our experiments demonstrated Cancer Stem Cells (CSC) population is a target for AP-induced cytotoxic effects. The aim of this work was to investigate the in vivo effects of AP extract, alone or combined with the chemotherapeutic drug, 5-Fluorouracil (5-FU). Therefore, male BALB/c mice, 8–12 weeks old were randomly divided into six groups. All of them were subcutaneously inoculated at the right dorsal flank with 2x106 CT26 cells. Once the tumor was detected, animals were treated twice a week (days 14, 17, 21, 24 and 28) with an intraperitoneal injection of different AP dilutions: AP1, AP2, AP3, 5-FURA (5 mg/kg) previously determined as poorly effective in vivo, AP2+5-FURA in a sub-effective dose or remained without treatment. Animals that were not inoculated with CT26 cells were used as control. Tumor size was determined twice a week. After 30 days, the mice were sacrificed. The histopathology of tumors and liver sections was performed by staining with hematoxylin and eosin. We found that while 5-FURA was not effective for inhibiting the tumor growth, its combined treatment with AP2 significantly inhibited the tumor growth in all the treated animals ($p<0.05$; Tukey test). Moreover, we found that AP treatments were capable to inhibit the tumor growth respect to control, even in the absence of 5-FURA chemotherapy ($p<0.05$; Tukey test). Concerning the histopathological analysis of tumor sections, some necrosis areas were observed only in animals treated with AP2 and AP2+5-FURA and the hepatic parenchyma showed a preserved architecture, similar to that observed in normal control animals. These results strongly suggest that AP extracts could be not only a promissory and useful tool to increase chemotherapy sensitivity, but also an anti-cancer treatment by applying alone.

0088 - THE ACTIVATION OF NICOTINIC RECEPTORS IMPAIRS THE RESPONSE OF TRIPLE NEGATIVE BREAST CANCER CELLS TO PACLITAXEL

Jaqueline OBREGON | Agustina SALEM | Zulma JUCHANI MAMANI | Valeria PANIAGUA | Mariana CLOZZA | Maria Elena SALES | Alejandro Javier ESPAÑOL

CENTRO DE ESTUDIOS FARMACOLÓGICOS Y BOTÁNICOS (CEFYBO-CONICET), FACULTAD DE MEDICINA, UBA

We previously demonstrated the role of cholinergic receptors in tumor progression. Particularly, muscarinic receptors are expressed in different types of breast tumors and are absent in normal mammary cells. The presence of the agonist carbachol enhances the effect of conventional chemotherapeutic agents in the treatment of these tumors. The expression of nicotinic acetylcholine receptors (nAChRs) has also been reported in these same tumors and in non-tumorigenic mammary cells. Since the consumption of nicotine (NIC) in smokers promotes the progression of lung cancer, we analyzed the effect of NIC in different types of human breast tumors by measuring cell viability with the MTT reagent and the modulation that NIC exerts on the response of these cells to the cytotoxic agent paclitaxel (PX). Treatment with added NIC for 48 h increased the proliferation of breast cells in a concentration-dependent manner. The maximal effect (Emax) obtained was significant with respect to control without treatment in different types of tumor cells (MCF-7: 111 ±

9; MDA-MB231: 89 ± 2; and MDA-MB468: 44 ± 5 %; p<0.0001). NIC also increased the proliferation of non-tumorigenic mammary cells (MCF-10A: 45 ± 7; p<0.0001). In addition, treatment with PX reduced in a concentration-dependent manner the viability of all cell lines with Emax values close to 100 % (p<0.0001). In MDA-MB231 cells, derived from a triple negative tumor, the Emax of NIC was significantly reduced to the control value in the presence of mecamylamine (10^{-4} M) confirming the participation of nAChRs. Moreover, the addition of NIC 10^{-10} M or 10^{-9} M significantly reduced the Emax of PX to 30 ± 5 or 36 ± 6 % respectively (p<0.001). We conclude that NIC increases the proliferation of both tumor and non-tumor mammary cells, indicating that it could be promoting or inducing breast malignancy and reduces the efficacy of PX treatment in triple negative breast tumors.

0097 - GLYPICAN-3 (GPC3) INHIBITS METASTASIS DEVELOPMENT AND PROMOTES DORMANCY IN BREAST CANCER CELLS THROUGH P38 MAPK PATHWAY

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We previously showed that GPC3 prevents metastatic spread of breast cancer cells as well as it activates p38 pathway. We hypothesize that GPC3 acts as a metastasis suppressor. The aim of this study was to examine whether GPC3 is inhibiting metastasis through p38 activation. We used the murine mammary LM3 cancer cell line reexpressing GPC3. Since it has been proposed that metastasis suppressors promote dormancy, we evaluated dormancy markers. We showed that the pErk/pp38 ratio was lower in LM3-GPC3 cells, while p21, p27 and SOX2 protein levels were higher, suggesting a dormant phenotype. We did in vivo experimental metastasis assays, confirming that LM3-GPC3 cells reduced their metastatic ability (p<0.005 Kruskal-Wallis). Interestingly, the presence of LM3-GPC3 cells was demonstrated in primary cultures of lungs from mice inoculated with those sublines, despite that metastatic foci were not detected. The GPC3 role was specific to dormancy since it did not affect s.c. tumor growth, but lungs of LM3-GPC3 primary culture tumor bearing-mice had no metastasis. So, dormant LM3-GPC3 cells can reactivate their proliferative capacity, remain viable, tumorigenic, but they reenter in dormancy upon reaching secondary colonization site. We analyzed whether GPC3 inhibits metastasis through p38 activation. Cells were s.c. inoculated into mice, and the p38 inhibitor SB203580 (or DMSO) was i.p. administered. The in vivo inhibition of p38 did not affect the tumor growth, but it induced an increase in LM3-GPC3 tumors local invasion (p<0.05 chi-square), as well as in spontaneous metastatic dissemination (p<0.005, Kruskal-Wallis). We did experimental metastasis assays, where cells were simultaneously inoculated with SB203580, confirming that the treatment reverses the inhibition on the metastatic spread induced by GPC3 (p<0.05, Kruskal-Wallis). Our results prove that GPC3 inhibits the metastatic ability of breast cancer cells and induces dormancy at secondary site, through the p38 activation.

0261 - EFFECTS AND UNDERLYING MECHANISMS OF GENE THERAPY WITH SUICIDE SYSTEM CYTOSINE DEAMINASE/ 5-FLUOROCYTOSINE IN SPONTANEOUS CANINE MELANOMA

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Cancer occurs spontaneously in humans and dogs, and its progression is often similar in both species. As a pre-clinical study aimed to improve the local effects of suicide genes on oncologic veterinary patients, we explored the responses of different spontaneous canine melanoma cell lines to yeast Saccharomyces cereviseae cytosine deaminase/ uracil phosphoribosyl transferase fusion protein (Ycd::Yuprt) non-viral gene transfer in combination with 5-fluorocytosine (5-FC). Ycd catalizes the passage 5-FC to 5-fluorouracil (5-FU) that interferes with DNA replication, while Yuprt drives to the synthesis of 5-FUTP that inhibits rRNA and mRNA processing. We determined the survival rates of 3D cultures on agar coated wells 12 days after treatment by the acidic phosphatase assay. We found that the spheroids formation is inhibited and the survival rate decreased to 50 % (0.05 < p < 0.0001). We explored the mechanisms related to cytotoxicity by the colony formation assay, and the senescence-associated beta-galactosidase (SA-βgal) activity in monolayer cultures. Ycd::Yuprt/5-FC reduced colony formation, and with high concentration of 5-FC colony forming ability almost disappeared (0.01 < p < 0.0001) while senescent cells increased to 70 % (p<0.0001). Furthermore, 50 % of treated cells were in subGo fraction as analyzed by flow cytometry (0.01 < p < 0.001). Finally, the cytotoxicity produced inside the cells was able to diffuse into the extracellular environment, generating the same cytotoxicity in cells that were not exposed to gene transfer, indicating a strong bystander effect (0.01 < p < 0.0001). Our encouraging results support further research on the use of this suicide system for local treatment of melanoma tumors in companion animals.

0378 - EFFECT OF VASOPRESSIN ANALOGS ON ANGIOGENESIS AND NEUROENDOCRINE DIFFERENTIATION IN AGGRESSIVE PROSTATE CANCER

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Prostate cancer (PC) is the second cause of cancer-related death in males worldwide. Invariably after treatment, disease progresses into a castration-resistant PC (CRPC) which implies no therapy response and poor prognosis. This tumor progression correlates with a loss of epithelial characteristics (EMT), promoting metastatic progression and development of cell foci with neuroendocrine differentiation (NED). Angiogenesis is a key factor for PC progression and has been linked with NED and androgen deprivation therapy. For years, our group has studied desmopressin (dDAVP), a first generation vasopressin (AVP) analog, agonist of V_2 receptor (AVPR2) and [V4Q5]dDAVP, a second generation analog with enhanced cytostatic activity. Both peptides showed antiproliferative effects in vitro and in vivo on several tumor models including aggressive PC and, interestingly, reduced the expression of NE markers. Given this evidence, and the increasing incidence of aggressive PC with NE features, this work aims to evaluate the effect of AVP analogs on key processes related to cancer progression on aggressive NE PC-3 model. The cells were treated with AVP analogs for 7 days, subsequently studying its effect on the expression of NE markers and genes associated to EMT by RT-qPCR, and the sensitivity to the chemotherapeutic agent Cisplatin was measured by MTS assays. Sustained exposure to analogs reduced the NE markers expression, and modulated the expression of genes associated to EMT in vitro. Furthermore, we assessed angiogenesis in vivo with Matrigel® plug modified assay in nude mice. Treatment with each analog reduced PC-3 cell-induced angiogenic response by nearly 50 % versus control. These results position AVP analogs as potential and interesting angiostatic agents, with the ability to modulate aggressiveness for CRPC, a disease with few therapeutic alternatives.

0392 - DOWNREGULATION OF MUSCARINIC RECEPTORS GENE EXPRESSION IN HUMAN BREAST CANCER CELLS REGULATES ANCHORAGE-INDEPENDENT CELL GROWTH IN VITRO AND ANGIOGENESIS IN VIVO.

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Muscarinic receptors (M) expression, activation and signalling play important roles in regulating many cellular process and cancer progression. It has been reported that human breast cancer MCF-7 cells express muscarinic receptors M3 and M4 subtypes and its activation promotes tumoral progression. We previously reported that the silencing of both M3 and M4 in MCF-7 cells significantly reduced neovascularization capacity of tumoral cells in vivo. The aim of this work was to evaluate the specific contribution of each M receptor on different tumoral progression parameters like anchorage-independent cell growth and angiogenesis in vivo. Here, we silenced M3 or M4 subtypes in MCF-7 cells by specific RNAi. After 5 days we used the different experimental groups (siM3, siM4 and MCF-7 cells with and without carbachol (Carb, -8M)) in the following assays. Briefly, for soft agar colony assay we seeded $2X10^4$ cells of each group into medium with soft agar. After 2 weeks, the colonies larger than 60 µm in diameter were counted. We observed that cholinergic stimulation of siM cells showed a significant reduction in colony number when compared with MCF-7+Carb, however this effect was greater in siM3 cells than in siM4 cells (siM3: 99.97 ± 9.50 %, siM4: 289.4 ± 5.3 % vs. MCF-7: 509.1 ± 11.8 %; p<0.0001). Angiogenesis was measured by inoculation of $2x10^5$ cells in female nude mice. After 5 days, the animals were sacrificed and angiogenesis was quantified in the sites of inoculation as vessel density. We found that silencing of both M receptors decreased the neovascular response in vivo of siM cells treated with Carb compared with MCF-7+Carb (siM3: 3.6 ± 0.1, siM4: 3.7 ± 0.3 vs. MCF-7: 6.4 ± 0.7; p<0.0001). According to our results, M receptors expression downregulation can modulate the malignant phenotype of MCF-7 cells, having a high inhibitory effect on anchorage-independent cell growth and angiogenesis.

