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SAIC AWARDS

IRENE FARYNA RAVEGLIA: ONCOLOGY AWARD

IN VIVO HEMIN PRE-CONDITIONING TARGETS THE VASCULAR AND IMMUNOLOGICAL COMPARTMENTS AND RESTRAINS PROSTATE TUMOR DEVELOPMENT

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Pre-conditioning strategies constitute a relatively unexplored and exciting opportunity to shape tumor fate. In this study we used hemin, an inducer of Heme Oxygenase-1 (HO-1), in an in vivo pre-conditioning model to assess prostate cancer (PCa) development. The stroma of immunocompetent C57BL/6 mice (n=5) was conditioned by subcutaneous administration of hemin prior to TRAMP-C1 (murine PCa cell line TC1) tumor challenge. Pre-conditioning resulted in increased tumor latency and decreased initial growth rate ($P<0.05$). Histological analysis of the tumors revealed impaired vascularization. Hemin-treated HUVEC exhibited decreased tubulogenesis in vitro only in the presence of TC1-derived conditioned media ($P<0.001$). Regarding the crosstalk between tumor and endothelial cells, TC1 cell motility and adhesion to the endothelium were significantly impaired in the presence of hemin pre-treated HUVEC conditioned media ($P<0.05$). An in vivo Matrigel plug assay confirmed that s.c. hemin pre-conditioning hinders tumor neo-vascularization in C57BL/6 mice (n=5; $P<0.01$). Furthermore, we also analyzed the effect of hemin treatment on the immune compartment. Hemin boosted CD8+ T-cell proliferation and degranulation in vitro, and antigen-specific cytotoxicity in vivo ($P<0.01$). Interestingly, a significant systemic increase in the frequency of CD8+ T lymphocytes was observed in hemin pre-conditioned tumor-bearing mice ($P<0.05$). Tumors from hemin-conditioned mice also showed reduced expression of Galectin-1 (Gal-1; $P<0.01$), key modulator of tumor angiogenesis and immunity, evidencing a clear and persistent remodeling of the microenvironment. Finally, data obtained at the mRNA and protein levels revealed a subset of PCa patients and PCa patient-derived xenografts, respectively, with mild HO-1 and low Gal-1 expression. Taken altogether, these data showcase a novel function of an already human-used drug as a novel means of boosting the endogenous anti-tumor response.

PROGNOSTIC IMPACT OF MINIMAL DISSEMINATION DETECTION IN NON-METASTATIC RETINOBLASTOMA WITH HIGH-RISK PATHOLOGY FEATURES

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Introduction: Metastatic relapse may occur in children with retinoblastoma and high-risk pathology features (HRPF) and it is a major cause of mortality worldwide. Detection of minimal dissemination (MD) may be a tool for risk estimation. We previously reported the use of CRX mRNA for MD determination in metastatic retinoblastoma

but no data in non-metastatic children with retinoblastoma and HRPF are available. Objectives: To evaluate whether MD is detectable in children with non-metastatic retinoblastoma who have HRPF and to assess the prognostic impact of MD on disease-free survival (DFS). Material and methods: It was a prospective study carry on from 05-2007 to 10-2013. We studied 84 patients with non-metastatic retinoblastoma and HRPF (isolated massive choroidal invasion in 14, post laminar optic nerve invasion (PLONI) in 51, 12 with scleral invasion without PLONI and 7 with tumor at the resection margin of the optic nerve) were evaluated at the time of primary or secondary enucleation. CRX mRNA was evaluated by RT-qPCR in bone marrow (BM) and cerebrospinal fluid (CSF) at diagnosis and during follow-up. In 14 cases, GD2 synthase was used instead of CRX for CSF evaluation. The main outcome measure was the metastatic relapse. Results: MD was detected in 9 cases (BM=7, CSF=2). MD was significantly associated with tumor extension beyond the resection margin of the optic nerve (p=0.0005) and scleral invasion (p=0.002). MD occurred in 18.6% of the group E eyes with glaucoma and in 8/80 and 1/16 of initially and secondarily enucleated children, respectively. Children with MD had a significantly lower 3-year DFS (0.78 (95% CI= 0.37-0.94) versus 0.98 (95% CI= 0.93-1) p=0.004). Conclusions: We identified a very high-risk population of children with retinoblastoma and HRPF who have MD in whom DFS is significantly lower despite intensive adjuvant therapy. Children with group E retinoblastoma and glaucoma have a significantly higher risk of MD at diagnosis.

ID4 ACTS AS A TUMOR SUPPRESSOR IN ER+ BREAST TUMORS

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Inhibitor of differentiation proteins 1, 2, 3 and 4 (ID1–4), are dominant negative regulators of the basic helix-loop helix (bHLH) family of transcription factors. In human tumors, an increased expression of ID proteins has been associated with reversion to an embryonic-like state, loss of differentiation, high rates of proliferation, migration and neo-angiogenesis. In breast cancer there are controversial findings regarding the role of ID4 during tumorigenesis. For instance, ID4 silencing by promoter hypermethylation is a frequent event in ER+ (estrogen receptor) breast tumors and is associated with an increased risk of lymph node metastasis. However, in ER- breast tumors ID4 increased expression has been associated with the ability of cancer cells to exhibit anchorage-independent growth. Our group has previously shown that ID4 promoter's unmethylation is associated with the aggressive Triple Negative Breast cancer subtype. It seems then, that ID4 has a dual role in breast cancer. Here, we hypothesize that ID4 acts as a tumor suppressor in ER+ breast tumors. To test our hypothesis we performed data mining analyses from the TCGA database and cell culture experiments. In silico analyses, in a cohort of 872 invasive ductal breast carcinomas, reveal that ID4 is downregulated in ER+ breast tumors (p<0,001) due to promoter methylation. To test the effect of ectopic ID4 expression, we performed ID4 transient transfection on MCF-7 and T47D breast cancer cell lines. Both are ER+, present ID4 promoter methylation and do not express ID4. Ectopic ID4 expression on MCF-7 and T47D cells lead to decreased proliferation and increased apoptosis. Cell cycle analysis indicated that ID4 transfected cells accumulated in G1 phase. ID4 overexpression also reduced migration rate respect to cells treated with an empty vector. The results presented suggest that ID4 acts as a tumor suppressor in ER+ tumors by leading to reduced proliferation and migration in breast cancer cell lines

DR. CARLOS LANTOS – FUNDACIÓN HONORIO BIGAND: ENDOCRINOLOGY AWARD

SEX DIFFERENCES IN THE DEVELOPMENT OF PROLACTINOMA IN MICE OVEREXPRESSING HUMAN CHORIONIC GONADOTROPIN (HCGβ+): ROLE OF PITUITARY TGFβ1

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TGFβ1 is an inhibitor of lactotroph proliferation and prolactin secretion, and the reduced TGFβ1 activity found in prolactinomas has been proposed to be involved in tumor development. hCGβ+ females, but not males, develop prolactinomas. In a previous work we found that hCGβ+ female pituitaries present decreased active TGFβ1 levels,

TGFβ1 biological activity and TGFβ1 receptors expression compared to their WT counterpart. This weaker TGFβ1 system was proposed to be involved in the development of prolactinomas in this group. The aim of the present work was to complete the previous work analyzing other components of the pituitary TGFβ1 system. As dopamine increases pituitary TGFβ1 expression and activity, acting through the dopamine D2 receptor (Drd2) expressed in lactotrophs, we also evaluated pituitary Drd2 expression, and hypothalamic tyrosine hydroxylase (TH) expression. Levels of LTBP1, Smad4 and Smad7, measured by qRT-PCR, were found increased in male pituitaries compared to females, without differences among genotypes, but were found decreased in tumoral hCGβ+ female pituitaries compared to the WT siblings. The lower expression of TGFβ1 system found in hCGβ+ female pituitaries was accompanied by a lower dopaminergic tone in this group, reflected by decreased hypothalamic TH and increased pituitary Drd2 expression. The high levels of progesterone present in hCGβ+ females could be involved in the decreased expression of hypothalamic TH found in this group. We did not find disturbances, neither in the pituitary TGFβ1 system, nor in hypothalamic TH in hCGβ+ males. We conclude that decreased TGFβ1 expression and activity found in hCGβ+ female pituitaries are undoubtedly involved in the development of prolactinoma in this group. Meanwhile, the stronger TGFβ1 system found in male pituitaries could be protecting them from excessive lactotroph proliferation and prolactinoma development, even in the presence of high levels of hCG.