0686 - ADENYLATE CYCLASE GENES ARE EXPRESSED IN BASAL CELL CARCINOMA AND NORMAL SURROUNDING SKIN OF NEVOID BASAL CELL CARCINOMA SYNDROME (NBCCS) PATIENTS

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NBCCS – also known as Gorlin-Goltz syndrome – is an autosomal dominant entity caused mainly by mutations in the PTCH1 gene. NBCCS is characterized by multiple basal cell carcinoma (BCC) development due to the Hedgehog (HH) pathway hyperactivation. We have previously described that the genes encoding components of the HH pathway are overexpressed in BCC and phenotypically normal skin of these patients (Martinez MF, et al. Cells, 2019). Taking into account that the HH pathway can be inhibited through proteolysis of its effectors by a cAMP-driven process that involved protein kinase A, we looked for the expression profile of the nine adenylate cyclase genes (ADCY 1 to 9). We performed quantitative RT-PCR in BCC and normal surrounding tissue (NST) of 4 NBCCS patients with PTCH1 mutations, and 3 control skin samples (CSS). We failed to detect ADCY6 mRNA in any tested samples. ADCY8 is only expressed in BCCs and the remaining ADCY genes are expressed in BCCs and NST of NBCCS patients. Any adenylate cyclase genes were expressed in the CSS. Additionally, we found a 2-fold increase in ADCY1 and a 10-fold decrease in ADCY5 mRNA levels in BCC compared to NST (p<0.05). These results reveal that adenylate cyclases are involved in NBCCS and suggest that the gene expression levels of cAMP pathway components could be modified directly or indirectly by the HH pathway hyperactivation. Our finding can improve the knowledge of phosphodiesterase inhibitors mechanism, another component of the cAMP pathway, in the treatment of BCCs and also be the initial study to delineate new ones.

0703 - HO1 PLAYS AN IMPORTANT ROLE IN IRON METABOLISM ALTERATION IN BREAST CANCER CELLS

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Heme Oxygenase-1 (HO1) catalyzes heme degradation, yielding biliverdin, carbon monoxide and iron. When iron is in excess produces oxidative stress through reactive oxygen species (ROS) generation. Since both HO1 and iron metabolism disruptions have been related to breast cancer progression, we sought to investigate how tumor cells regulate iron metabolism when HO-1 expression is altered. For this purpose, we first investigated the correlation of HO1 with several iron proteins by using in silico analyses and corroborated the strongest hits by using immunohistochemistry (IHC) performed on human biopsies (n= 33). In addition, a syngeneic model of LM3 and a xenograft model of MDA-MB-231 cells stably overexpressing HO1 were used to study these hits. We further performed in culture analyses using LM3 breast cancer cells treated with hemin (H), vehicle or the combination with an antioxidant, and studied iron storage (Prussian Blue), ROS levels (DFCA) and cell cycle progression (flow cytometry). In silico analyses showed that HO1 correlated with DMT1 (p= 9.8e-05), ZIP14 (p= 4.2e-06), Prohepcidin (p= 1.4e-12) and L-ferritin (p= 2.2e-16). In order to study the correlation between HO1 and DMT1 in breast cancer we analyzed by IHC their expression in biopsies. We observed an inverse correlation between DMT1 and HO1 expression (p<0.05). The IHC studies showed an increase in ZIP14 and prohepcidin expression and a slight decrease in L-ferritin and DMT1 expression in hemin-treated and HO1-overexpressing cells in both animal models. In culture studies showed that the iron storage was increased in hemin- treated LM3 cells and was associated to a decrease in cell viability (p<0.05), an increase in the apoptotic rate (p<0.05) and high ROS levels (p<0.01). NAC treatment reverted the apoptotic effect of H (p<0.05). Altogether these results indicate that HO1 induction plays a role in carcinogenesis through free iron accumulation, ROS production and oxidative stress.

0716 - BONE MARROW DERIVED MONOCYTES MEDIATE THE DELIVERY OF CONJUGATED POLYMER NANOPARTICLES IN A PLECLINICAL GLIOBLASTOMA ORTHOTOPIC MODEL

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Photodynamic therapy (PDT) has recently gain attention as alternative treatment of glioblastoma (GBM). Due to their superb light absorption and photostability, conjugated polymer nanoparticles (CPNs) are promising photosensitizers in PDT. However, GBM represent a challenge to current treatments due to the preferential location within Central Nervous System and the presence of the blood-brain barrier (BBB) hindering the arrival and accumulation of drugs into the tumor upon systemic administration. Trojan horse therapy, using cells with homing

capabilities for GBM, was explored to facilitate the arrival of chemotherapeutic prodrugs; but to the best of our knowledge there are no reports on the preparation of CNPs-loaded monocytes and its evaluation in cellular delivery. We hypothesize that bone marrow derived monocytes (BMDMs) incorporate CPNs without affecting cell functionality and cross BBB to reach GBM, as stealth carriers. To this end, we isolated BMDMs from C57BL/6 mice using conditioned medium (CM) with M-CSF from L929 cells. The identity of monocytes (more than 90 % cells) was confirmed by double staining with anti-CD11b and anti-F4/80 after 5 days of proliferation with CM. CPNs uptake by BMDMs was assayed taking advantage of the intrinsic fluorescence of CPNs using flow cytometry. The percentage of CPNs-loaded BMDMs increased over time (68.5 ± 1.1 % at 24 h) and the amount of CPNs per cell, measure as fluorescence intensity (MFI), also increased over time ($p<0.0001$). GBM orthotopic model was developed injecting GL261 cells into C57BL/6 mice. After 14 days, BBB disruption was confirmed by gadolinium T1-enhanced MRI. Once CPNs-loaded BMDMs (2 x 10^6 cells, n= 6) were injected intravenously, pharmacokinetics and tumor arrival were monitored by in vivo imaging system (IVIS) up to 48 h. The MFI of CPNs increased in tumors injected with loaded BMDMs compared with those injected with saline. Taking advantage of monocytes tropism induced by GMB cells we demonstrated that BMDMs can effectively deliver CPNs into GBM in vivo.

0721 - HYPOXIA-INDUCED AUTOPHAGY INCREASED RESISTANCE TO VEMURAFENIB TREATMENT ON SENSITIVE, BUT NOT RESISTANT MELANOMA CELLS

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Melanoma is the most aggressive type of skin cancer. Although recent therapies have shown an impressive success, unfortunately patients develop resistance after a short period of disease control. Autophagy has been indicated as a mechanism of resistance in tumor cells. Autophagy is a process regulated by numerous factors, including hypoxia, a distinctive feature of the tumor microenvironment. Our hypothesis is that hypoxia or prolonged treatment with Vemurafenib (Vf, BRAF inhibitor) augment autophagy that in turn further increases Vf resistance. To this end, we cultured Lu1205 human melanoma cells with or without $CoCl_2$ during 24h to induce hypoxic condition that was confirmed by HIF-1α; expression. Certainly, $CoCl_2$ treatment increased LC3BII level, a marker of autophagy, but it did not affect Lu1205 cell viability. To investigate the role of hypoxia in Vf resistance, Lu1205 cells were cultured in presence or absence of $CoCl_2$ during 24h and then treated with Vf for another 24h. As assessed by MTT assay, cells that were pre-treated with CoCl2 were more resistant to Vf (5-10 µM). Interestingly, the inhibition of autophagy with chloroquine or NH_4Cl diminished this resistance. Hypoxia did not modify the ability of Vf to reduce pERK1/2 but decreased the pAKT levels induced by the inhibitor. Moreover, Lu1205 cells with acquired resistance to Vf generated in our lab showed increased survival and higher levels of LC3BII and pERK1/2 than sensitive cells when were treated with Vf. On the other hand, treatment of Vf resistant cells with $CoCl_2$ did not induce significant changes in LC3BII, pERK1/2 and pAKT levels. Altogether, these results suggest that hypoxia promotes a pre-resistant cell state with increased autophagy that enhances the develop of Vf resistance. Moreover, this mechanism is exacerbated in cells with acquired Vf resistance by long period treatment.

0722 - POTENTIAL ANTI-MELANOMA EFFECTS OF TERPENES DERIVED FROM THE ESSENTIAL OIL OF ORIGANUM VULGARE

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In recent years, there has been a growing interest in the biological activities of plants essential oils. Origanum vulgare L. species, belongs to the Lamiaceae family and is distributed in Europe, Mediterranean Basin and Asia and are mainly used for gastronomic purposes. The objective of this study was to evaluate the anti-melanoma action of Oreganum vulgare essential oils (OvEO) and the major terpenes that derive from it. In order to investigate the potential anticancer effects of OvEO in vivo, mice were injected subcutaneously with 1×10^6 B16 melanoma cells in the right flank. At 11 day, the mice were treated intratumorally with 5 % OvEO in isotonic saline solution. OvEO inhibited tumor growth showing a reduction of 35-40% for tumor size on days 15, 17 and 19, which revealed a significant anti-melanoma potential. Then, we analysed the chemical composition of OvEO by gas chromatography and Ý-Terpinene (13.7 %) and Terpinen-4-ol (11.2 %) were the main compounds. In vitro antiproliferative activity of these terpenes was assayed on culture of A375 and Lu1205, melanoma cells with BRAF V600E mutation, by MTT assay. Terpinen-4-ol showed anti-proliferative properties at 0.05% concentration and induced death rate between 50-60% at concentrations of 0.1 % on the several melanoma cells tested but did not decrease the elevated pERK1/2 levels on these cells. On the contrary, Ý-Terpinene did not show activity at these concentrations. On the other hand, the co-treatment with 0.05 % Terpinen-4-ol increased the BRAF inhibition-induced cell death of Vemurafenib (Vf). Moreover, this terpene diminished the viability of A375 and Lu1205 Vf-resistant cells, which indicated that Terpinen-4-ol could improve the treatment of these types of melanomas that do not respond to therapy with BRAF inhibitors. Altogether, these results suggest that OvEO has anti-tumor activity and Terpinen-4-ol could be an important active principle of this essential oil to induce the death of tumor cells.

0738 - IGF-1R NUCLEAR LOCALIZATION IN PAEDIATRIC GLIOBLASTOMA: PHENOTYPIC CHARACTERIZATION AND USE OF IGF-1R/IR INHIBITOR OSI906 AS A TARGETED THERAPY

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In gliomas, the most frequent solid tumors in children, IGF1R nuclear localization is associated with high grade and increased risk of death. Chemotherapy after surgical resection is the mainstay of therapy. However, the best regimen needs to be determined. Aim: To characterize the impact of IGF1R nuclear localization in glioblastoma cells and the response to treatment with OSI906 (IGF1R/IR dual inhibitor) alone or in combination with Temozolomide (TMZ), a current adjuvant therapy in children. U87Mg cells were used to obtain clones expressing wild type GFP-IGF1R (WtU87) or GFP-IGF1R1025X1100X1120X to avoid IGF1R nuclear translocation (MutU87). Proliferation, wounding assays and qPCR were carried out with/without 50 nM IGF1. Cells were cultured in complete media (10% FBS) with addition of OSI906 (0.5 uM), TMZ (40 or 100 uM) or the combination of drugs. Nude mice were injected sc with WtU87 or MutU87 cells. When tumors reached 150 mm^3, OSI906 (25mg/kg, 3d) or TMZ (250mg/kg, single dose) were given by gavage. IGF1 had no effect on WtU87 or

MutU87cells proliferation or apoptosis after 5 days of culture. On the contrary, IGF1 stimulation increased motility, GLUT-1 & FASN expression, LDH activity and PDHc activation in WtU87compared to MutU87cells ($p<0.05$). All effects were blocked by preincubation with OSI906. Proliferation of WtU87 and MutU87cells decreased under TMZ40 or OSI906, and TMZ40+OSI906 had an additive effect only in WtU87 ($p<0.02$). TMZ100 had a strong inhibitory effect on both cell lines ($p<0.001$). In vivo studies showed similar trends. IGF1R nuclear translocation contributes to glioblastoma aggressiveness by increasing motility and metabolism of tumor cells. It also renders glioblastoma cells sensitive to IGF1R targeted therapy alone or in combination with TMZ, in vitro and in vivo. These results suggest that the use of IGF1R inhibitors in patients with nuclear localization for IGF1R, could be useful to reduce TMZ doses in children.

0777 - INHIBITION OF ESTABLISHED METASTASES GENERATED BY A MURINE MELANOMA AND A MURINE FIBROSARCOMA BY TREATMENT WITH META-TYROSINE

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CEDIE

Concomitant tumor resistance (CR) is the phenomenon according to which a tumor-bearing host inhibits or retards the growth of secondary tumor implants. The importance of the study of CR relies on its relevance to elucidate mechanisms of metastases control since metastases may be considered natural secondary tumor implants. Tyrosine isomers, in particular meta-tyrosine (m-Tyr), have been claimed to be mediators of CR and in previous reports we demonstrated that m-Tyr could inhibit the growth of established metastases from three murine metastatic mammary carcinomas. In this communication we evaluated whether this anti-metastatic effect could be extended to other murine tumors very refractory to other treatments: a highly metastatic spontaneous melanoma (B16) and a highly metastatic methylcholanthrene-induced fibrosarcoma (MC-HM). Both tumors growing subcutaneously generate lung metastases starting at day 15 after tumor inoculation with 1×10^5 cells. Mice bearing B16 or MC-HM tumors were divided into two groups at 18 days after tumor inoculation. One group (experimental group) received a daily inoculation of m-Tyr (67 mg/Kg/day) by the intravenous route for the following 21 days and the other group (control) received saline. After that time, all mice were sacrificed and lung metastases counted. Metastases for B16 (median [range]): Experimental group: 14 [0 - 64], (n= 12). Control group: 43 [5 -206], (n= 11); $p<0.02$. Metastases for MC-HM (median [range]: Experimental group: 4 [0 - 33], (n= 6). Control group: 54 [8-100], (n= 7); $p<0.01$. In both tumor models, when total metastatic load (number of metastases x individual size) were compared the difference was strikingly greater than that observed by comparing number of metastases only. Our results demonstrated that m-Tyr can inhibit the growth of established metastases of very refractory tumors.

0779 - EFFECT OF PLATELETS IN THE AGGREGATION OF HUMAN MELANOMA CELL LINES

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Malignant melanoma is one of the most aggressive cancers and can disseminate from a relatively small primary tumor and metastasize to multiple sites. Hematogenic tumor cell dissemination is one of the main causes of death in patients with malignant melanoma. Although several studies had linked platelets to this process in different malignancies, the exact role of platelets remained controversial. Therefore, the aim of our work was to study the effect of platelets in the aggregation of human melanoma cell lines. First, we evaluated the platelet rich plasma (PRP) cytotoxic effect on tumor cells by the acidic phosphatase assay (APH) on 5-days melanoma and PRP cocultures. We found that viability of melanoma cells was not altered by PRP addition in most cell lines, while others presented increased viability. Next, we focused on the effect of PRP in the aggregation of cells. To elucidate this, PRP was added to non-attached cells of melanoma in 3D culture conditions, at two different times of the spheroid formation. The kinetics of the interaction was followed by optical microscopy up to 7 days. The addition of PRP at the beginning of spheroid formation was responsible of the acceleration and solid compaction of spheroids on all melanoma cell lines tested. However, on already formed spheroids, PRP only helped hM1 and hM10 cell lines, which usually do not form 3D structures, to form spheroids. Finally, we aim to discriminate whether the modulation by PRP was due to platelets presence or soluble mediators. Thus, we co-incubated melanoma cells with PRP, platelet poor plasma (PPP) or washed platelets on 3D culture conditions and followed the cultures as described before. Our results showed that PPP was not able to improve spheroid formation while washed platelets and PRP enhanced the aggregation and compaction of melanoma multiple cell spheroids since the first day of interaction. In summary, our results demonstrate that platelets are able to modulate the aggregation of human melanoma cell lines in vitro.

0782 - DIFFERENTIAL EXPRESSION OF CPB1 IN BREAST CANCER MODELS WITH DIFFERENT LEVELS OF PROGESTERONE RECEPTOR ISOFORMS

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Seventy percent of breast cancers are susceptible to an endocrine therapy currently aimed to target the estrogen receptor alpha. We have recently shown that breast cancer tissue cultures with higher levels of progesterone receptor (PR) isoform A (PRA) than isoform B (PRB) were inhibited by the antiprogestin mifepristone. This highlights the relevance of identifying patients that may benefit from this therapy. An RNA-seq study revealed 139 genes that were differentially expressed in 14 patients with higher levels of PRA than PRB (PRA-H) as compared with those with the opposite ratio (PRB-H). In this analysis, we focused in CLEC3A and SCGB1D2 genes upregulated in PRA-H and CPB1 upregulated in the PRB-H tumors. We validated the expression of the three genes using real time PCR (qPCR) using human breast cancer samples and we only found significant values with CPB1. The aim of the study was to further investigate the expression of CPB1 in human and murine breast cancer models with different PR isoform ratios. We used two PRA-H tumors: C4-HD and C4-HI and two PRB-H tumors: C42-HI and C4-HIR from the murine medroxyprogesterone acetate -induced breast cancer model. We evaluated the expression of CPB1 by qPCR and by immunohistochemistry (IHC). CPB1 was upregulated in both PRB-H tumors as compared with the two PRA-H tumors at the mRNA level (qPCR; $p<0.05$) and at the protein level (IHC; $p<0.05$). CPB1 was mainly localized in the cytosol of PRB-H tumor cells. Preliminary studies using the human MDA-iPRAB cells suggest a similar trend ($p= 0.07$) when comparing tumors induced to express PRA or PRB. Since CPB1 is a known useful serum marker in acute pancreatitis, studies are underway to explore if it can be used as a follow up marker in breast cancer patients. We conclude that CPB1 might be a candidate gene used to discriminate PRA-H and PRB-H tumors.

0797 - 4-METHYLUMBELLIFERONE PROMOTES AN ANTITUMOR IMMUNE RESPONSE BY IMPROVING THE RECOGNITION OF CANCER STEM CELLS AND MODULATING THE PROFILE OF ANTIGEN PRESENTING CELLS.

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Tumor microenvironment involves the interaction of different components including tumor cells, cancer stem cells (CSCs) and immune cells, such as macrophages (Mø) and dendritic cells (DCs). These interactions could promote tumor immune escape, growth and dissemination. Previously, we demonstrated that 4-Methylumbelliferone (4Mu) decreases the expression of the "not eat me" molecule CD47 and facilitates phagocytosis of CSCs by Mø, supporting an antitumoral response in a murine model of hepatocellular carcinoma (HCC). The aims of this work were to evaluate whether the effects that 4Mu exerted on CSCs also enhance phagocytosis by DCs, analyze the role of immune response in the antitumoral efficacy observed in tumors treated with 4Mu, and study changes in the Mø profile. First, we performed a phagocytosis test using bone marrow derived-DCs from C3H mice. As a result, Hepa129 cells treated with 4Mu were more phagocytated by DCs than non-treated cells ($p<0.05$). Then, we used immunosuppressed or immunocompetent mice and inoculated them with 4Mu treated-Hepa129 CD133+ cells. Tumor grew in immunosuppressed mice while there was a significant delay in tumor progression in immunocompetent mice. In addition, we pulsed DCs with 4Mu treated Hepa 129 cells (DCs/Hepa/4Mu) and inoculated them in C3H mice with established s.c tumors. Mice received DCs/Hepa/4Mu showed a significant decrease in tumor growth when compared to DCs/Hepa mice ($p<0.05$). We confirmed the results using BALB/c mice and BNL cells (hepato-cholangio cell line). Finally, we analyzed the Mø profile. Mice with liver fibrosis were inoculated orthotopically with 1.25×10^5 Hepa 129 cells (day 0). On day 5, animals received saline or 4Mu orally (200 mg/kg). On day 9, we observed by flow cytometry and qPCR that intratumoral, peritumoral and non-tumoral Mø showed an M1 type profile when mice were treated with 4Mu. We suggest that the ability of 4Mu to promote an antitumor immunity was based on the stimulation of CSCs recognition, and modulation of the immune profile of antigen presenting cells.

0799 - METFORMIN IN COMBINATION WITH GLUCOSE METABOLISM INHIBITORS PROMOTES AUTOPHAGY AND APOPTOSIS IN CANINE AND FELINE MELANOMA CELLS

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We have previously demonstrated that Metformin (MET), the most wideworld prescribed hypoglycemic drug, has cytotoxic effects on different melanoma cell lines. Furthermore, we described that not only the inhibition of the glycolytic but also the pentose phosphate pathway (PPP) potentiates the effect of MET. The aim of the present work was to study the mechanism involved in MET effectiveness alone or in combination with 2-deoxyglucose (2DG, HK inhibitor, first and limiting enzyme of glycolysis) and 6-aminonicotinamide (6AN, G6PD inhibitor, the main and limiting enzyme of the PPP) on two melanoma cell lines, Sc (canine) and Dc (feline) derived from spontaneous tumors. In order to confirm the autophagic nature of the acridine orange positive vesicles (previously described by our group), Sc and Dc cells were lipofected with a plasmidic construction containing LC3B-RFP. After 48 h of treatment, we found that MET, MET-2DG and MET-6AN resulted in a cytoplasmic accumulation of LC3B-RFP protein also confirmed by immunofluorescence. In addition, MET-6AN autophagic vesicles were inhibited by chloroquine (an autophagy inhibitor) ($p<0.05$). Since MET inhibits the mitochondrial complex I, we found an increased in glucose consumption and lactate production in Sc ($p<0.01$), whereas MET-6AN increased glucose consumption in Dc ($p<0.05$) probably as compensatory mechanism. Next, by means of a double staining with acridine orange/ethidium bromide (AO/EB) we found that, after 48 h of MET-2DG and MET-6AN treatments, both melanoma cells increased the number of late apoptotic/necrotic events. Finally, we found that MET-2DG induced G0/G1 whereas MET-6AN induced G2/M phase cell cycle arrest in both cell lines after 48 h of treatment ($p<0.01$ and $p<0.05$, respectively). These studies suggest that the efficacy of metformin in combination with glucose metabolism inhibitors involves not only autophagy but also apoptotic mechanism.

0801 - INCREASING EVIDENCE ABOUT THE CROSSTALK BETWEEN WNT/B-CATENIN AND NOTCH SYSTEM IN OVARIAN CANCER

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Wnt/β-catenin and Notch interact in different physiological processes. Both signalling pathways are now known as highly related to tumor biology by regulating hallmarks like proliferation, apoptosis, resistance and angiogenesis among others. We analyzed the role of both systems in ovarian cancer using specific inhibitors. For this purpose, we performed an in vivo experiment. A human ovarian adenocarcinoma cell line (IGROV-1) was subcutaneously injected in 6-8 weeks-old female nude mice. Once the tumours were palpable, we injected a Wnt/β-catenin inhibitor, ICG-001 (5 mg/kg) and a Notch inhibitor, DAPT (5 mg/kg). Mice were injected every two days three times and they were euthanized 3 days after the last injection. Our results showed a significant decrease in tumour size when mice were treated with ICG-001 or DAPT compared to non-treated animals. The co-treatment produced no changes respect to the individual ones. Similar results were obtained for Ki67 proliferation marker and ciclin D1 protein. Regarding angiogenesis, we detected a decrease in endothelial cell area in DAPT and ICG-001+DAPT treated tumors, as well as in periendothelial cell area. However, HIF1α; did not change between treatments. Nrarp, a downstream effector of Notch signalling, decreased significantly in all treatments compared to control animals but without differences between them. Also, Hes-1 Notch ligand had the same profile as Nrarp. By these means, we demonstrated that Wnt/β-catenin as well as Notch inhibitor produced antiproliferative and antiangiogenic effects. When simultaneously administered, they maintained the same effects but these were not potentiated. In conclusion, Wnt/β-catenin and Notch signalling interact somehow in ovarian tumour growth and angiogenesis but the mechanism of the interaction remains to be elucidated.

0823 - A HIGH THROUGHPUT APPROACH TO DELINEATE PROTEIN COMPLEXES IN FORMALIN-FIXED PARAFFIN-EMBEDDED PROSTATE ADENOCARCINOMA AND BENIGN PROSTATIC HYPERPLASIA TISSUES

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Prostate cancer (PCa) is a progressive disease involving multiple gene alterations; however, little is known at the proteome level. Most of the functional information of the cancer-associated genes relies in the proteome, a complex biological system with dynamic interactions. To identify differential protein complexes associated with PCa we carried out an in-depth proteomics analysis. PCa and

benign prostatic hyperplasia (BPH) samples were obtained from the Hospital de Clínicas "José de San Martín" (with written informed consent and institutional review board approval). Proteins were obtained using phase-transfer surfactant-aided extraction/tryptic digestion of formalin-fixed, paraffin-embedded (FFPE) tissue sections mounted on microscope slides. Samples were subjected to mass spectrometry analysis (ESI-MS/MS). We found 109 proteins enriched in PCa compared with BPH samples. To identify protein complexes differentially expressed we used CORUM, a database of human protein complexes. Utilizing biological complexes as a cluster vector, we calculated a proteomics signature profile for each sample based on the hit rates of their reported proteins, in the absence of fold change thresholds, against the cluster vector. We identified the following differentially expressed protein complexes in PCa compared with BPH ($p<0.05$): Septin, MLL1, MLL2, Set1A, Set1B. Gene ontology analysis revealed that PCa enriched complexes were associated with histone methylation, regulation of transcription, chromatin organization and actin filament reorganization involved in cell cycle. String database was used to build the protein interaction models. Results showed that two of these PCa protein complexes (MLL and SET1) directly interact with the androgen receptor (AR). Furthermore, our data unveiled the septin complex as a potential interactor of HSP90, an AR activator. In summary, we identified distinct biological complexes in PCa, that may act as novel targets for innovative therapeutic interventions.