HYPERMETHYLATION OF HOXA10 BY NEONATAL ENDOSULFAN EXPOSURE: AN EPIGENETIC MECHANISM FOR IMPAIRED EMBRYO IMPLANTATION

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The homeobox gene, Hoxa10, is crucial for uterine development during embryogenesis and for embryo implantation at adulthood. In previous work we demonstrated that neonatal exposure to endosulfan alters uterine Hoxa10 expression in prepubertal rats, and decreases the number of implanted embryos at adulthood. This work investigates the effects of neonatal endosulfan exposure on Hoxa10 uterine expression and DNA methylation status during the pre-implantation period. Newborn female rats were treated by s.c. injections every 48 h, from postnatal day 1 (PND1) to PND7, with corn oil (vehicle, Control), 6 µg endosulfan/kg (Endo6, reference dose EPA) or 600 µg endosulfan/kg (Endo600, no observed effect level, EPA). On PND90 females were pregnant and on gestational day 5 (pre-implantation period) uterine samples were collected. The expression of Hoxa10 was determined at protein and mRNA levels by immunohistochemistry and real time RT-PCR, respectively. mRNA relative expression of the DNA methyltransferases (DNMT) 3a and 3b was also evaluated. Upon Hoxa10 gene we searched for CpG islands and restriction sites for the BstUI enzyme, to evaluate the methylation status of its regulatory regions by Methylation-Sensitive Restriction Enzymes-PCR technique (MSRE-PCR). Predicted binding sites for transcription factors were also investigated. Both doses of endosulfan decreased the expression of Hoxa10 mRNA, while only Endo600 down-regulated Hoxa10 at protein level. Endo6 and Endo600 groups showed increased DNA methylation levels in regulatory regions of Hoxa10 gene, that are potentially regulated by critical transcription factors associated with the implantation process. An up-regulation of DNMT3a and DNMT3b was detected in endosulfan-treated rats. Neonatal exposure to endosulfan decreases uterine Hoxa10 expression during the pre-implantation period, via hypermethylation of regulatory regions of the gene. These alterations could account for the endosulfan-induced implantation failures.

TESTOSTERONE FAVORS A HIGHER RECRUITMENT OF NEUTROPHILS WITH REDUCED EFFICIENCY IN KILLING BACTERIA

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Although androgens have been suggested to exert modulatory functions on adaptive immunity, there is scarce evidence about their role on the innate immune/inflammatory response. Considering that neutrophils are es-

sential effector cells against bacterial pathogens, the aim of this work was to evaluate the effects of testosterone on neutrophils infiltrating the prostate gland. Adult male Wistar rats were first orchiectomized and immediately replaced with testosterone at physiological level (T) or vehicle (OX), and then subjected to acute prostatitis by intra-prostatic inoculation of E. coli (for 5 days, T+BP and OX+BP groups) or LPS (for 24 hs, T+LPS and OX+LPS groups). T+BP animals showed a higher neutrophil infiltration compared to OX+BP, with intense E. coli immunostaining, correlating with the presence of phagocytosed bacteria in active neutrophils by electron microscopy. In LPS-induced prostatitis, testosterone treatment also promoted a higher neutrophil recruitment (Gr+) cells per gland by flow cytometry, which was correlated to an increased mRNA expression of CXCL1 and CXCL2), with the cells having a lower myeloperoxidase (MPO) activity. Interestingly, sorted Gr+ infiltrating neutrophils showed a higher mRNA expression of IL10 and IL6 in T+LPS by qPCR. Finally, testosterone also increased thioglycollate-induced neutrophil recruitment in the peritoneum, with the cells exhibiting a reduced bactericidal ability when coincubating ex vivo with E. coli. These findings reveal a intriguing role for testosterone on the early inflammatory response in the prostate, with neutrophils being a main target. Testosterone increases local chemokine expression, leading to a higher recruitment of neutrophils to the site of infection. However, testosterone favors an IL10high MPOlow phenotype, with reduced efficiency in killing bacteria. This immunomodulatory effect of testosterone represents a novel factor to consider in alternative approches for inflammatory diseases.

INITIAL EXPERIENCE OF MOLECULAR STUDIES IN DIFFERENTIATED THYROID CANCER

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Differentiated thyroid cancer is the most common endocrine neoplasia. The Fine Needle Aspiration (FNA) of thyroid nodules, followed by cytological analysis is the standard preoperative diagnostic procedure, however the indeterminate FNA cytopathology is 10-30%. Molecular studies of indeterminate FNAs reduce the rate of diagnostic surgery and may define the extension of the surgery.

Aims: To evaluate the sensitivity and diagnostic accuracy of molecular studies of thyroid FNAs. To correlate these results with cytological and histological studies of the same patients. To confirm the utility of molecular studies to define diagnostic cytopathology.

The 7 most frequent genetic alterations in differentiated thyroid cancer were studied: mutations in BRAF, K/H/N-RAS genes and RET/PTC 1 and 3, and PAX8/PPARG gene fusions. The diagnosis was made by High Resolution Melting (HRM) for exons with most frequent mutations. Also, commercial kits were used to validate HRM results. Gene fusions were diagnosed by RT-nested PCR.

To date, were analyzed prospectively 80 thyroid FNA samples belong to the gray areas of Bethesda by molecular techniques. Of these, 29 underwent surgery and histological confirmation could be obtained. Of 80 FNAs, 17 were positive for a mutation in 1 of the 7 genes analyzed. Of the 29 patients operated, 13 were positive for 1 of these mutations. Histological analysis confirmed the presence of neoplasia in all positive samples and carcinoma in 11 of 13 molecular positive (PPV 84.6%). Of the 14 samples without mutations, only 2 were papillary carcinoma, the rest patients had benign histology (NPV 87.5%). So far, based on the sample analyzed we have a sensitivity, specificity and diagnostic accuracy above 84%.

The results confirm the high sensitivity and specificity of molecular studies. This methodology is already used in our hospital to improve the preoperative diagnostic accuracy, contributes to risk stratification and helps selecting the appropriate surgery.

AMERICAN SOCIETY OF MICROBIOLOGY AWARD

INFECTOLOGY AND PARASITOLOGY

CESTODE PARASITES SECRETE EXTRACELLULAR VESICLES CARRYING ANTIGENIC PROTEINS AND MICRORNAS

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Cestode parasites are platyhelminths passively transmitted between the hosts involved in their life cycles and can infect almost all vertebrate species. Some of the zoonoses they cause are among the most severe neglected tropical diseases in humans prioritized by the World Health Organization. Lately, several studies described the secretion of extracellular vesicles (EV) as a path of intercellular communication in many organisms and also as a new mechanism of inter-species cross-talk in the host-parasite interplay. The term EV groups varying types of membranous structures which mainly differ in their biogenesis, morphology and protein content. EV can also carry lipids and nucleic acids, including DNA, mRNAs and small RNAs. It has been shown that nematode and trematode parasites secrete EV, which can be internalized by host cells. These EV contain proteins and small RNAs, among which microRNAs were identified. Here, we aimed to determine whether cestode parasites secrete EV and characterize their content. For this, we chose the larval stages of the model cestodes Taenia crassiceps and Mesocestoides corti. First, we demonstrated the in vitro secretion of membranous structures compatible with EV by transmission electron microscopy. Then, we characterized their protein content by LC-MS/MS. As a result we identified expected eukaryotic EV markers and also, among others, proteins tested for immunodiagnosis of cestode infection as well as host immunoglobulins. Finally, we proved by capillary electrophoresis that cestode EV carry small RNAs and then microRNAs were detected by RT-(q)PCR. This is the first report of EV as well as microRNAs secretion in cestode parasites and could represent a new cross-species communication mechanism with the host. We also provide evidence on a new route used by cestode parasites for the secretion of formerly studied proteins. These results provide relevant information for the improvement or development of new diagnosis methods of cestodiases.

CHLAMYDIA TRACHOMATIS NEITHER EXERTS DELETERIOUS EFFECTS NOR FIRMLY ATTACHES TO IN VITRO CAPACITATED SPERMATOZOA

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Chlamydia trachomatis is the most prevalent sexually transmitted bacterial infection among sexually active young adults. Yearly, approximately 100 million new cases are diagnosed worldwide. Moreover, up to 90% infections in women and 50% in men are asymptomatic favoring bacterial spread. However, whether Chlamydia trachomatis has detrimental effects on sperm quality and male fertility is still a controversial issue. In the present study, we analyzed the effects of Chlamydia spp. on in vitro capacitated human and mouse spermatozoa. By in vitro and in vivo assays, we also analyzed the ability of Chalmydia spp. to firmly interact/attach to spermatozoa. Human and mouse sperm were obtained from healthy donors and cauda epididimys from C57BL/6 mice, respectively. Highly motile in vitro capacitated human or mouse spermatozoa were exposed, at different times, to increasing concentrations of elementary bodies of C. trachomatis (serovar E or LGV) or C. muridarum, respectively. Then, several sperm quality parameters were analyzed. In addition, confocal microscopy and in vitro and in vivo approaches were performed to analyze whether Chlamydia spp. firmly attach to spermatozoa. In vitro capacitated human or murine sperm exposed to increasing bacterial concentrations or soluble factors from C. trachomatis or

C. muridarum, respectively, did not show differences in the levels of sperm motility and viability, apoptosis, mitochondrial membrane potential, DNA fragmentation, ROS production and lipid peroxidation, when compared with control sperm ($p>0.05$). Moreover, Chlamydia spp. did not firmly attach to either human or mouse spermatozoa.