0824 - ROLE OF TGF-BETA; PERK AND T3 IN THE DEVELOPMENT OF HEPATOCARCINOMA

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Hepatocarcinoma is the most frequent liver tumor. Hexachlorobenzene (HCB) is a dioxin-type cpmpound that promotes the preneoplastic foci formation. We shown that HCB alters thyroid hormones, TGF-β levels in vivo and deregulates cell growth in Hep-G2. Endocrine deregulation would be an important process in hepatocarcinogenesis. In the present work our objective was to evaluate key mechanisms of action in the HCC development in pre-neoplastic stages using HCB as a tumor promoter. We use in vivo, an I/P model [diethylnitrosamine (DEN) 100 mg/kg) / HCB (100 mg/kg)] in rat liver and a nude mouse model treated with HCB i.p. (0.3 and 3 mg/kg) and inoculated with Hep-G2. We evaluate: a) PCNA (Western blot (W)); b) TGF-β (RT-PCR, W) c) T3 tissue concentration (RIA). In nude mice skin angiogenesis. In vitro, in Hep-G2 cell line: a) role of TGF-β (W, RT-PCR) using a specific inhibitor and b) role T3 (10^{-7} M), in the effect of HCB (5 μM) on PCNA and TGF-β. In EA-hy926 cell line the effect of HCB and the conditioned medium (MC) of the HCB (5 μM) Hep-G2 treated a) cellular migration b) tubulogenesis and c) PCNA. In the effect of HCB on PCNA a) role of pERK kinase b) role of TGF-β and c) T3 (10^{-7} M) with specific inhibitors and exogenous T3. In the I/P model, PCNA increased (33 %, $p<0.01$), TGF-β (33 %, $p<0.01$) and decreased T3 (39 %, $p<0.01$). In Hep-G2, PCNA increase (25 %, $p<0.05$) with HCB and not varied with HCB/I/TGFβ. TGF-β and PCNA did not alter when T3/HCB administering. In EA-hy926 increase PCNA and cell migration with HepG2 MC/HCB (31 %, $p<0.01$; 26 %, $p<0.05$). These parameters did not alter when pretreating with I-ERKp. PCNA did not alter with HCB/T3 (10^{-7} M) or HCB/InhTGF-β. In nude mice with HCB (3mg/kg) vascularity increased (32 %, $p<0.01$). We conclude that in the models of in vitro hepatocarcinogenesis, inhibitors of TGF-β and pERK or exogenous T3 reverse the proliferative and cell migration stimuli promoted by HCB, demonstrating their critical role in this process.

0835 - IMPLEMENTATION OF BETA RADIATION EMITTING DEVICES (BE) AS A COMPLEMENTARY TOOL TO BORON NEUTRON CAPTURE THERAPY (BNCT) FOR THE TREATMENT OF SUPERFICIAL CANCER

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The BNCT clinical trials in Argentina started in 2015 after changes in the beam in the RA-6 reactor. BNCT is based on the nuclear reaction 10B (n, alpha) 7Li. Due to the characteristics of the neutron beam, the maximum dose is not on the surface of the tumor, but at 1 cm deep. Some materials (like Rhodium, Indium and Silver) have a high effective neutron capture section, rapid decay activation products and high energy beta particles emission. Since beta radiation has a short range of tissue penetration, these devices called Beta Enhancers (BE) can be used to compensate for the BNCT surface dose gradient or even to significantly increase it. In this work we analyze in vivo the therapeutic efficacy of the different types of BE and the advantages of adding them to BNCT therapy. Materials and Methods: NIH nude mice were implanted with cells from the HT-29 colon cancer human cell line, developing tumors at day 15. The animals were divided into 5 groups Control, BNCT, BNCT-Rh, BNCT-Ag and BNCT-In. The mice were irradiated 45 minutes with a neutron flux of 4.96×10^8 n/cm^{2}sec. Animal monitoring after irradiation does not show any signs of radiotoxicity. Tumor growth decreased in all the groups treated by BNCT. Histological studies had a correlation between the area of tumor necrosis and the total physical dose absorbed (between 7 and 9 Gy). A smaller amount of cancer stem cells (CSC) CD133+ was observed in all the BNCT groups compared with the control ($p<0.01$) after three weeks of treatment. Among the BNCT groups, the persistence of CSC was lower in the BNCT-Rh group. All the BNCT treatments showed an efficacy in controlling tumor growth post irradiation during a month. However, the BE Rh exhibited a smaller number of CSC CD133+ at 21 days post treatment, indicating an advantage over the others treatments.

0847 - PHARMACOLOGICAL INHIBITION OF JUMONJI HISTONE DEMETHYLASES ALLOWS TO OVERCOME RADIATION RESISTANCE IN NON-SMALL CELL LUNG CANCER.

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Toxicity from and resistance to ionizing radiation therapy constitutes a major obstacle to curative treatments for non-small cell lung cancer (NSCLC). Regimens for radiation therapy are often limited by toxicity to normal tissues and the development of resistance. Thus, strategies to reduce the total amount of ionizing radiation (IR) used are required. IR results in a wide variety of chromosomal DNA damage including DSB. Epigenetics refers to a set of mechanisms that regulate chromatin accessibility and therefore DNA-Based process such as DNA repair. Particularly, Jumonji (JmjC) histone lysine demethylases (KDM) play roles in DNA repair pathways. Our aim is to study if pharmacological inhibiton of JmjC could be used as a targeted therapy to radiosensitize NSCLC. Liquid colony formation assay was performed to determine IC50 of JIB-04, a JmjC pan-inhibitor, and radioresponse curves in Human NSCLCs cell lines (H1299, A549, HCC95 and HCC1719) and immortalized non-cancerous human bronchial epithelial cells (HBEC3KT and HBEC30KT). For in vivo experiments NSCLC cells were injected subcutaneously (H1299 and A549) into the right posterior leg of female athymic nude mice. Mice were treated for a total of 12 doses EOD with JIB-04 (50 mg/kg/day) by oral gavage or with vehicle; radiation was administered 4 hours after treatment. Tumor

growth delay, survival and the dose enhancement factor (DEF) were then determined. Pharmacological inhibition of JmjC KDM using JIB-04 resulted in a strong sensitization of radio-resistant NSCLC (H1299, A549, HCC95) ($p<0.001$) but not radio-sensitive NSCLC (HCC1719) to radiation. In addition, we found that JIB-04 does not radiosensitize normal cells (HBEC3KT and HBEC30KT). In vivo, treatment with JIB-04 plus IR inhibit tumor growth compared with control mice and either treatment alone ($p<0.001$, DEF>6). Even more, mice treated with JIB-04 and IR survived significantly longer than mice treated with either agent alone or with vehicle even after the end of treatment. In conclusion, our study suggests that the epigenetic inhibitor JIB-04 could help to overcome radioresistance both in vitro and in vivo.

0855 - EVALUATION OF CIRCULATING LYMPHOCYTES SUBPOPULATIONS DURING THE GROWTH OF M-406 TRIPLE NEGATIVE MURINE MAMMARY TUMOR

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Inbred mice models provide an interesting tool for identifying factors that control susceptibility to breast cancer. M-406 mammary adenocarcinoma appeared in an inbred CBi mouse. CBi- mice were artificially selected from CBi. Cells of the immune system play an important role in tumor development. In order to determine their participation on tumor growth in genetically different hosts, CBi, CBi- and F1 reciprocal hybrids (F1A: CBi x CBi- and F1B: CBi- x CBi) were s.c. challenged with M-406, tumors were measured, and blood samples were taken on days 0, 7 and 14 in CBi and F1 and on days 0, 5, 8 and 12 in CBi- mice. Circulating CD4+, CD8+, Treg and Th17 cells were quantified (flow cytometry). Tumors grew exponentially in 100% of CBi (susceptible) and F1 female and male mice. However, in CBi- (resistant) after a short period of growth, reaching the maximum size on day 8 (female) and 12 (male), 100 % of tumors were rejected. CBi, F1A and F1B mice, did not differ in tumor volume doubling time (TVDT) for both sexes, while in CBi-, TVDT in males was higher than in females ($p<0.05$). We determined the ratio CD8+/Treg in CBi males: day 0 > day 14; ($P<0.05$); CBi females: day 0 day 12 ($p<0.01$) without differences in CBi- females; F1A males and females: day 7 > day 14 ($p<0.0001$; $p<0.001$, respectively); F1B: without differences between days or sexes. Conclusions: 1) The susceptible phenotype is dominant over the resistant. 2) CD4+ and Th17 lymphocytes could not explain tumor growth/rejection behavior in genetically different hosts. 3) CBi males and females utilize different antitumor immune mechanisms leading to tumor escape and growth, without modifying tumor growth rate. 4) The decrease in CD8+/Tregs ratio in CBi- males could be partly responsible for the observed delay in tumor growth. 5) The similar values in CD8+/Tregs ratios for F1A and F1B (males and females) could explain, in part, the absence of differences in tumor growth rate.

0865 - ADRENERGIC RECEPTORS IN BREAST CANCER: DIFFERENTIAL EFFECTS OF ALPHA 2A AND 2C-ADRENERGIC RECEPTOR EXPRESSION ON TAMOXIFEN SENSITIVITY IN STABLY TRANSFECTED LUMINAL MCF-7 CELLS.

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Breast cancer is the most frequently diagnosed and leading cause of cancer death among women worldwide. Epinephrine and norepinephrine, released during stress, bind to 9 different adrenoceptors. Our group has already described (SAIC 2015, poster 660) by in silico analysis in a great database that patients with high expression of Alpha2A-adrenoceptors (A2A-AR) have better disease-free survival than those with lower expression, mainly in luminal tamoxifen-treated ones. Contrarily, a high expression of Alpha2C-AR was associated with worse outcome in luminal B but not in luminal A patients. The aim of the present work was to study the sensibility of tamoxifen on A2A or A2C-AR-overexpressing cells. The human luminal breast cancer MCF-7 cells were stably transfected with A2A or A2C-AR or the empty vector. The expression of A2-AR and Estrogen Receptor Alpha (ER) was measured by RT-qPCR, the sensitivity to tamoxifen by tritiated thymidine incorporation and ER, progesterone receptor and pERK relative to ERK, by Western Blot. They were all performed in the absence of adrenergic stimulation because catecholamines released during stress bind to all receptors and no specific ligand for individual A2-AR exists yet. We successfully over expressed alpha2A and alpha2C on MCF-7 cells: 65 (A2A) and 28 % (A2C) increase compared with empty vector (pCDNA, $p<0.05$ and $p<0.01$, respectively). When analyzing the sensitivity to tamoxifen treatment, the A2A cells exhibited an EC50 of $2.867e^{-}10$ vs. 4.250 e-10 of pCDNA, $p<0.01$; while A2C of $1.202e^{-}9$, $p<0.001$.This was accompanied by a decrease in both cases of ER levels measured by RT-qPCR, $p<0.05$ and WB. A2A cells also showed diminished cell proliferation ($p<0.01$) in the absence of any stimulation when compared with pCDNA and A2C. We suggest that the increase of tamoxifen sensitivity in A2A cells could be due to the combined effect of inhibiting ER expression and cell proliferation.

0871 - 4-METHYLUMBELLIFERONE INDUCES SENESCENCE, INHIBITS MIGRATION AND MODULATES CD44 AS WELL AS RHAMM IN HUMAN GLIOBLASTOMA CELL LINES

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4-methylumbelliferone (4MU) is a non-toxic coumarin derivative used as inhibitor of hyaluronan (HA) synthesis, but there are reports about independent effects of this inhibition. Currently, this drug is being studied on several neoplasms. Nevertheless, little is known about its effects on glioblastoma (GBM), the most frequent and malignant primary tumor of the central nervous system. HA is strongly involved in tumor progression, favoring cell proliferation and migration through its main receptors, CD44 and RHAMM, both associated with poor prognosis. GBM shows higher levels of HA than normal brain tissue. Given that current therapy for this tumor is ineffective and highly toxic, new drugs are needed for GBM treatment. Our hypothesis is that 4MU is a potential new drug for GBM therapy. Therefore, the aim of this work was to evaluate the effects of 4MU on cell proliferation, migration, senescence induction, expression of CD44 and RHAMM, and the receptors involved in HA-induced migration on LN229 and U251 human GBM cell lines. Cell proliferation was evaluated by BrdU incorporation assay, migration by the wound healing assay, senescence by SA-ß-gal assay and expression of receptors by Western blot (WB) and immunofluorescence (IF). We found that 4MU inhibited cell proliferation and migration in a dose-dependent manner in both cell lines ($p<0.05$). These effects were not prevented by the co-treatment with HA. Besides, 4MU increased the percentage of SA-ß-gal+ cells in a dose-dependent manner in U251 cell line, but in LN229 cells only at the higher dose ($p<0.05$). Furthermore, 4MU modulates the expression of RHAMM and CD44 ($p<0.05$). Regarding the implication of CD44 and RHAMM in HA-induced

migration, we evaluated this process using blocking antibodies which prevented the effect of HA ($p<0.05$). In conclusion, we demonstrated that 4MU inhibited all studied processes involved in GBM malignancy, thus being a promising therapy for GBM.

0883 - IMIQUIMOD TREATMENT OF TRANSFORMED CELLS: NF-KB AND TLR-7/8 SIGNALLING INDEPENDENT DEATH.