In conclusion, our results demonstrate that C. trachomatis does not directly exert deleterious effects on in vitro capacitated spermatozoa. Also, we provide evidence indicating that Chlamydia spp. does not firmly attach to spermatozoa shedding light on an old open question with significant implications for assisted reproduction.

MICRORNAS IN TAENIA CRASSICEPS AND TAENIA SOLIUM: CHARACTERIZATION AND FUTURE APPLICATIONS

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The cestode parasite *Taenia solium* is the etiological agent of neurocisticercosis, one of the 17 neglected tropical diseases prioritized by the WHO. Taenia crassiceps, another cestode from the same genus, is used as a laboratory model of *T. solium*. microRNAs (miRNAs) are small non-coding RNAs considered master regulators of gene expression with key roles in diverse cellular processes. At present there is no evidence of miRNAs from these parasites. High-throughput characterization of small RNAs (sRNAs) in *T. Crassiceps* larval stage (cisticercus) is presented. RNA was purified from cysticerci (3 biological replicates), sRNA library was constructed and sequenced with HiSeq 2500. The mirDeep2 software was used for miRNA identification. The *T. Solium* genome was used for bioinformatics analyses, since it is the closest genome available, allowing also to identify *T. solium* miRNAs at the genome level. Experimental validation of selected sequences was performed by Northern blotting. The results obtained showed that miRNAs were the most abundant category of sRNAs accounting for 83% of mapped reads. A final high confidence set of 42 miRNAs: 38 conserved and 4 novel, was obtained. Northern blot results showed bands compatible with miRNA biogenesis, allowing validating identified miRNAs. Expression analysis showed that few miRNAs accounted for most miRNA expression. Previous results of our group (Cucher et al, 2015; Macchiaroli et al, 2015, Basika et al, 2016) showed that these set of miRNAs is conserved and also highly expressed in other cestodes including *Echinococcus spp*, suggesting important roles in cestode biology. We are currently predicting genes regulated by the highly expressed miRNAs in cestodes, some of which are absent or divergent in the mammal hosts. This is the first report of miRNAs in *T.crassiceps* and *T. solium*. Highly expressed parasite miRNAs absent or divergent in the hosts were indentified and could be candidates for drug and diagnosis targeting.

RUBÉN CHERNY AWARD

HEME OXYGENASE -1 (HO-1) IN THE FOREFRONT OF A MULTI-MOLECULAR NETWORK THAT GOVERNS CELL-CELL CONTACTS AND FILOPODIA-INDUCED ZIPPERING IN PROSTATE CANCER

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Prostate Cancer (PCa) cells display abnormal expression of cytoskeletal proteins resulting in an augmented capacity to resist chemotherapy and colonize distant organs. We have previously shown that heme-oxygenase 1 (HO-1) is implicated in cell morphology regulation in PCa. Here, through a multi “omics” approach we define the HO-1 interactome in PCa, identifying HO-1 molecular partners associated with the integrity of the cellular cytoskeleton.

The bioinformatics screening for these cytoskeletal-related partners reveal that they are highly misregulated in prostate adenocarcinoma compared to normal prostate tissue. Under HO-1 induction, PCa cells present reduced frequency in migration events, trajectory and cell velocity and, a significant higher proportion of filopodia-like protrusions among neighboring cells. Moreover forced-expression of HO-1 was also capable of altering cell protrusions in transwell co-culture systems of PCa cells with MC3T3 cells (pre-osteoblastic cell line). Accordingly, these effects were reversed under siHO. Transcriptomics profiling evidenced significant modulation of key markers related to cell adhesion and cell-cell communication under HO-1 induction. The integration from our omics-based research provides a four molecular pathway foundation (ANXA2/HMGA1/POU3F1; NFRSF13/GSN; TMOD3/RAI14/VWF; PLAT/PLAU) behind HO-1 regulation of tumor cytoskeletal cell compartments. The complementary proteomics and transcriptomics approaches presented here promise to move us closer to unravel the molecular framework underpinning HO-1 involvement in the modulation of cytoskeleton pathways, pushing towards a less aggressive phenotype, showcasing its relevance as a key homeostatic factor against the aggressive disease.

MULTILAYERED VALIDATION OF ABCC4/MRP4 AS A THERAPEUTIC TARGET FOR PANCREATIC CANCER

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most severe types of cancer, and because of its early development of resistance to standard therapeutic agents, and its late diagnosis, it results imperative to identify and validate new and key therapeutic targets. A lot of evidence implicates disturbances in cAMP cascade with PDAC, suggesting the oncogenic potential of this signaling pathway in this setting. In addition to the known classical mechanisms of regulation of cAMP, it was recently described its extrusion to the extracellular compartment mediated by MRP4. The aim of the present work was to validate MRP4 as a therapeutic target and characterize the role of cAMP extrusion in PDAC progression. The analysis of MRP4 expression profiles in human PDAC samples indicated higher levels of expression in tumor cells (normal tissue vs primary tumor; $p<0,01$). We also determined an inverse relationship between MRP4 expression and the probability of survival of patients, establishing a clear association between the staining intensity of MRP4 and Ki67. This suggests the existence of a subset of cells within the tumor that are actively proliferating and express higher levels of MRP4. In vitro assays in PDAC cell lines (PANC-1, BxPC3 and HPAF-II) demonstrated a positive correlation between MRP4 expression, cell indifferentiation and cAMP extrusion. Pharmacological inhibition of MRP4, as well as its specific knockdown by shRNA, led to a significant decrease of cell migration and proliferation ($p<0,01$), the latter by regulating cell cycle progression in G1/S. Both effects were reversed by inhibition of the cAMP-dependant Epac/Rap1 cascade or by adding cAMP acting through a still unknown receptor. Furthermore, silencing MRP4 strongly reduced tumor growth and incidence in nude and NSG animal models. Altogether, these results validate MRP4 as a new therapeutic target for PDAC and demonstrate the oncogenic potential of cAMPextrusion.

ARE HIPPOCAMPAL NMDAR SUBUNITS RAISE AFTER LEARNING TASKS INVOLVED IN MEMORY TRACE?

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NMDA receptors (NMDAR) play a critical role in synaptic plasticity, memory encoding and storage. These receptors are heterotetramers composed by two obligatory GluN1 subunits and two regulatory subunits: GluN2 (A-D) or GluN3 (A-B), being GluN2A and GluN2B the major regulatory subunits in central areas related to cognitive functions. It was already shown that there is an increase on hippocampal GluN1 and GluN2A 70' after 5' exploration of a new environment (open field, OF) leading to habituation of 1,2 and 3 month old Wistar rats. We hypothesized that this NMDAR subunits increase could be related to memory tracing; hence, we investigated if those changes would take place following other learning tasks like an object recognition task (OR). Along 3 consecutive days rats were left to explore an OF for 10', to habituate to it. In the 4th day, rats were exposed to 2 identical novel objects (A-A') for 5' in that familiar OF (training session: Tr); there was no significant difference in exploration time spent at each object. In the 5th day, each rat was left into the arena for 5' with either a familiar and a novel object (A-B), or with two familiar objects (A-A') (test session: Te) and time spent at exploring each object was recorded. Rats spent significantly longer time exploring the novel object than the familiar one. Immediately or 70' after Tr or Te rats were euthanized and hippocampal extracts were analyzed by western blot. There was a significant increase only in hippocampal GluN1 and GluN2A only 70' post-Tr though not after Te, nor at the hippocampus neither at CPF. These results suggest that changes in hippocampal NMDAR subunits could be related to early consolidation of new spatial memories, being involved in memory trace.