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The immunotherapeutic agent imiquimod (IQ), an agonist of the Toll-like receptors (TLR) 7/8, has been reported to be effective in the treatment of several skin pathologies including melanoma and infantile haemangioma. In immune cells, the classic pathway for IQ signalling comprises TLR7 and NF-KB activation. Previously, we have demonstrated that IQ causes cell death, oxidative stress and loss of migratory ability in haemangioma and melanoma cells in vitro. In order to gain insight on IQ signalling mechanism in transformed cells, we studied TLR expression and the involvement of NF-kB in IQ-induced cell death. Murine melanoma B16F-1 and haemangioma H5V cells were treated with IQ (0, 5, 10 and 50 µg/mL) in the presence or absence of an NF-KB inhibitor (BAY 11-7082) during 24 hours. Cell viability was analysed by crystal violet staining and nuclear morphological changes were evaluated by a nuclear morphometric analysis (NMA) with ImageJ on Höescht 33258-stained nuclei. TLR-7/8 expression was assayed by RT-qPCR. Both H5V and B16F-1 cells suffered loss of viability (circa 50 %) at IQ 10 µg/mL but inhibition of NF-KB did not modify cell death levels. Likewise, NMA showed an increased number of small and regular nuclei (50-60 %, $p<0.05$) at IQ 10-50 µg/mL associated to apoptotic cells. The percentage was similar either with or without BAY 11-7082 treatment. In addition, after incubation with IQ+BAY, a slight tendency to the appearance of large regular nuclei, compatible with senescent cells, was detected in both cell lines accompanied by cytoplasmic vacuolization. With respect to TLR7 expression, low levels were obtained for H5V cells (0.13 ± 0.10) compared to ganglion and resulted undetectable for B16F1, as well as TLR8 expression in both cell lines. Consequently, these results suggest that IQ would be exerting its cytotoxic effect without involving NF-kB and TLR-7/8 signalling.

0893 - ASSESSMENT OF BACULOVIRAL VS ADENOVIRAL VECTORS FOR GENE DELIVERY IN EXPERIMENTAL BRAIN CANCER

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We aimed to compare the transduction efficiency and neuropathology of adenoviral (AdV) vs baculoviral (BV) vectors in order to develop therapeutic strategies for the treatment of brain cancer. Although AdVs can be produced in high titers and yield good transduction efficiency in the brain, the population exhibits pre-existing anti-AdV immunity, leading to transient transgene expression. BVs primarily infect insects at larval stage, but they also transduce cells from other species. Even though BVs are less stable than AdVs after long-term storage, their advantage is that pre-existing immunity against BVs has not been reported in humans. Our general hypothesis is that BVs may lead to more stable transgene expression than AdVs upon injection into naïve and neoplastic brain. We constructed AdV and BV encoding tdTomato under the control of the CMV promoter. Human and rat GB cell lines were incubated with different doses of AdV or BV for 48 h and transduction efficiency was assessed by microscopy. AdV (MOI 50-500) and BV (MOI 500-2000) transduced GB cell lines with similar efficiency. AdV (~10^7 UFP) and BV (~10^6 UFP) were injected by stereotactic surgery into orthotopic GL26 GB growing in the brain of C57Bl/6 mice and 5 d later, mice were perfused/fixed and brains were sectioned in cryostat. We detected comparable expression of tdTomato within tumors injected with either vector. AdV or BV were also injected into the striatum of naïve mice and 5 d later, brains were processed for immunohistochemistry to identify glial cells, showing that transduced brain cells were GFAP+. CD45 staining showed similar immune cell infiltration around BV and AdV injection sites and no signs of neurotoxicity were observed. Our findings indicate that both vectors transduce GB and glial cells with similar efficiency without evident neurotoxicity. Given that humans do not present pre-existing immunity against BVs, BV may constitute a valuable tool for delivery of therapeutic genes in the brain.

0896 - LOOKING FOR DRUG SYNERGY AGAINST CANCER THROUGH POLYAMINE METABOLISM IMPAIRMENT: INSIGHT THE METABOLIC EFFECT OF INDOMETHACIN OVER KRAS-MUTATED LUNG CANCER CELLS.

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Non-small cell lung cancer (NSCLC) is the most lethal and prevalent lung cancer type. Mutations in the Kirsten rat sarcoma viral oncogene homolog (KRAS) gene are present in approximately 25 % of patients with NSCLC. The levels of polyamines (putrescine, spermidine, and spermine) are increased in cancer, being pivotal for tumor proliferation. Indomethacin (INDO) increases the abundance of an enzyme termed spermidine/spermine-N1-acetyltransferase (SSAT, encoded by the SAT1 gene), a key player in the catabolism of polyamines. Consequently, our aim was to investigate the effect of INDO in two NSCLC cell lines, with different KRAS mutation status. A549 and H1299 NSCLC cells (KRAS-mutated and wild-type, respectively) were exposed to INDO. Evaluations included SAT1 expression, SSAT levels and GC/MS metabolomics. Also, levels of polyamine synthesis enzymes and the synergistic effect of INDO and inhibitors of these enzymes were investigated. INDO increased the SAT1 expression and SSAT levels in both cell lines. In A549 cells, INDO significantly reduced the levels of putrescine and spermidine and increase the metabolic features upstream of the polyamine pathway (i.e., ornithine and methionine). However, in H1299 cells, the metabolic impact of INDO was non-significant. Regarding the polyamine synthesis enzyme levels, we found that ornithine decarboxylase (ODC) is increased in A549 cells, whereas S-adenosylmethionine-decarboxylase (AMD1) and polyamine oxidase (PAOX), are increased in H1299 cells. This observation correlated with relative resistance to the drugs DFMO, SAM486, and MDL72527 (inhibitors of ODC, AMD1, and PAOX, respectively). Finally, INDO had synergistic effect with MDL72527 in A549 and with SAM486 in H1299 cells. These results indicate that INDO alters polyamine metabolism in NSCLC cells and enhances the effect of polyamine synthesis inhibitors. However, these effects could vary depending on the genetic background of each NSCLC cell type.

Supported by FONDECYT 1160807 grant.

0917 - EVALUATION OF MUSCARINIC RECEPTORS GENE EDITING BY CRISPR/CAS9 ON MIGRATION, SPHEROID GROWTH AND ANGIOGENESIS IN HUMAN BREAST CANCER CELLS.

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CENTRO DE ESTUDIOS FARMACOLÓGICOS Y BOTÁNICOS (CEFYBO), UNIVERSIDAD DE BUENOS AIRES-CONICET (1); CEFYBO, UBA-CONICET (2)

It has been reported that muscarinic receptors (M) are absent in normal breast cells and are up-regulated in tumor cells. Particularly, human breast cancer MCF-7 cells express M3 and M4 subtypes and its activation promotes cancer progression. We demonstrated that M expression in non-tumorigenic human mammary cell lines triggers malignant transformation. To confirm the contribution of M on tumoral progression, we developed new cell lines by genome editing of M3 and/or M4 receptors in MCF-7 cells using specific CRISPR-Cas9-gRNA complexes (cM3, cM4 and cM3M4). We analyzed the effect on cell migration capacity by wound healing assay, on the ability to generate three-dimensional structures (spheroids) in vitro and on induced angiogenesis in vivo. All cM cell lines significantly decreased their migration capacity (cM3: 67 ± 2; cM4: 77 ± 0.4 and cM3/4: 36.5 ± 6.7 %; n= 3) in comparison with MCF-7 cells (considered as 100 %, p<0.0001). Spheroids were formed using the hanging droplet method. All cM cell lines were able to form spheroids, however their growth kinetics were slower than MCF-7 spheroids, especially cM3M4. Tumor induced angiogenesis was quantified inoculated $2x10^5$ cells of different experimental groups in female NUDE mice. After 5 days, the animals were sacrificed and angiogenesis was quantified in the sites of inoculation as vessel density. The pretreatment with carbachol increase angiogenic response of inoculated MCF-7 cells in comparison to control (6.4 ± 0.7 vs. 3.3 ± 0.7, p<0.0001). The specific gene editing of M receptors in MCF-7 cells treated with carbachol significantly reduced neovascularization capacity of tumoral cells (cM3: 4.8 ± 0.5*; cM4: 3.8 ± 0.6**; cM3M4: 3.4 ± 0.5** vs. MCF-7 *p<0.01, ** p<0.0001). In conclusion, the specific M gene edition by CRISPR-Cas9 system in tumoral MCF-7 cells can effectively reduce the effects of muscarinic activation on migration, spheroid growth and angiogenesis.

0920 - IMPLICATIONS OF THYROID HORMONES (TH) IN REXINOIDS ANTI-LYMPHOMA ACTIVITY: INTEGRIN ALPHA V BETA 3 INHIBITION IN THE TREATMENT OF T CELL LYMPHOMA (TCL) WITH BEXAROTENE

Maria Mercedes DEBERNARDI | Johanna Abigail DÍAZ ALBUJA | Helena Andrea STERLE | Maria Celeste DIAZ FLAQUE | Graciela Alicia CREMASCHI | Maria Florencia CAYROL

BIOMED. UCA

Bexarotene (Bex), a RXR agonist used for cutaneous TLC treatment, is associated with clinical hypothyroidism, thus requiring the concomitant administration of levothyroxine (T4). We found that physiological levels of TH contribute to the malignant phenotype of TCL via the TH membrane receptor (mTR), the integrin alphaVbeta3. Here we investigated the consequence of T4 on the antineoplastic effect of Bex in different TCL subtypes. We confirm the presence of the RXR by WB in our panel of TCL subtypes. BEx effect in vitro on apoptosis and proliferation of CUTLL1, OCI-Ly12 and OCI- Ly13.2 TCL cells was higher in the absence than in the presence of TH supplementation (p<0.05). Also, we studied the impact of T4 addition to Bex treatment (BexT4+) in mice bearing a syngeneic TCL solid tumor and found that Bex decreased EL-4 tumor growth (p<0.001 vs. vehicle), being this effect even higher in the absence of T4 (p<0.05 vs. Bex). However, Bex alone decreased the anti-lymphoma immunity, as shown by a decrease of activated CD8+T-cells and of IFNg and TNFa tumor production (p<0.05 vs. BexT4+), thus T4 replacement is necessary to avoid a negative immunity. In a metastatic TCL murine model, we found that Bex alone decreased the number of experimental metastasis in the liver (p<0.001 vs. vehicle) and kidneys, this effect tends to be less pronounced in the presence of T4. Integrin alphaVbeta3 is overexpressed in TCL cells, so we investigated if its inhibition with cilengitide (Cil) would impair the pro-survival effect of TH and its role in Bex treatment. We demonstrated that mTR inhibition resulted in improved Bex-induced effects on apoptosis and cell proliferation in vitro in all TCL subtypes (p<0.05). Moreover, in vivo Bex+Cil combination render significantly smaller tumors (p<0.001 vs. vehicle and p<0.05 vs. BexT4+), while maintaining the anti-lymphoma immunity. Our results provide a rational method for evaluating the addition of Cil to treatments based on Bex and T4 supplementation for TCL.

0941 - PROGNOSTIC IMPACT OF IMMUNOHISTOCHEMICAL CHARACTERIZATION OF MARKERS OF BIOLOGICAL SUBTYPES IN RETINOBLASTOMA. PRELIMINARY RESULTS

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HOSPITAL DE PEDIATRIA JUAN P GARRAHAN - CONICET (1); INSTITUT CURIE (2); HOSPITAL DE PEDIATRIA JUAN P. GARRAHAN (3)

Retinoblastoma (RB) is the most frequent ocular tumor in childhood, its prognosis is based on the identification in the enucleated eye of high risk factors in pathology (HRPF) such as the invasion of the choroid, sclera and optic nerve. However, the risk of relapse is variable in the cohort of patients with HRPF. Results from our group obtained in studies of exome, transcriptome and methylome suggest the existence of two tumor subtypes called cone and mixed, which may be identified by the expression of two markers ARR3 and TFF1 by immunohistochemistry (IHC). Low or no expression of TFF1 is observed in cone-precursor derived tumors whereas high expression is observed in mixed tumors. Mixed tumors show more tumor heterogeneity, higher genomic instability, MYCN gains and copy number variations. However, it is unknown if any of these subgroups is associated to higher risk of metastasis. Evaluation of ARR3 and TFF1 expression in 3 patient groups: intraocular with no HRPF (n= 42), with HRPF (n= 93) and extraocular (n= 17). The quick score (QS) system was used to grade the expression of these markers and was reviewed by 3 observers. We established the correlation between the IHC results and genomic data in 23 cases (12.2 %) with 100 % concordance. Mixed type tumors were significantly more frequent in extraocular (17/17) and HRPF patients (53/93) compared to those with no HRPF (14/42), (p<0.01). Median QS was significantly higher in extraocular group (mean 180) compared to HRPF (mean 120), and to no HRPF (mean 25), (p<0.05). Biopsies of metastatic sites and tumor-derived cell lines from metastatic patients were positive for TFF1, suggesting that mixed subgroup is more aggressive than cone tumors. The use of IHC technique based on biological differences allowed us to identify the mixed subgroup as a more aggressive retinoblastoma subtype. This classification warrants its confirmation as a prognostic factor for guiding treatment intensity.