DNA REPLICATION IS REQUIRED FOR THE TRANSCRIPTIONAL SWITCH DURING MOUSE EMBRYONIC STEM CELL DIFFERENTIATION

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A central question in developmental biology is how cells adopt different fates during differentiation. Mouse embryonic stem cells (mESCs) provide a good in vitro model to study this, since their differentiation recapitulates early embryonic development. Here, we aimed to gain insight on how transcriptional programs are switched during differentiation, with the hypothesis that the epigenetic transformation underlying gene expression changes is coupled to processes that normally reorganize the structure of chromatin, such as DNA replication. We have previously shown that inhibition of DNA replication when synchronized cultures of mESCs are set to differentiate to epiblast-like cells (EpiLCs) severely abrogates the transcriptional switch (TS) associated with this cell transition. However, inhibition of DNA synthesis is known to activate the DNA damage response (DDR), raising the possibility that failure to differentiate was connected to this process and not to replication itself. In this work, we evaluated the role of DDR in the TS repression upon DNA replication inhibition. We show that inhibition of DNA synthesis with mechanistically unrelated drugs activates DDR, as judged by Chk1 phosphorylation, p53 stabilization and upregulation of the p53 transcriptional target Mdm2. To comprehensively dissect the role of DDR, we used the CRISPR/Cas9 system to generate a mESC knockout line for p53 (p53 KO). After validation of several clonal lines by DNA sequencing and Western blotting, we studied the effect of replication inhibition in synchronized cultures of p53 KO cells differentiating to EpiLCs. Although we observed a partial rescue in the TS to EpiLCs, KO cells never reached the wild type control levels. We further inhibited DDR upstream of p53, targeting ATR and Chk1 proteins, and observed that TS was still inhibited even in the absence of an active DDR. Our results indicate that DNA replication is a critical process in the TS that takes place during cell differentiation.

HOMING AND THERAPEUTIC POTENTIAL OF MESENCHYMAL STROMAL CELLS AS VEHICLES OF ANTIFIBROTIC GENES IN ADVANCED LIVER FIBROSIS: KEY ROLE OF HEPATIC MACROPHAGES.

ESTEBAN JUAN FIORE (1), BAYO JUAN (1), MARIANA MALVICINI (1), ESTANISLAO PEIXOTO (1), CATALINA ATORRASAGASTI (1), ALEJANDRINA REAL (1), MARCELO RODRIGUEZ (1), SOFÍA GOMEZ BUSTILLO (1), MARIANA GARCÍA (1), JORGE AQUINO (2), GUILLERMO MAZZOLINI (1).

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Background: Hepatic macrophages (hMø) have a pivotal role in liver fibrogenesis. Mesenchymal stromal cells (MSCs) are actively recruited to injury sites, show immunomodulatory properties and can be a powerful tool as therapeutic gene carriers. We previously showed anti-fibrotic effects of in vivo application of MSCs engineered to exogenously express insulin growth factor like-I (IGFI MSCs). We aimed to characterize the main cytokines produced by the fibrotic liver involved in MSCs recruitment. We also analyzed the influence exerted by MSCs on hMø and if it could drive liver fibrosis resolution. Metodology: Experimental liver fibrosis was induced in BALB/c mice by 8 weeks administration of thioacetamide. For in vivo tracking of administrated MSC we used Xenogen InVivo Imaging System. Depletion of hMø was performed using clodronate. Results: MSCs in vivo and in vitro migration was higher to cirrhotic livers in comparison with healthy livers. Also, MSCs displayed a high migration to CM derived from liver of cirrhotic patient or cirrhotic mice or a hepatic stellate cell line (LX2). Analysis of cytokines expression by protein array of CM derived from patient and LX2 cells showed high levels of GRO, MCP-1 and IL-8. Incubation of MSCs with antibody against IL-8/GRO receptors resulted in a 50% reduction of their migration capacity toward LX2 CM. hMø isolated from IGFI-MSCs treated fibrotic livers showed reduced expression levels of pro-inflammatory and pro-fibrogenic genes and an up-regulation in pro-regenerative genes vs. control conditions. Similarly, hMø from cirrhotic patients showed a similar shift after incubation with CM from IGFI-MSCs. Factors secreted by MSCs preconditioned hMø reduced the activation status of hepatic stellate cells. Finally, hMø depletion abrogated the therapeutic effect and the pro-regenerative stimuli of IGF1 MSC therapy. Conclusions: Our data provide new early mechanisms which are required for MSCs homing and IGFI-MSCs liver fibrosis amelioration.

YOUNG INVESTIGATOR AWARD - FUNDACIÓN HONORIO BIGAND

NOVEL ROLES OF THE β-GALACTOSIDE-BINDING PROTEIN GALECTIN-1 IN HEPATOCELLULAR CARCINOMA DISSEMINATION

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Hepatocellular carcinoma (HCC) is the third cause of cancer-related deaths annually.

Although HCC treatment has improved during the past decade, its incidence still matches mortality. As metastasis is the most common cause of death among patients with this disease, it is important to explore the mechanisms underlying the spread of HCC cells for the development of new therapeutic agents. Galectin-1 (Gal-1) belongs to a family of lactose-binding lectins characterized by their affinity for β-galactoside moieties. Gal-1 is a multifunctional protein involved in different aspects of tumorigenesis. In human HCC tissues Gal-1 is up-regulated, and this overexpression correlates with HCC cell migration, tumor invasiveness, metastasis, and shortened patient survival. However, the role of Gal-1 in the molecular mechanisms leading to HCC dissemination remained uncertain. Further results obtained in our laboratory provided evidences of the involvement of Gal-1 in HCC cell adhesion, polarization, and epithelial-mesenchymal transition. Moreover, our findings revealed that Gal-1 overexpression, partly induced by transforming growth factor β1 (TGF-β1), promotes HCC cell proliferation, resistance to TGF-β1-induced growth inhibition and glycan-dependent adhesion to liver sinusoidal endothelial cells. Therefore, the novel contribution of Gal-1 to tumor hepatocyte dissemination highlights this glycan-binding protein as an interesting therapeutic target to restrain HCC progression.

RETINOID X RECEPTORS ON SURVIVAL AND MODULATION OF INFLAMMATORY RESPONSE IN A MOUSE MODEL OF RETINITIS PIGMENTOSA

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Retina neurodegenerative diseases, which have no effective treatments, share as a final common step the death of photoreceptors (PhR). Degeneration or altered functionality of retinal pigmented epithelium (RPE) cells may also be involved. Inflammation has a role in these pathologies as well, involving cell types having immunomodulatory capacity such as Müller glial cells and RPE cells. Therapeutic strategies aim to reducing neuronal death or decreasing the effect of inflammation in the initiation and/or progression of these diseases. Retinoid X receptors (RXR) have a unique role in modulation and integration of multiple cell functions. Their agonists have shown beneficial clinical effects in animal models of chronic inflammatory diseases. Since little is known on the roles of RXR in the retina, we investigated whether these receptors might prevent PhR death and control inflammation. Using in vitro models of retinal degeneration induced by oxidative damage, we demonstrated that RXR activation promotes PhR survival and protect RPE cells from apoptosis. Therefore, we turned to a mouse model of Retinitis Pigmentosa, the rd mouse, to analyze the roles of these receptors using primary neuro-glial culture and performing in vivo experiments. We investigate whether the pattern expression of RXR is altered in association with the PhR degeneration, and whether different RXR agonists could modify this pattern of expression and promote PhR survival and an anti-inflammatory response in retina cells with immunomodulatory capacity. To have a global view of the impact of RXR activation in modulating the immune response in these conditions, we also would like to understand how these compounds affect the antiviral drug-response in infected cells. To this purpose we use RPE cells infected with Herpes Simplex-1 virus. Moreover, we are interested in identifying the specific RXR isoform and dimers implicated in the above mentioned effects, so that a pharmaceutical intervention could be developed successfully.

CHEMOTACTIC PATHWAYS INVOLVED IN MESENCHYMAL STROMAL CELL RECRUITMENT TOWARDS HEPATOCELLULAR CARCINOMA AND LIVER FIBROSIS. ROL FOR AUTOCRINE MOTILITY FACTOR, IL-8, GRO AND MCP-1.

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Hepatocellular carcinoma (HCC) is the 2nd cause of cancer-related death worldwide and the majority of patients are diagnosed at advanced stages. New therapies are needed and those focused on the delivery therapeutic genes by mesenchymal stromal cells (MSC) are gaining interest. Our aim was to investigate the chemotactic pathways involved in MSC recruitment towards HCC. We have demonstrated that soluble factors present in the conditioned media (CM) derived from HCC tumors induced in vitro and in vivo MSC chemotaxis towards the tumor. Autocrine Motility Factor (AMF), a cytokine released by HCC cells, has been previously described to stimulate tumor cell motility. In vitro chemotactic assays demonstrated that MSCs migrated to recombinant AMF (rAMF) and AMF blockage with a specific antibody reduced their migration toward HCC CM. Moreover, MSCs-primed with rAMF showed increased in vitro and in vivo migration towards HCC. Recently, we have demonstrated that HCC CM shared the presence of GRO, MCP-1 and IL-8, being the latter with the highest concentration. Blocking and knockdown experiments of MCP-1, IL-8, CXCR1 and CXCR2 reduced >20 % MSC migration. Simultaneous blockage of AMF, CXCR1 and CXCR2 resulted in >60% inhibition of MSC migration to HCC. Stimulation of MSCs with HCC CM (sMSC) resulted in increased in vitro migration and differential expression of ~500 genes, being 46 genes related with cell migration and invasion. Factors produced by sMSC were able to increase fibroblasts, mononuclear and endothelial cells chemotaxis in comparison to factors produced by unstimulated MSCs. We also demonstrated that injection of MSCs, primed with AMF or with HCC CM, in tumor bearing- mice did not modify tumor growth. We conclude that AMF, IL-8, GRO and MCP-1 play a critical role in MSC recruitment to HCC, and stimulation of MSC with rAMF or HCC CM increased MSC homing to HCC, thus becoming a promising strategy to improve their therapeutic efficacy.