0943 - MULTI-ELEMENTAL ANALYSIS BY SYNCHROTRON X-RAY MICROFLUORESCENCE IN MAMMARY GLAND TUMOUR OF MICE TREATED WITH OMEGA-3 FATTY ACID

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IFEG CONICET (1); INICSA CONICET (2); UNIVERSIDAD NACIONAL DE CÓRDOBA (3)

The Salvia hispanica L., whose common name is Chia, is an annual herbaceous plant belonging to the Lamiaceae family. Chia seeds have been investigated and recommended due to their high levels of essential fatty acids with the highest proportion of omega-3 fatty acid (ω3-FA) compared to other natural sources known to date. It is well known that the consumption of ω3-FA can slow or stop the growth of cancer cells. This work contains the preliminary results of our study about the effects of Chia oil (ChO) in a murine mammary gland adenocarcinoma by micro-X ray fluorescence (micro-XRF) analysis. The histological and multielemental analysis by micro-XRF has been combined to infer information about the mechanisms related with the anti-tumorigenic actions of ω3-FA. It is an experimental study in which 24 mice BALB/C were used, randomly distributed between two dietetic groups: 12 with normal diet (control) and 12 with diet rich in ChO. Three months after starting the diet, they were inoculated with mammary gland cells with moderate metastatic capacity (M3). The animals were slaughtered 45-50 days after inoculation. Two adjacent cuts were made to each sample, one of 6 micrometers for conventional histological analysis and another of 30 micrometers placed in a Kapton film for measurement with micro XRF. Micro XRF measurements were performed at the Brazilian Synchrotron Light Laboratory (LNLS) in Brazil. Variation of the values of certain chemical elements of the samples treated with ChO with respect to the control was observed. Calcium (Ca) and Zinc (Zn) showed significant decreases in samples with ChO. Phosphorus (P) was located in regions of tumor cells. The results were analyzed by t-test. The distribution and concentration of Ca, Zn and P in samples can help us explain the anti-tumorigenic role of ω3-FA as biomarkers of metabolic functions.

0953 - MICROFLUIDIC-ASSISTED SYNTHESIZED OF OXALIPLATIN NANOVEHICLES COMBINED WITH CURCUMIN POLYMERIC MICELLES APPLIED TO CHEMORESISTANT COLORECTAL CANCER TREATMENT

Rodrigo LLOYD(1) | Elena María SANMARCO(2) | Dailenys ESPINOSA MARTINEZ(1) | Mario Alberto GADAN(3) | Julia GALLINO(2) | Florencia GIANNONI(2) | Marcela MORETON(4) | Diego CHIAPPETA(4) | Juan Martín CABALEIRO(2) | **Lucia POLICASTRO** (2)

LABORATORIO DE NANOMEDICINAS, COMISIÓN NACIONAL DE ENERGÍA ATÓMICA-ANPCYT (1); LABORATORIO DE NANOMEDICINAS, COMISIÓN NACIONAL DE ENERGÍA ATÓMICA-CONICET (2); COMISIÓN NACIONAL DE ENERGÍA ATÓMICA (CNEA) (3); DEPARTAMENTO DE TECNOLOGÍA FARMACÉUTICA, FACULTAD DE FARMACIA Y BIOQUIMICA, UBA-CONICET (4)

Resistance and metastatic recurrence are the main barriers for the effective treatment of cancer. Administration of antineoplastic drugs in 100 nm-size nanovehicles (NV) optimizes the localization of drugs in tumor tissue. This occurs manly due to the enhancement permeably retention effect that reduce peripheral toxicity and increase the tumor local therapeutic effectiveness. Oxaliplatin (Oxp), is a high efficiency chemotherapeutic drug but with severe adverse effects, thus this encapsulation in NV could significantly improve the effect. Moreover, the combination of this chemotherapeutic drug with sensitizing molecules, such as curcumin (Cur), could yet increase the possibilities of therapy success. This molecule is highly hydrophobic, so its encapsulation in NV also could improve its delivery. However, NV conventional synthesis technologies are inefficient with variations in the batch-to-batch procedures, which make it difficult for a rapid translation to patients. In recent years, microfluidic technology-assisted nanomedicines synthesis, where fluids are subtly controlled, significantly improve these processes and allow reproducibility between different batches. The aim of this work is to administrate Oxp in liposomes and Cur in polymeric micelles performed by microfluidic technology in order to improve chemoresistant colorectal cancer treatment. We developed a micromixer chip for the encapsulation of Oxp in liposomes, obtaining optimal conditions in size and % of encapsulation of Oxp. Both compounds encapsulated, alone or in combination, were tested in Oxp resistant subcutaneous tumor in nude mice developed by our group and administrated by intravenous injection. The administration of the combination of encapsulated drugs produced a high effect in tumor growth with only one dose application. These results could have a high impact on the synthesis and the encapsulation of oncologic drugs in liposomes in the national and regional pharmaceutical industry.

0970 - INHIBITION OF PORCUPINE HAS A DIFFERENTIAL IMPACT ON THE GENE EXPRESSION SIGNATURE OF BREAST CANCER CELL LINES OF DIFFERENT LEVEL OF AGGRESSIVENESS.

Sofia VALLA(1) | Gianina DEMARCHI(1) | Agustina CHIMENTO(1) | **Nadia BONADEO** (1) | María Lucía ROMANO(1) | Laura Daniela ALANIZ(1) | Martin GOTTE(2) | Carolina CRISTINA(1)

CENTRO DE INVESTIGACIONES BÁSICAS Y APLICADAS (CIBA) – CITNOBA (UNNOBA – CONICET) (1); KLINIK FÜR FRAUENHEILKUNDE UND GEBURTSHILFE, UNIVERSITÄTSKLINIKUM MÜNSTER (2)

Wnt pathway is involved in cellular processes which are dysregulated in cancer as cell renewal, proliferation and EMT. In particular, in breast cancer (BC) it has a role in both tumor initiation and progression. Porcupine's palmitoylation of Wnt ligands is required for their proper signaling and release and its inhibition showed anti-tumoral effects on different BC models. In order to study if the inhibition of Porcupine (PORCN) had different effects regarding to the aggressiveness of the BC cell lines, we analyzed the impact of IWP-2, a PORCN inhibitor, in the TNBC cell line MDA-MB-231 and in the less aggressive MCF-7 cell line. Cells were treated with 5 µM IWP-2 or DMSO as control. IWP-2 reduced the synthesis of Wnt ligands and target genes in MCF-7 but not in MDA-MB-231 cells (qPCR, 24 h, *p<0.05: WNT3A*, AXIN2*, CCND1*, C-MYC*, CCNB1*). We also found a decrement in CYCLIN B1 protein levels in MCF-7 cells. However, it reduced the expression of ß-CATENIN in both cell lines (WB, 48 h). Furthermore, cell number was also reduced in both cases (TB, 72 h). When analyzing stem cell markers, SOX2 mRNA was decreased and KLF-4 increased in MCF-7 and MDA-MB-231 suggesting that PORCN inhibition has a similar effect on the stem-cell phenotype in both cell lines. However, the number of colonies was reduced in MDA-MB-231 but not in MCF-7 cells (CFA, 8 d). In hanging drop assays (6 d), both cell lines formed bigger but less compact mammospheres than controls, with a stronger effect in MCF-7 cells, suggesting a loss of cell adhesion in this cell line. This was also consistent with reduced E-CADHERIN expression in MCF-7 cells. Regarding EMT, no differences were found in SNAIL-1, ZEB-1 and E-CADHERIN expression at the mRNA level with IWP-2 treatment although TGF-ß synthesis was decreased in MCF-7 cells. Our results suggest that the differences in the genetic background and phenotype of breast cancer cells can modulate the effect of PORCN inhibition over tumoral properties in vitro.

1017 - MUC4 IS THE PRINCIPAL MEDIATOR OF TNF-INDUCED TRASTUZUMAB RESISTANCE AND FOSTERS AN IMMUNOSUPPRESSIVE TUMOR MICROENVIRONMENT IN HER2+ BREAST CANCER

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IBYME-CONICET (1); FACULTAD DE CIENCIAS EXACTAS Y NATURALES - UBA (2)

About 13-20 % of breast cancer (BC) patients are HER2 positive (HER2+) and receive trastuzumab (T), an anti-HER2 monoclonal antibody, but 30-50 % of them relapse. We have demonstrated that tumor necrosis factor alpha (TNF) induces the expression of the transmembrane glycoprotein mucin 4 (MUC4) that shields T epitope in HER2, impairing its antitumor effects, and that the soluble and transmembrane TNFα (sTNFα, tmTNFα) inhibitor Etanercept (E) downregulated MUC4 expression and sensitized de novo T-resistant BC xenografts to T. Here, we studied the in vivo participation of MUC4 on T resistance and on antitumor innate immune response. T-resistant JIMT-1 cell line was transduced with a doxycycline (Dox)-inducible MUC4 shRNA construct (JIMT-shMUC4). To block TNFα, we used E or the dominant negative-TNF protein INB03 (DN), able to neutralize sTNF. Nude mice bearing JIMT-1-shMUC4 tumors were assigned to the experimental (+Dox 2 mg/ml in water) or control group (−Dox) and treated with IgG, T, E (5 mg/kg), DN (10 mg/kg), T+E or T+DN i.p. twice a week. Tumor volume was monitored routinely. Tumor-infiltrating immune cells were evaluated by immunofluorescence and flow cytometry. In control groups, only T+E and T+DN inhibited tumor growth (72 and 75 %, respectively, $p<0.0001$ vs. IgG). MUC4 knockdown sensitized tumors to T at comparable levels to T+E and T+DN (62, 78 and 76 % respectively, $p<0.0001$ vs. IgG). This was accompanied by an increase in NK cells activation and degranulation and an increase in M1/M2 macrophages ratio in the tumor microenvironment (TME). Upon MUC4 downregulation, myeloid-derived suppressor cells infiltration was reduced and macrophage recruitment was enhanced in the tumor bead of IgG+Dox vs. IgG-Dox groups. We conclude that MUC4 is the major player in TNF-induced T resistance in vivo. In addition, MUC4 favors an immunosuppressive TME. We propose that patients with HER2+ and MUC4+ tumors should be treated with T and TNF-blocking agents to avoid resistance.

Medicina Regenerativa y Terapia celular/ Regenerative Medicine and Cell Therapy II

Chairs: Marcelo Choi | Alejandra Duarte

0584 - PLURIPOTENT STEM CELLS EDITION IN PLAKOPHILIN-2 GENE FOR ARRHYTHMOGENIC CARDIOMYOPATHY IN VITRO MODELING

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FLENI-CONICET

The arrhythmogenic cardiomyopathy (ACM) is an inherited heart muscle disease characterized by the progressive replacement of contractile myocardium by fibro-fatty adipose tissue, generating ventricle arrhythmias and sudden death. The ACM has a genetic origin with mutations in desmosomal genes. Among these, one of the most commonly disrupted gene is Plakophilin-2 (PKP2), including non-sense mutations. Our aim was to evaluate PKP2 expression during cardiomyocyte differentiation, and to generate a PKP2 knockout (KO) iPSCs line by CRISPR/Cas9 in order to model the disease in-vitro. RNAseq data showed increased PKP2 expression during cardiomyocyte differentiation and this result was confirmed by Western blot (WB) analysis. We observed an increase of 8 times of PKP2 protein expression between day 0 and day 21 of differentiation ($n= 2$; $p<0.05$). In order to generate an early stop codon in the PKP2 gene, we designed 2 RNA guides (gRNA1 and gRNA2) directed to the exon 1 of the gene. We co-transfected 1µg of the CRISPR system with gRNA1 and 1µg with gRNA2 to $2x10^5$ iPSCs. After puromycin selection, the cells were clonally expanded and analyzed for PKP2 expression by WB. From 13 clones that were evaluated, 2 of them were negative (15.4 %), obtaining PKP2 KO-iPSCs lines. In summary, the increase of PKP2 gene expression during cardiomyocyte differentiation shows the importance of this protein in the cardio-system. In addition, the generation of 2 KO iPSCs lines for this protein will allow us to model the ACM in-vitro after differentiating this cell lines to cardiomyocytes.