IDENTIFICATION OF BIOMARKERS FOR CANCER DEVELOPMENT AND PROGRESSION.

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All types of cancer are caused by the existence of genetic abnormalities within the cancerous cells. These abnormalities consist of the cumulative acquisition of genetic changes that make cells to bypass the cell cycle checkpoints that regulate the normal cell proliferation and physiology. These DNA anomalies are usually translated into mutations or altered expression of RNAs and proteins. In Oncology, it is extremely important that tumors are diagnosed during the early stages and to administer the most appropriate treatment because every time treatments fail, tumors become more aggressive and resistant to therapy, jeopardizing patients´ life. Up to date, there are only few validated biomarkers that are strong predictors of tumor development and progression; for example, the hormonal receptors in breast cancers. However, the availability of development/ progression predictors is non-existing for most types of cancer, and there is an urgent need to discover such biomarkers. The main aim of the projects is to identify genetic abnormalities (mutations, polymorphisms, gene expression patterns) that allow a better characterization of tumors in order to improve the diagnosis and to develop tailored treatments. We previously reported that polymorphisms in GSTP1, GSTT1 and GSTM1 were associated with the risk of disease relapse and shorter relapse-free survival in prostate cancer and childhood acute lymphoblastic leukemia. Currently, we are seeking mutations and gene expression profiles that help to stratify patients into: a) good/bad responders to therapy, b) low/high risk of disease progression, c) low/high risk of developing severe acute therapy-related toxicity. The identification and inclusion of these molecular biomarkers into the clinic will help to improve the diagnosis and prognosis of prostate cancer and acute lymphoblastic leukemia which, in turn, will increase survival and quality of life of the patients.

OCT4 EXPRESSION MEDIATES PARTIAL CARDIOMYOCYTE TRANS-DIFFERENTIATION OF MESENCHYMAL STEM CELLS: POTENTIAL ROLE IN THE CROSS-TALK WITH THE MICROENVIRONMENT AND REGENERATION CAPACITY

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Cell therapy using mesenchymal stem cells (MSCs) has shown their capacity to facilitate both myocardial repair and angiogenesis in models of cardiac injury. Mechanisms mediating tissue regeneration remain unclear and are likely multifactorial. Current opinion favors invoking paracrine actions of MSCs to explain hemodynamic improvement. While trans-differentiation of donor cells into cardiomyocytes is no longer considered a likely possibility, it is unclear whether or not partial cardiomyocyte differentiation enhances the release of soluble growth factors and cytokines in mediating tissue regeneration. Consequently, MSCs interaction with the cardiac microenvironment may be important to engender cardiac repair. OCT4 is a well-known transcription factor that regulates the self-renewal and pluripotency of embryonic stem cells. We recently showed that umbilical cord-derived MSCs have a higher OCT4 expression and an enhanced differentiation potential compared with bone marrow derived-MSCs. Moreover, we found a link between partial cardiomyocyte reprogramming and paracrine effects, as the improvement in cardiac function was significantly higher only after intramyocardial injection of UC-MSCs vs BM-MSCs in a mice model of acute myocardial infarction. Whether OCT4 is involved in this process or mediates MSC multipotency, however, has not been demonstrated. Thus, we investigated the role of the pluripotency factor OCT4 in partial cardiomyocyte reprogramming of MSCs by using our established co-culture system with rat embryonic cardiomyocytes. We found that MSCs must first gain OCT4 (de-diferentiate) before being able to trans-differentiate into cardiomyocytes, a mechanism that resembles the reprogramming process. Moreover, the specific silencing of OCT4 negated not only partial cardiomyocyte reprogramming but also MSCs differentiation into other lineages demonstrating that this factor is essential for the multipotent capacity of MSCs. Our findings suggest new mechanisms that may mediate MSC plasticity and their crosstalk with the microenvironment.

SAI AWARD

DR. LEONARDO SATZ AWARD

DEVELOPMENT OF INNATE T CELLS IN THE THYMUS UNDER INFECTIOUS / INFLAMMATORY SYSTEMIC CONDITIONS

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Our previous work demonstrated that during the acute stage of certain infections (*Trypanosoma cruzi* or *Candida albicans*) with a strong Th1 component, the number of $CD8^+CD44^{hi}$ T cells in the thymus increased. These cells are named "innate T cells" and belong to a different lineage from conventional SP CD8 thymocytes that give rise in the thymus. They express the transcription factor EOMES, produce high levels of IFNg and differentiate in the presence of IL4 and IL15. This effect is mediated by the inflammatory process since we obtained similar data when we induced systemic expression of IL12 and IL18 by hydrodynamic injection of their cDNAs. The aim of our work is to determinate the origin and function of these cells. Our flow cytometry data demonstrated that these cells express the killing receptor NKG2D and have high cytotoxic activity measured by CD107a expression assay ($p<0,05$). Moreover, when mice are adoptively transferred with thymocytes from OT-I *T. cruzi*-infected or IL12 + IL18-treated mice previous to *T. cruzi* infection, a protective effect can be observed since the overall survival is increased and parasitemia is decreased compared to non-transferred control mice ($p<0,05$). When we injected $CD45.1^+$ control thymocytes directly into the thymuses of *T. cruzi*-infected $CD45.2^+$ mice, we observed that $CD45.1^+$ cells up-regulated CD44 and EOMES expression compared to $CD45.1^+$ cells injected in non-infected $CD45.2^+$ recipient mice ($p<0,05$). Moreover, when we co-cultured $CD45.1^+$ control thymocytes whit $CD45.2^+$ *T. cruzi*-infected thymocytes we observed up-regulation of CD44 and EOMES in a IL4 and IL15-dependent manner ($p<0,05$). Our results indicate that under systemic infectious/inflammatory processes, innate $CD8^+$ T cells are generated in the thymus by local IL4 and IL15 production. The presence of non-conventional $CD8^+$ T cells suggests a deviation in the normal thymic ontogeny that may have implication in the output and the repertoire of T cells in secondary immune organs.

GALECTIN-1 (GAL1) AND COMPLEX N-GLYCAN COORDINATELY REGULATE SPONTANEOUS DEVELOPMENT OF AUTOIMMUNITY

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Galectin-1 (Gal1), an endogenous glycan-binding protein, plays a critical role in immune cell homeostasis. Here, we studied the relevance of endogenous Gal1 in the development of spontaneous autoimmunity. We found that aged Gal1-deficient mice (*Lgals1*$^{-/-}$; ~1-2 y) had increased titers of autoantibodies in circulation and more pronounced signs of inflammation and tissue modification in salivary glands (SG) compared to age-matched WT mice. Moreover, *Lgals1*$^{-/-}$ SG showed increased number of infiltrating cells, with a higher frequency of $CD8^+$ T cells. As Gal1 binds to N-acetyllactosamine residues in complex N-glycans, we analyzed signs of inflammation in β1,6N-acetylglucosaminyltransferase V-null (*Mgat5*$^{-/-}$) mice. Similar to *Lgals1*$^{-/-}$ mice, aged *Mgat5*$^{-/-}$ SG showed increased number of ducts/mm^2 and infiltrating cells, with higher proportion of $CD8^+$, $CD4^+$ and $B220^+$ cells, than WT SG. Seeking for possible mechanisms underlyig this effect, we studied migration and activation factors in $CD8^+$ T cells. We found increased expression of CXCL9 and CXCL10 in *Lgals1*$^{-/-}$ SG and higher percentage of $CD8^+CXCR3^+$ and $CD8^+PD1^+$cells in draining lymph nodes (DLN) and SG when compared to WT mice ($P<0.05$). Moreover, *Lgals1*$^{-/-}$ DLN had lower frequency of $CD11c^+$ dendritic cells (DCs), and these cells exhibited altered functionality. When co-cultured with $CD4^+$ cells, *Lgals1*$^{-/-}$ DCs induced higher IFN-g and lower IL-10 production ($P<0.05$), ulti-

mately leading to enhanced CD8+ T cell proliferation *in vitro,* compared to WT DCs. Furthermore, *Lgals1*$^{-/-}$ SG showed lower expression of PD-L1, supporting heightened CD8+ T-cell responses. Finally, we found downregulated Gal1 expression in NOD mice developing spontaneous sialadenitis and administration of rGal1 attenuated SG infiltration (P<0.05). Thus, Gal1 and complex *N*-glycans play critical roles in the control of salivary glands homeostasis, modulation of CD8+T-cell responses and development of autoimmune disease.

ROLE OF PROTEIN S AND TYRO3 RECEPTOR TYROSINE KINASE IN THE PATHOGENESIS OF MULTIPLE SCLEROSIS AND THE TYPE-2 PROTECTIVE ENVIRONMENT.

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Multiple sclerosis (MS) is a chronic inflammatory and autoimmune disorder causing central nervous system demyelination and axonal injury by infiltration of autoreactive Th1/Th17 cells. The inflammatory environment is the main driver of damage and loss of neurologic function. Interestingly, helminth-infected MS patients showed lower number of relapses, lesion activity and minimal changes in disability scores compared with uninfected individuals with MS. Parasite-driven protection was associated with a reduction of pro-inflammatory cytokines via SOCS3, induction of Tregs and IL-10. TYRO3, AXL and MERTK (TAM) tyrosine kinase receptors and its ligand Protein S (PROS1) are critical regulators of the immune response and we have recently demonstrated that Th2 environment enhances PROS1 and TYRO3 expression. We hypothesize that the engagement of PROS1-TYRO3 axis will be enhanced during helminth infection negatively regulating an associated inflammatory response Th1/Th17. We evaluated TAM receptors and PROS1 expression in peripheral blood monocytes, dendritic and T cells compartment of patients with diagnosed MS (N=18), helminth-infected patients (HP, N=8) and healthy controls (HC, N=13-20). We found a significant increased level of PROS1 and MERTK in blood CD4 T cells of MS patients compared to HC. However, after in-vitro TCR activation, CD4 T cells from MS induced lower levels of PROS1 (100.3 ± 5.6) vs HC (120.7± 2.6) or HP (137.8 ± 6.0) measured as mean fluorescence intensity. Furthermore, PROS1 levels in CD4 + /IFNg + and CD4 + /IL13 + was higher in HP > HC > MS. Interesting, CD1c dendritic cells showed higher TYRO3 expression in HP (10.4 ± 0.9) vs HC (6.5 ± 0.3) and MS (4.9 ± 0.3) measured as fold increase referred to isotype. Our results suggest that the enhanced PROS1/TYRO3 axis in HP could be favoring a more efficient engagement of this anti-inflammatory pathway and could contribute to explain the parasite-driven protection observed in helminth-infected MS.

PROTUMORAL PROPERTIES OF NEUTROPHIL EXTRACELLULAR TRAPS (NETS) IN CHRONIC LYMPHOCYTIC LEUKEMIA.

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We previously reported that PMN from CLL patients are prone to release extracellular traps (NETs) in response to PMA. In the present work we extended these findings using ionomycin (1µg/ml) or TNF-α (10 ng/ml) plus LPS (100 ng/ml) to induce NETosis. We found higher concentrations of DNA and increased elastase activity in supernatants from CLL-PMN compared to those from healthy donors (HD) PMN (n=6, p<0.05 for ionomycin, n=5, p<0.05 for TNF-α+LPS). These differences in NETs formation were corroborated by fluorescent microscopy using propidium iodide and anti-elastase Ab. Our previous findings showed that plasma from CLL patients prime neutrophils to form NETs. Given that levels of IL-8, a cytokine involved in NET induction, are higher in CLL plasma, we supplemented HD plasma with IL-8 (0.15 ng/ml, the average concentration in our CLL patient cohort) and preincubated

HD PMN before triggering NETosis. IL-8 supplemented HD plasma promoted a higher response to PMA (n=5, p<0.05). Moreover, CLL plasma induced the activation of HD neutrophils as assessed by cell size increase and upregulated the expression of the IL-8 receptor, CXCR2 (n=6, p<0.05) without modifying CXCR1 levels. Given that both, stimulating and deleterious effects of NETs have been reported depending on the experimental model, we determined if NETs could modify the survival of leukemic B cells from CLL patients. Using NETs prepared with ionomycin or TNF-α+LPS we found protection from spontaneous apoptosis (n=10, p<0.05) and upregulation of the activation markers CD80, CD86 and CD69 (n=10, p<0.05). Of note, NETs were unable to delay apopotosis of B cells purified from HD blood. Our study provides new insights into the immune dysregulation in CLL and suggests that the chronic inflammatory environment typical of CLL probably underlies this inappropriate neutrophil priming.

SAFE AWARDS

BEST PRESENTATION AWARD

FLAVONOIDS ISOLATED FROM DALEA ELEGANS INHIBIT MELANOGENESIS IN MOUSE B16 MELANOMA CELLS

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Tyrosinase inhibitor compounds have importance in the treatment of hyperpigmentation diseases and are used as whiteners agents in cosmetics. Several currently marketed whiteners have adverse effects; for example Kojic acid (KA) is genotoxic, hepatocarcinogenic and produces dermatitis. For this reason is important the researching for new inhibitors of tyrosinase. Previously we have reported an important inhibitory activity on mushroom tyrosinase by a prenylated flavanone (8PP) and a chalcone (Triangularin) isolated from roots and aerial parts of Dalea elegans Gillies ex Hook. & Arn. In order to investigate this condition in cell line, we evaluate the melanogenesis inhibition of these compounds on mouse B16 melanoma cells through the tyrosinase intracelular inhibition and the extracelular melanin inhibition by spectrophotometrically measuring of the adduct formation between 3-methyl-2-benzothiazolinone and dopaquinona. The cytotoxicity assay was performed by MTT methodology. The maximum non-cytotoxic concentration (MNCC) for 8PP, Triangularin and KA were of 10 µM, 100 µM and 5000 µM, respectively and according with these results we evaluated the melanogenesis inhibition. The results demonstrated that these compounds have the ability to penetrate the membrane of B16 cells and inhibit the tyrosinase intracelular at non-cytotoxic concentrations. Comparing the inhibition % of each compound with reference inhibitor KA, 8PP and Triangularin would be two hundred-fold and five-fold more active than KA, respectively. In addition, it has been observed that these compounds decreased extracellular melanin. The 50 % of inhibition for 8PP and Triangularin were 1 and 25 µM, respectively. For KA, the 50 % of inhibition was 2000 µM. So, 8PP and Triangularin would be two thousand and eighty-fold more active than KA, respectively. It would be needed PCR and Western blot studies to establish the mechanisms by which these compounds act on melanin biosynthesis.

DEVELOPMENT OF A DRUG DELIVERY SYSTEM (DDS) BASED ON POLYMERIC NANOPARTICLES: THE POSSIBILITY OF AN ORAL ADMINISTRATION ROUTE FOR INTERFERON ALPHA

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Interferon alpha (IFNa) is a protein drug used to treat oncological diseases and viral infections. Owing to its sensitivity to enzymatic degradation and limited absorption in the gastrointestinal tract, pegilated IFNa is administered via parenteral route once weekly which is associated with pain, allergic reactions and poor patient compliance. To

overcome these problems, the design of a suitable drug delivery system (DDS) able to protect the drug in order to administer it orally would lead not only to greater acceptance and adherence to the treatment but also to a better quality of life for patients. In this context, we prepared IFNa2b loaded chitosan nanoparticles (IFN CS NPs) by ionotropic gelation method. Infrared spectra supported the formation of CS NPs. The amount of CS that formed NPs, colorimetrically determined, was 95.5%. Size, determined by dynamic light scattering (DLS), showed a bimodal distribution; the mean sizes were 381.7 ± 35.2 nm and 50.17± 6.96 for blank CS NPs, 353.0 ± 31.2 nm and 42.49 ± 23.75 for IFNa-loaded ones. The polidispersity index was 0.472 ± 0.030 and 0.407 ± 0.010, while the zeta potential (Z-Pot) 31.4 ± 4.6 mV and 31.8 ± 1.7 mV, respectively. The Z-Pot value suggests not only a net positive surface charge but also physical stability of the DDS as was confirmed at 4 and 25°C for 30 days by DLS results. The encapsulation efficiency was 99.5%. Transmission electron microscopy confirmed the size obtained by DLS results. The antiviral activity of encapsulated IFN determined in Vesicular Stomatitis Virus (VSV) infected MDBK cells, was comparable to commercial IFN. Preliminary pharmacokinetic studies in Balb/C mice showed absorption of IFNa2b after oral administration of IFN loaded CS NPs in opposition to different studies in which the drug was not detected in plasma following administration of free drug. These promising CS NPs show great potential for application in oral delivery of IFNa2b allowing an enhancement of patient compliance.