0610 - APPLICATION OF THE BGP-CS SOLUTION IN CRYOPRESERVATION OF RAT HEPATOCYTES

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Hepatocyte transplantation is an alternative to organ transplantation for the treatment of some liver diseases. Hepatocyte cryopreservation is the tool to maintain functionality from the isolate to the requirement. Currently, there are controversies about optimal cryopreservation protocol and the utilization of commercial cryopreservation media represents a significant cost increase. A modification of BES-gluconate-polyethylene glycol (BGP)-HMP solution, conceived for hypothermic perfusion of gastrointestinal organs, turns it into in a more versatile preservation solution, renamed BGP-CS. In this work, we investigated its potential application in a cryopreservation protocol of isolated rat hepatocytes by slow cooling. Cells suspensions were cryopreserved using BGP-CS or HTK commercial solution with DMSO 10 % as cryoptrotectant agent (CPA) by cooling at 4.2 ºC/min, until -80 ºC followed by direct immersion in LiqN2. Subsequent steps were taken to improve cryopreservation protocol using BGP-CS. Three slow cooling rates (1.3, 2.4 and 4.2 °C/min) and two CPA removal protocols (drip dilution 1:13 and 1:25) were tested. A mathematical model based on Kedem-Katchalsky formalism was used for to check osmotic stress in the CPA removal process. Viability and functionality were evaluated by TB, IP-H, FDA-IP and MTT. BGP-CS has shown similar performance when it was compared to a commercial solution ($p>0.05$, $n=n5$). Cooling rates tested have not shown a difference in cell viability and functionality ($p>0.05$, $n= 5$). We have selected the one that optimizes the time-cost balance. Dilution of the CPA with greater volume has shown significant cell loss ($p<0.001$, $n= 6$), although mathematical modeling showed no signs of osmotic stress in either protocol suggesting that we could be losing the cells in the dilution volume. Further refinement of resuspension protocol together with simulation results allowed us to design an optimal CPA removal protocol increasing viable cell yield ($p<0.05$, $n= 5$). To summarize, the use of BGP-CS improves cryopreservation outcome reducing costs and increasing viable cell yield.

0625 - HUMAN MUSE CELLS GENETICALLY-MODIFIED BY BACULOVIRAL VECTOR FOR THERAPEUTIC ANGIOGENESIS

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Muse cells are endogenous repair stem cells identified by SSEA3+. If Muse cells are not enough, the administration of exogenous Muse cells offers a prominent functional recovery. However, genetically improved Muse cells have not yet been developed. The objective was to generate human Muse (hMuse) cells that overexpress the double mutated HIF-1alpha gene through a baculoviral vector, in order to improve its angiogenic capacity. hMuse were isolated from abdominal lipoaspirates using severe cellular stress procedure. Mesenchymal stem cells (ASC´s) were harvested from matched donor samples. Recombinant AcMNPV baculovirus expressing the green fluorescent protein (Bv.CMV-GFP) or the therapeutic gene (Bv.CMV-dmHIF-alpha) were developed. The efficiency transduction (TE) of the different multiplicities of

infection (MOI): 0, 25, 50, 100, 200 and 400 were determined with Bv.CMV-GFP vector by cell counting using fluorescence microscopy. The selected MOI was subsequently used to transduce hMuse with the Bv.CMV-dmHIF-1alpha vector under the same conditions. Comparative gene expression analysis for angiogenic factors and S1P receptor 2 (S1PR2) was performed by RTqPCR. TE obtained for each MOI was 0, 17.3, 24.7, 47.8, 58.6 and 59.8%, respectively. The MOI 200 was selected because it yielded an ET of 58.6 %. Although a MOI 400 increased TE to almost 60%, we did not consider that this increase justified duplicating the viral load of the cells. Using MOI 200, HIF-1alpha gene expression increased 5.9 fold in hMuse and 2555 fold in hMuse-HIF compared to its expression in ASCs. VEGF and FGF expression showed similar behavior; VEGF 3.3 and 7.9 and FGF 3.5 and 5.9 fold increases, respectively. Expression of S1PR2 (1.5 and 1.1) showed no changes between groups. hMuse cells could be effectively transduced with a baculoviral vector doubling their angiogenic potential without altering their migratory capacity mediated by S1PR2.

0680 - CHANGES IN MESENCHYMAL STEM CELLS DERIVED EXOSOMES CAUSED BY UV-C RADIATION

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Exosomes are extracellular vesicles ranging from 30 to 150 nm that originate from endosomal pathway. They contain proteins, lipids and nucleic acids, suggesting a possible role in cellular communication. In our study, we sought to elucidate the effect genomic damage provoked by UV-C may have on exosomal secretion of mesenchymal stem cells derived from induced pluripotent stem cells (iPS-MSC). Here, iPS-MSC were irradiated with three different UV-C intensities (0.001, 0.01 and 0.1 J/cm^2) and the expression of genes involved in the exosomal pathway was evaluated by RT-qPCR. TSAP6, ALIX, Syntenin, Rab27a and P21 did not show significant difference in the three conditions. Given these results, exosomes secreted by irradiated and not irradiated IPS-MSC were isolated from conditioned media using a size exclusion chromatography column and the concentration of extracellular vesicles was measured by Tunable Resistive Pulse Sensing. No significant differences were shown between conditions. To assess if there is a change in intercellular communication due a distinct exosome content, functional assays were performed. A wound Healing assay showed that cells incubated with not irradiated IPS-MSC derived exosomes had significant more wound closure than those incubated without exosomes, and the effect is lost when cells are incubated with irradiated IPS-MSC derived exosomes. We were able to verify that these results are not due to changes in cell replication, as analysis with propidium iodide showed no differences in cell cycle between the conditions. Taken together, our results suggest that exosomal secretion could be altered due to genomic damage caused by UV-C. While genes of exosomal pathway and the number of total exosomes secreted by irradiated and non irradiated IPS-MSC does not show differences, changes are evidenced in exosomal function. We hypothesize that there may be a distinctive exosome composition between the conditions that remains to be explored.

0690 - LARGE-SCALE EXOSOME ISOLATION FROM MESENCHYMAL STEM CELLS CULTURED IN SPINNER FLASK.

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Due to their potential for differentiation and their immunomodulatory capacity Mesenchymal Stem Cells (MSC) have been widely used in preclinical and clinical studies for various diseases and tissue regeneration. Multiple evidences show that their regenerative effects are mainly due to their paracrine function mediated by their secretome, which is composed of both, soluble factors and factors released within extracellular vesicles (EV). Exosomes are a type of EV that are thought to mediate cellular communication. Our group have previously shown that within the exosomal content there are lots of proteins involved in regeneration and immunomodulation processes, moreover, we observed positive effects on cell migration when using medium with MSC exosomes in wound healing assays. From the translational point of view, these microvesicles are very interesting since they could be produced in big amounts and at a lower cost than MSC, stored for later use and would allow developing cell-free therapies of easier application. The aim of this work was to isolate MSC exosomes on a large scale and characterize them for later use in functional tests. First, we needed to scale up MSC culture. For this, we set up the conditions to grow them in Spinner flasks, obtaining a big number of cells in a short time. These cells were checked and characterized by evaluating the expression of typical membrane integrins and extracellular matrix proteins by Flow cytometry and Real-Time PCR. Once we set up the culture conditions, we proceeded to isolate exosomes from it. After 24 hours of medium conditioning, we performed a series of centrifugations and separation of vesicles by Size-Exclusion Chromatography. The eluted fractions were incubated with anti-CD9 and anti-CD81 following incubation with anti-CD63 magnetic beads. The flow cytometry results confirmed that we could effectively isolate exosomes, and TRPS's quantification showed a large number of isolated vesicles. In the future we plan to assess a quality check of the isolated exosomes, as well as to analyze their effect in functional assays.

0766 - EVIDENCES POINTING THAT BONE MARROW ORIGINATING TUMOR CELLS WITH LUNG COLONIZING ABILITY, RATHER THAN TUMOR CELLS REMAINING WITHIN THE BONE MARROW, ARE RELATED TO A MESENCHYMAL STEM CELL PHENOTYPE

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A critical challenge in the clinical management of osteosarcoma (OS) is the appearance of lung metastasis. This bone marrow – associated tumor represents the most frequent bone tumor in pediatric and young adult populations. In this context, 20% of OS patients are diagnosed with metastatic OS, but a high percentage of the remaining cases diagnosed without metastasis could already present micrometastasis undetectable through conventional methods. Our previous results indicated that a differential gene expression distinguished OS cells with higher ability to home into the lungs. Interestingly, molecular differences were subtle at the level of cellular content but more prominent at the level of the secretory compartment. These molecular features were reproduced by a functional behavior relevant to a colonizing ability to the lungs. In order to gain insight into spatial arrangements of OS cells that diverged in their lung colonizing ability, that may contribute to understand advantages to home into the lungs and

could relate to metastatic mechanisms, we approached 3D OS cultures. We observed that OS cells that remain at the primary tumor site had lesser ability to establish 3D growth, while cells leaving the tumor and colonizing the lungs established 3D growth successfully; this last feature was shared by mesenchymal stem cells (MSC). This would point that cell-cell contact is a prominent feature in lung colonizing cells. Since our previous results demonstrated that the secretome of divergent OS cells is the compartment that mostly distinguished the ability to home into the lungs, we analyzed GOs in the secretory fraction in divergent OS cells and bone marrow MSC. MSC share the original niche where the bone tumor arises, and related to possible closeness between MSC and OS cells, we demonstrated that the cells that leave the primary tumor rather than the cells remaining at the primary niche of residence for OS, share similarity with MSC. This points at the necessity to target stem-like tumor cells that leave the tumor and colonize secondary sites. Conventional therapies to treat OS, which are directed to the primary tumor OS site, may be unsuccessful considering those stem-cell like OS populations residing in the primary site.

0770 - PARACRINE EFFECT MEDIATED BY EXTRACELLULAR VESICLES DERIVED FROM IGF-I OVEREXPRESSING HUMAN UMBILICAL CORD PERIVASCULAR CELLS IN A MURINE MODEL OF EXPERIMENTAL LIVER FIBROSIS

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Cirrhosis is the result of chronic liver damage/regeneration cycles and fibrosis accumulation. Human umbilical cord perivascular cells (HUCPVC) are mesenchymal stromal cells that could allow tissue regeneration by secretion of soluble factors and extracellular vesicles (EV). We previously demonstrated that HUCPVC engineered to produce insulin growth factor like-I (IGF-I-HUCPVC) ameliorate liver fibrosis in mice. Our aim is to evaluate the role of EVs in the therapeutic effect of IGF-I-HUCPVC in liver fibrosis. Conditioned media (CM) or EV depleted CM (DCM) were collected from HUCPVC infected with adenovirus codifying with IGF-I or green fluorescence protein (GFP). EVs were isolated from CM by differential centrifugation and characterized by electron microscopy, dynamic light scattering and flow cytometry to test shape, size and EV markers expression respectively. IGF-I levels were assayed in EV after lysis and/or dialysis by ELISA. Fibrosis was induced in BALB/c mice by administration of thioacetamide for 8 weeks (600 mg/kg/week). On week 6, IGF-I-HUCPVC and GFP-HUCPVC derived EV, CM or DCM were intravenously administrated (3 doses, 15 µg/dose/mice, every 5 days) and at week 8 liver samples were collected. Hepatic Stellate Cells (CFSC-G2 cell line) and hepatic macrophages (Mø) were incubated in vitro with EV, CM or DCM, and gene expression evaluated by qPCR. CFSC-G2 incubation with CM or EV derived from IGF-I-HUCPV downregulates the expression of COL1A2 and a-SMA in comparison with DCM-IGF-I-HUCPVC ($p<0.001$). Then, we found that lysis of dialyzed EV-IGF-I-HUCPVC results in an increase of IGF-I levels ($p<0.001$). In vivo, CM and EV derived from IGF-I-HUCPVC reduced collagen deposit while EV-depleted CM does not ($p<0.001$). In vitro, iNOS, IL-6 and TNF-a; are downregulated in Mø after treatment with EV-IGF-I-HUCPVC compared to controls ($p<0.001$). Our results showed that EV mediate the therapeutic effect of IGF-I-HUCPVC and ameliorates liver fibrosis.

0785 - TBX20 OVEREXPRESSION INDUCES CELL PROLIFERATION AND ANGIOGENESIS IN VITRO

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In the last years, several therapies aimed at myocardial regeneration have been investigated to treat acute myocardial infarction (AMI). To reach this aim, the main approaches are to induce neovascularization (angiogenesis) and myocardial self-regeneration (myocardiogenesis). It has been shown that the overexpression of TBX20 transcription factor in transgenic mice induces cardioprotective effects, suggesting that the overexpression of TBX20 could be a therapeutic alternative for cardiac regeneration. Our objective was evaluated the effect of the overexpression of TBX20 over cell proliferation and angiogenic induction in vitro. Baculoviral vectors overexpressing human TBX20 (BvTBX20) and control vectors (BVNull) were generated. Then, H9c2 cells were transduced with these vectors using a MOI of 300. At 2- and 5-days post-transduction cell proliferation was evaluated by MTS assay and cell count. Supernatants from transduced cells were used to perform a tubulogenic assay in HMEC cells. Cell proliferation rate was highest at 5 days post-transduction in the BvTBX20 group vs BvNull assessed by MTS assay (BvTBX20: 107 ± 1; vs. Bvnull: 100±1 % cell proliferation, $p<0.01$, t-test), and cell count (BvTBX20: 136,500 ± 15,256 cells; vs. Bvnull: 92,166 ± 11,250 cells, $p<0.05$, t-test). At 2 days post-transduction no significant differences were found. In the tubulogenic assay a higher amount of rings was found in the BvTBX20 group vs BvNull at 2 (4.34 ± 0.40 vs. 1.43 ± 1.28) and 5 days (7.35 ± 2.40 vs. 2.77 ± 1.04, $p<0.05$, t-test). Conclusion: The overexpression of TBX20 transcription factor increases cell proliferation in rat myoblast cell line H9c2 and promotes angiogenesis in vitro. These results suggest that TBX20 overexpression could be a therapeutic alternative for tissue regeneration.