SELF-GELLING ELASTIN AND SILK-ELASTIN RECOMBINAMERS FOR TIMOLOL OPHTHALMIC ADMINISTRATION IN THE TRATAMIENT OF THE GLAUCOMA

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The development of topical ophthalmic formulations for the treatment of eye diseases such as glaucoma, present a challenge, since most drugs are hardly absorbed, having bioavailabilities from 1-10%. An alternative to increase the residence time of formulations is the use of bioadhesive systems. Taking this into account, here we developed an elastin-like and a silk-elastin like recombinamers, named respectively as ELR and SELR, for their incorporation in an ophthalmic formulation against glaucoma. Our hypothesis is that due to the thermo-sensitive behavior of these materials, the formulation could be administered topically as drops, and once sensing the temperature of the eye, suffer a shift to a gel system, which could help to prolong the permanence of the formulation in the eye, and therefore, could enhance the therapeutic effect.The thermosensitive gels each system Timolol (T) was charged and the release of T and erosion of ELR and SELR were evaluated for 8 hs. In vivo studies were performed on New Zealand rabbits. Each formulation was placed into the conjuntival fornix and the intraocular pressure (IOP), Irritation and Adhesion were measure.Both recombinamers display clearly differences on the release kinetics. It was determined using the model Korsmeyer, The results indicated the percent release at 8 hours was 80.39% and 40.04% of T for ELR and SELR, respectively. The rate constant, incorporating structural and geometric characteristics of the system was higher for ELR indicating a faster rate of drug release. In both cases the release mechanism T responded to an anomalous diffusion. This behavior can be inferred that the same erosion/dissolution of the matrix predominates in drug release, which coincides with erosion studies indicate greater resistance and mechanical stability of the gels SELR. *In vivo* tests evidenced that formulations containing the recombinamers further decreased the IOP when compared with the control solution formulation. Furthermore, SELR-formulation was found to be more effective that its counterparts ELR-formulation, which agrees with the enhanced mechanical properties provided by the presence of the silk moieties. These results evidenced that the self-gelling ELR and SELR have great potential for its use as components of ophthalmic pharmaceutical formulations.

EVALUATION OF SCHEDULE-DEPENDENT EFFECTS OF CHEMOTHERAPY IN VITRO AND IN ONE IN VIVO RETINOBLASTOMA MODEL

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Current treatment of retinoblastoma involves using the maximum dose of chemotherapy that induces tumor control and is tolerated by patients. The effect of metronomic chemotherapy treatment has not been assessed for retinoblastoma and may aid to decrease the incidence of adverse events by using lower doses. Our aim was to evaluate the cytotoxic and antiangiogenic effect of chemotherapy used in the clinics using different in vitro and in vivo models. Two patient-derived retinoblastoma cell types (007 and 008) and two human vascular endothelial cell types (HUVEC and EPC) were exposed to increasing concentrations of melphalan or topotecan in a conventional (single dose) or metronomic (7-day exposure) treatment scheme. The concentration of chemotherapy causing a 50% decrease in cell proliferation (IC50) was determined by MTT. The effect of treatments on endothelial cells was assessed by the ability of tube formation using matrigel assay. We also evaluated the in vivo response to conventional and metronomic topotecan in a retinoblastoma xenograft model. We compared the vascular density of tumors after treatments using CD31.

Melphalan and topotecan were cytotoxic to both retinoblastoma and endothelial cells after the two treatments schemes. Whereas the IC50 for melphalan and topotecan significantly decreased after metronomic compared to conventional treatment in endothelial and 008 cells ($p<0.05$), no change in the sensitivity to both agents was evident for 007 ($p>0.05$). Metronomic topotecan or melphalan significantly inhibited in vitro tube formation in HUVEC and EPC compared to vehicle treated cells ($p<0.05$). In mice, continuous topotecan lead to significantly lower tumor volumes compared to conventional ($p<0.05$). CD31 expression was lower after metronomic compared to conventional treatment ($p<0.05$). We propose that metronomic chemotherapy may be a valid option for retinoblastoma treatment based on the observed cytotoxic and antiangiogenic effect.

INCREASED INFLAMMATORY CELL PROFILE TOGETHER WITH MERTK UP REGULATION AND T CELL-DERIVED PROTEIN S REDUCTION IN IBD PATIENTS

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Background: Crohn's disease (CD) and Ulcerative Colitis (UC) are Inflammatory Bowel Diseases (IBD) characterized by chronic inflammation and tissue damage. Protein S (PROS1), an agonist of TYRO3, AXL and MERTK (TAM) receptors, is expressed upon T cell activation reducing dendritic cell activation. Genetic ablation of Axl and Mertk increases the inflammatory signature and loss of tissue repair macrophage phenotype in a mouse model of IBD. Purpose: Our goal was to characterize the immune compartments, TAM receptors and PROS1 expression in monocyte/macrophage and lymphocytes of IBD patients. Results: Blood mononuclear cells from 33 IBD patients (16 CD/17 UC) classified as active disease (AD) or in remission by CDAI and Mayo scores, and 35 healthy controls were analyzed by FACS. Increased % of $CD14^{high}CD11b^{high}CD11c^{+}$ monocytes were observed in active IBD (21.1 ± 2.2 N=20) vs remission (9.6 ± 1.0 N=13) and controls (8.7 ± 0.6 N=19) and positively correlated with the CDAI ($r=0.67$ $p<0.01$) or Mayo scores ($r=0.82$ $p<0.001$). Interestingly, we observed an increased % of $CD11b^{low}CD64^{+}CD206^{-}$ cells in active IBD (48.2 ± 9.7 N=9) vs remission (13.3 ± 4.2 N=12) and controls (7.2 ± 1.2 N=14). Although AXL is highly expressed in circulating monocytes, no differences between IBD vs controls were observed. However, we do detect a significant up regulation of MERTK comparing IBD vs controls ($p<0.01$). TYRO3 was differentially expressed in monocytes of only CD vs controls. In-vitro TCR stimulation with a-CD3/CD28 leads to a significant lower level of PROS1 in CD4 T cells of active CD. Conclusions: Our results show a clear inflammatory cell profile with increased level of circulating monocytes and $CD64^{+}CD206^{-}$ cells in active IBD. Interestingly, MERTK that is associated with tolerogenic responses was consistently up regulated in monocytes of active IBD. Moreover, CD4 T cells showed reduced levels of PROS1 after activation reflecting a more inflammatory memory profile in IBD patients.

SUBLINGUAL ADMINISTRATION OF TACROLIMUS IN PEDIATRIC LIVER TRANSPLANT PATIENTS

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Tacrolimus (FK) has led the market during the last ten years, as the calcineurin inhibitor of choice for prevention and treatment of rejection in solid organ transplantation. FK products include capsules and intravenous formulations. However, young children have difficulties in swallowing the capsules immediately post transplantation. Moreover, intravenous FK is very toxic. Therefore sublingual (SL) administration is an alternative to achieve therapeutic levels and avoid early graft rejection.

Objective: to study safety and efficacy of SL FK administration in pediatric patients who could not swallow FK capsules due to age, mechanical ventilation and/or sedoanalgesia, during their stay in the Intensive Care Unit post-transplantation.

Methods: pediatric patients with biliary atresia transplanted in 2014-2015 were studied. Trough FK levels, adverse events, clinical parameters and drug-drug interactions were recorded. Wilcoxon matched pairs test was used. Efficacy was evaluated by the occurrence of acute rejection (AR).

Results: 22 patients were included, with a median (range) follow-up and age of 2 days(6-68) and 0.9 years (0.6-6.3), respectively. Three AR and 3 adverse events (nephrotoxicity, hypomagnesemia and neurotoxicity) occurred during the study period. The median (range) daily dose and trough FK levels was 0.11mg/kg(0.02-0.31) and 6.4ng/ml(2.0-23.2), respectively. During concomitant administration of clarithromycin, a significant increase was observed in dose normalized FK trough levels ($p<0.05$).

Conclusion: Safety and efficacy parameters of SL-FK administration were studied in pediatric liver transplant patients who had difficulties in swallowing the capsules, under mechanical ventilation and/or sedoanalgesia. According to FK blood levels achieved, the SL route was effective. FK-clarithromycin interaction may affect safety of SL-FK administration. This study emphasized the role of therapeutic monitoring to maintain FK blood levels within the therapeutic range.