0795 - STEM - ASSOCIATED FEATURES IN TUMOR CELLS ABLE TO COLONIZE SECONDARY TUMORAL SITES

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Osteosarcoma (OS), the most frequent bone tumor in pediatrics, presents critical clinical challenges in lung metastasis and chemoresistance emergence. Understanding OS switch into a metastatic phenotype and the interaction OS-stromal cells relevant in the new niche, would help in developing better diagnostic and therapeutic tools. In order to distinguish aspects that would allow OS cells to leave the bone niche and survive in a new tissue environment, we evaluated behavioral features acquired by OS cells with ability to establish secondary tumor growth in the lungs, approaching the degree of differentiation, doxorubicin (doxo) exclusion and distribution properties and molecular signatures. Our results indicate that lung-colonizing OS cells diminished its osteoblastic potential while modified the intracellular localization of chemodrugs. In this way, doxo switched from a nuclear to a

cytoplasmatic distribution in cells with lung colonizing ability (0.884 ± 0.015 SAOS2; 0.546 ± 0.131 LM7). These features coincided with a higher level of expression of stem-related genes and lower expression of differentiation-associated markers even at basal conditions in the metastatic cells. On the other hand, the higher osteogenic activity of OS cells with non-colonizing features was even reflected as a paracrine osteo-inductive effect. In addition, OS cells with high and low lung-colonizing capacities have opposite impact in mesenchymal stem cells (MSCs). Further, OS cells colonized-mouse lungs had a greater chemoattractive induction on MSCs. A major acquisition in tumor cells with metastatic features is a switch into a stem-like state that could favor their survival in the pulmonary niche, opening new possibilities for specific chemotherapeutic schemes. We provide new insights on OS cells differing in lung homing ability, with particular emphasis on multidrug resistance and interaction with MSC, which would impact in early diagnosis and therapeutic management.

0841 - BACULOVIRAL VECTOR ENCODING MUTANT HIF-1ALPHA AS A POSSIBLE TREATMENT FOR PERIPHERAL ARTERIAL DISEASE

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Peripheral artery disease (PAD) is an ailment characterized by decreased arterial blood flow to the lower limbs. Given the lack of effective treatments, angio-arteriogenesis has been proposed. We hypothesized that the administration of a baculovirus encoding mutant HIF-1ALPHA (Bv.mHIF-1A) would induce collateral vessels neoformation in the ischemic limb. Methods: A. In vitro studies: 1) Transgene expression: Skeletal myoblast (SkM) isolated from rabbit adductor muscle were transduced with Bv.mHIF-1A at MOI= 100, and HIF-1A expression was evaluated by RT-qPCR and Western blot. 2) Angiogenic potential of transduced cells: the tubulogenic assay was performed with supernatants of SkM and SkM-mHIF-1A cultures. B. In vivo studies: 12 rabbits underwent sterile excision of the femoral artery of the left posterior limb. Seven days later, rabbits were randomized to receive in the ischemic muscle 10 injections containing 1E9 copies of the Bv.mHIF-1A vector (treated group, n= 6) or 1E9 copies of Bv.null (control group, n= 6). Two weeks post-treatment digital angiography was performed in both posterior limbs. HIF-1A mRNA levels in SkM-mHIF-1A cells was 1,000-fold higher than in non-transduced cells ($p<0.05$, t-test). HIF-1A protein levels were also overexpressed. Supernatants derived from SkM-mHIF-1A formed more tubular networks than those from non-transduced SkM cells (7.38 ± 0.69 vs. 4.99 ± 1.01 rings/mm², $p<0.01$; t-test). Finally, at 14 days post-treatment the density angiographyically visible collaterals was higher in Bv-mHIF-1A - traeated rabbits (8.12 ± 0.42 colaterales/cm²) than in those treated with Bv-null (6.13 ± 1.15 colaterales/cm²; $p<0.05$, t-test). Conclusion: The Bv.mHIF-1A induced angiogenesis in vitro and collateral vessels neoformation in the ischemic muscle of rabbits at 14 days after treatment. Further safety and efficacy studies at longer follow up periods are needed to estimate the potential usefulness of this approach in the clinical setting.

0845 - 3-D PRINTING OF OF BIODEGRADABLE SCAFFOLDS TO RESTORE SCAR TISSUE: PLA PROOF OF CONCEPT.

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Regenerative medicine (RM) has become relevant particularly because more than 150 million people worldwide have functional problems in tissues or organs. One of the strategies to restore function is the application of biomaterials with pre-administered cells. Materials to be used constitute one of the fundamental points in this process, since they positively or negatively influence the survival of the cells. One of the best known biomaterials is polylactic acid (PLA), a polyester that in the body degrades into lactic acid, which is easily removed. 3D printing is being applied in RM to address the need for tissues and organs suitable for transplantation, and implies additional complexities, not only because of the possibility of combining materials and cells, but also because of the possibility of incorporating growth and/or differentiation factors. We approached the feasibility of printing biologically plausible tissue structures for organ replacement using 3D printers and PLA as biomaterial. To this end we printed tubes as scaffolds that would substitute scar tissue. By Fusion 360 we designed tubes of 14.0 mm long (tolerance of ± 0.1 mm) and 5.4 mm in diameter (tolerance of ± 0.05 mm) of 1.72 mm thick PLA, that were sterilized by UV light. The printing was done on a modified Makerparts2 printer with a 0.2 mm diameter spout. Size, macrostructure and porosity would suit recellularization. We are currently approaching the use of fibroblasts to adhere to the scaffold to help in the regeneration process of the target tissue and provide anchoring for epithelial cells. Cells from excess, discarded tissue samples (surgeries at the Dermatology department, HIBA) will be used to anchor on the generated structures. The choice of resorbable materials that shape suitable artificial prosthesis (scaffold) is essential, allowing the biological system to function as a bioreactor using the scaffold as the structure from which the new organ will be reorganized.

0848 - HLA TYPING AS A QUALITY CONTROL FOR PURITY IN CELLULAR THERAPY PRODUCTS.

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Research on advanced therapies holds great therapeutic potential. Among them, cell therapy, based on transplantation of live cells, frequently relies on in vitro cultures with or without genetic modifications. Cross-contamination with unrelated cells and/or with microorganisms are among the most frequent risks related to cell culture. According to local and international standards, cellular therapy products characterization, even those intended for pre-clinical research, should include testing for identity, purity, potency, and viability. Short tandem repeats (STR) analysis is recommended for genetic cell line authentication for research but there is no clear consensus on the analysis for products intended for cell therapy. Our goal is to evaluate whether human leukocyte antigen (HLA) typing, by sequence-specific oligonucleotide (SSO) technology, could be use as a quality control to guarantee cell purity, particularly lack of cross-contamination with unrelated cells that will not be revealed by phenotypic characterization or morphological differences. We chose HLA typing because it is a highly polymorphic set of genes and this technology is commonly used in many typing laboratories including ours. Genomic DNA from 4 mesenquimal cell lines (MSC) was obtained and used to performed SSO-PCR for HLA-A; HLA-B and HLA-DRB1 and Luminex analysis according to standard protocols. To test the ability to detect cross-contamination, we performed gDNA mixes between samples (1:1 - 1:100). For the analysis, we compared the number of positive and negative beads in each sample and in the mixes. We set a minimum of 3 different beads as criteria to define

contamination. All 4 cell lines showed a unique HLA profile for HLA-A; HLA-B and HLA-DRB1. Also, we were able to detect cross-contamination in all the mixes assayed up to 1:4 by means of at least 3 different beads for each gene. Cell contaminations lower than 20 % were not detected. HLA typing by SSO-PCR technology is effective to determine lack of cross-contamination with unrelated cells and may be a suitable assay for cellular therapy products.

0885 - COMPARISON OF DEPROTEINIZED BOVINE BONE, BIOGLASS AND, SYNTHETIC HYDROXYAPATITE IN THE BONE HEALING OF A CRITICAL SIZED BONE DEFECT. PRELIMINARY STUDY IN RATS.

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Bone grafts are important for alveolar bone height and volume preservation necessary for dental implant placement. The development of biomaterials for bone grafting with comparable characteristics and biological effects than those renowned internationally is necessary. Deproteinized bovine bone putty (BB), bioglass (BG) and, synthetic hydroxyapatite (SH) are frequently indicated as bone grafting materials due to their osteoconductive properties. We compared the bone healing response of BB, BG and SH (Odontit Implant Systems, Argentina) in a critical sized bone defect. We created a bone defect of 4 mm diameter in rat tibiae for implantation with each biomaterial (N=30 rats). Samples were collected at 2 and 4 weeks for histological and histomorphometrical analysis of new bone formation (NBF) and remaining particles of each device (RP). Radiographic analysis was done at T=0 and at 2 and 4 weeks. Results: % of NBF (mean ± SD): 2 weeks: Control group: 6.60 ± 3.71; BB group: 23.23 ± 3.89*; BG group: 18.35 ± 5.23*; SH group: 26.27 ± 9.30*; 4 weeks: Control group: 6.69 ± 2.38; BB group: 24.37 ± 3.66*; BG group: 17.45 ± 6.64*; SH group: 32.25 ± 3.80*. % of RP: 2 weeks: Control group: 0 ± 0; BB group: 5.04 ± 1.39*; BG group: 3.03 ± 2.31*; SH group: 4.46 ± 2.87*; 4 weeks: Control group: 0 ± 0; BB group: 4.45 ± 2.35*; BG group: 2.87 ± 1.14*; SH group: 3.78 ± 1.68* (*p<0.05 versus control group; p= NS between BB, BG and SH groups). Although SH exhibited a trend towards increased NBF we did not find statistical significance among the three biomaterials. Bone healing at the implanted sites, was accompanied by a progressive inflammatory response consistent with the expected histological stages of bone repairing. The 3 biomaterials exhibited an increased in radiopacity at 2 and at 4 weeks vs. control group indicating NBF. All biomaterials were associated with trabecular bone formation. Although further studies need to be done, our results indicate that BB, BG and SH exhibit similar osteoconductive properties.

0919 - LIPOSOME LOADED COLLAGEN BASED BIOMATERIALS WITH ANTIMICROBIAL ACTIVITY

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Hydrogels are promising materials in the field of biomedicine due to its biocompatibility and biodegradability. Liposomes (L) have proved to be an effective vehicle as they are not toxic, biodegradable and can encapsulate and release different drugs in a controlled way. Silver nanoparticles (AgNPs) have proved antibacterial activity. In order to overcome the challenge of developing a long-term antimicrobial material, we report a non-conventional biomaterial with prolonged bactericidal effect based on the incorporation of liposomes encapsulating silver nanoparticles in collagen hydrogels. AgNPs were synthesized by a reduction method and then incorporated in liposomes (L-AgNPs) by the lipid film hydration and extrusion technique. Collagen hydrogels were prepared by exposing a collagen solution extracted from rat tails to a saturated atmosphere of ammonia to induce gelation. Antimicrobial collagen-based scaffolds were prepared by adding AgNPs-containing liposomes suspensions to the collagen gels (Col-L-AgNPs). The optical properties of AgNPs were monitored by UV–vis spectroscopy. The morphology of AgNPs and L-AgNPs was studied by TEM and the structure of collagen gels before and after incorporation of L-AgNPs was analyzed by SEM. The antibacterial efficiency of Col-L-AgNPs was then evaluated on Gram-positive (S. aureus) and Gram-negative (P. aeruginosa) bacteria and cytotoxicity towards mammalian cells was studied. As a result, AgNPs exhibited well dispersed spherical morphology with an effective diameter between 5 and 20 nm. The L-AgNPs showed an effective diameter of approximately 410 nm. Col-L-AgNP showed an important bactericidal activity against both bacteria and did not affect cell viability. Based on these results, Col-L-AgNPs is promising as a new material that conserves a strong bactericidal activity and biocompatible properties for 72 h which is especially attractive for wound dressing as it does not need to be replaced repetitively.

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