CD207+/CD1A+ CIRCULATING CELLS ARE PRESENT IN PATIENTS WITH ACTIV LANGERHANS CELL HISTIOCYTOSIS

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Background: Langerhans Cells Histiocytosis (LCH) is a disorder characterized by an abnormal accumulation of $CD207^{+}CD1a^{+}$ myeloid cells in almost any tissue. The precursors of these pathogenic cells were not yet clearly defined in vivo, however, it has been shown in vitro that monocytes and $CD1c^{+}$ dendritic cells can achieve high levels of CD207 and CD1a when exposed to TGFb and TSLP. We hypothesized that precursor cells expressing CD207 and/or CD1a should be circulating in active multisystem LCH patients. Methods: Pediatric patients with confirmed diagnosis of LCH were stratified as unisystem or multisystem; unifocal or mutifocal; with active disease (AD) or non-active disease (NAD). CD1a and CD207 expression was analyzed in blood mononuclear cells of LCH children and compared with same compartment of cells from healthy adults and umbilical cord blood by FACS analysis. Plasma TSLP and TGFb were analyzed by ELISA. **Results:** The myeloid compartment showed a significant increase of CD11b fraction including $CD11b^{high}$ plus $CD11b^{+}$ (36.4 ± 3.7, N=11) in AD vs NAD patients (18.9 ± 1.7, N=12) and healthy adults (24.9 ± 1.3, N=12). We have also identified the presence of high percentage of circulating $CD11b^{high}CD11c^{+}CD207^{+}$ cells (39 ± 9.4, N=12) in AD vs NAD (3.1 ± 0.5, N=11), healthy adults (0.6 ± 0.4, N=9) and umbilical cord blood (2.1 ± 0.5, N=5). Moreover, circulating $CD11c^{high}CD207^{+}CD1a^{+}$ cells (19 ± 7.4, N=12) are present in active multisystem patients compared with NAD (2.1 ± 0.4, N=12), healthy adults (0.7 ± 0.3, N=8) and umbilical cord blood (2.8 ± 1.1, N=4). TSLP and TGFb levels were significantly increased in active LCH compared to non-active patients. **Conclusions:** Circulating monocytes expressing CD207 as well as DCs expressing CD207/CD1a were found in multisystem active disease. In concordance with this result we observed increased plasma levels of TSLP and TGFb in active LCH patients suggesting that these cytokines could be key drivers of pathogenic LC in vivo.

BENEFICIAL PROPERTIES OF METHYL GALLATE ON EXPERIMENTAL COLITIS

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Chronic inflammation of the gastrointestinal tract is observed in inflammatory bowel disease (IBD). This condition encompasses two major conditions, known as Crohn's disease and ulcerative colitis. To date, no complete response has been achieved with conventional therapies, therefore, the development of new therapies is an important goal in the IBD therapy. Methyl gallate (MG) is a gallotannin that is widely distributed in herbal medicines, food plants and mushrooms. Previous studies reported that this bioactive phenolic compound presents antioxidant, anti-inflammatory, antimicrobial and anti-tumor activities. The aim of this study was to test the activity of MG on an experimental colitis model. Doses of MG (100 and 300 mg/Kg, vo) and mesalazine (100 mg/kg, vo), reference drug, were tested in colitis model, inducing by intracolonic instillation of a 2 mL of 4% (v/v) acetic acid solution. MG induced a significant reduction in the colon weight/length ratio, expressed in mg/2cm (control: 155.1±9.4, colitis: 545.2±36.3, MG 100 mg/Kg: 528.5±67.0, MG 300 mg/Kg: 374.0±34.7, mesalazine: 329.3±36.9), macroscopic lesion score (control: 0.14±0.08, colitis: 3.4±0.2, MG 100 mg/Kg: 2.5±0.6, MG 300 mg/Kg: 2.2±0.5, mesalazine : 1.8±0.2), GSSG/GSH ratio (control: 0.04±0.02, colitis: 0.70±0.2, MG 100 mg/Kg: 0.05±0.02, MG 300 mg/Kg: 0.06±0.02, mesalazine: 0.09±0.02), showing a similar pattern in TBARs levels. Na+K+ATPase activity were recovered in treated groups (control: 827.2±59.6, colitis: 311.6±54.8, MG 100 mg/Kg: 642.2±175.0, MG 300 mg/Kg: 809.7±100.6, mesalazine: 525.3±81.7). Futhermore, MG reduced the overexpression of COX2, IL-6, TNF and the severity of microscopic tissue damage induced by acetic acid intracolonic. The recovery of number of globet cells and mucin production in treated groups was also observed. Therefore, this study could demonstrate that methyl gallate possesses beneficial properties in a preclinical model of inflammation bowel disease.

PHARMACOKINETIC/PHARMACODYNAMIC ANALYSIS OF A THERAPEUTIC REGIMEN OF MARBOFLOXACIN IN GOATS WITH MASTITIS BY MONTE CARLO SIMULATION

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Goat mastitis generates economic losses and threatens public health. Coagulase-negative staphylococci (CNS) and *S. aureus* are principal pathogens. Marbofloxacin (MFX) is a fluoroquinolone approved for veterinary use indicated for mastitis. The objectives of this study were to evaluate the pharmacokinetics and milk bioavailability of MFX by IM route in lactating goats, determine MIC and MPC from regional pathogens make a PK/PD analysis by Monte Carlo simulation, and correlate the PK/PD results with clinical response and milk culture. Seven goats with mastitis by CNS were included. MFX was administered by IM route at 10mg/kg/24hx5days. Milk production, pH and culture were performed once a day. Serum and milk samples were quantified by microbiological method, and a non-compartmental model was used. Milk bioavailability of MFX was evaluated by AUC24milk/serum ratio. MICs and MPCs were obtained from regional strains of CNS (*n*=106) and *S. aureus* (*n*=8) isolated from caprine mastitis in Córdoba, Argentina. 10000-subjects Monte Carlo simulation was carried out. PK/PD endpoints were AUC/MIC>40, 50 and 60, C_{max}/MIC>10, AUC/MPC>13.5 and Cmax/MPC>1.2. Probability of target attainment (PTA) and cummulative frequency of response (CFR) were calculated. AUCmilk/serum ratios were >1. During treatment, milk production increased and pH decreased in infected udders, consistent with lower values of AUC in serum and milk. No pharmacokinetic differences in milk between healthy and infected udders were observed. All animals completed the study with negative cultures. The proposed dose regimen of MFX presented a PTA>90% only for MICs of 0.2-0.4µg/ml for all PK/PD endpoints. CFR was >90% for all endpoints. For AUC/MPC endpoint a PTA>90% were achieved only for MPCs of 0.8-1.6µg/ml. CFR was <25% in all cases. For Cmax/MPC endpoint, a PTA>90% was observed with MPCs of 0.8-3.2µg/ml, with a CFR<75%. The results indicate that the proposed dose regimen of MFX in goat mastitis has good efficacy, but may promote the emergence of resistance.

INCREASED EXPRESSION OF TYRO3 AND PROTEIN S IN CIRCULATING CD11B+ CELLS OF PATIENTS WITH ACTIVE LANGERHANS CELL HISTIOCYTOSIS

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Background: TYRO3, AXL and MERTK (TAM) tyrosine kinase receptors and its cognate agonist Protein S (PROS1) have been identified as negative regulators of the immune response as well as non-classical proto-oncogenes, aberrantly expressed in multiple haematological and epithelial malignancies. Langerhans Cell (LC) Histiocytosis (LCH) is a disorder characterized by an abnormal accumulation of CD207+CD1a+ myeloid cells in almost any tissue. The etiology of this disease is still under scientific discussion and it is not clear if LCH results from malignant transformation orunbalanced immune response that leads to the proliferation of pathogenic LC-like cells. Our aim is to explore the role of the TAM axis in the pathogenesis of pediatric LCH. Methods: We analyzed the expression of TAM receptors and PROS1 in peripheral blood mononuclear cells of pediatric patients with confirmed diagnosis of LCH stratified as unisystem or multisystem with active disease (AD) or non-active disease (NAD) and adult controls. The expression levels of PROS1 and TAM receptors were determined by flow citometry and expressed as fold increase of mean fluorescence intensity (MFI) compared to the isotype control. Results: Circulating total $CD11b^+$ fraction was significantly expanded in AD (36.4 ± 3.7% N=11) vs NAD (18.9 ± 1.7% N=12) and adult controls (24.9 ± 1.3% N=12). Interestingly, this fraction that is consider the main source of inflammatory myeloid cells, showed higher levels of PROS1 in AD (14.2-fold N=4) compared with NAD (6-fold N=5) and adult controls (6.7-fold N=6). TYRO3 was also up regulated in circulating CD11b+ cells in AD (10.6-fold N=8) compared with NAD (5-fold N=9) and adult controls (4.5-fold N=6). Conclusion: Our results show that higher levels of TYRO3 and PROS1 are associated with active and multisystem LCH suggesting that this axis could be involved in the expansion of precursor and pathological LC-like cells.

ENHANCEMENT OF THERMAL NOCICEPTION AND ASTROCYTE REACTIVITY IN SOMATOSENSORIAL CORTEX INDUCED BY AMPHETAMINE INVOLVES CENTRAL AT1 RECEPTOR ACTIVATION

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The use of psychostimulants, such as amphetamine (Amph), is associated with inflammatory processes over glia and vasculature. Brain Angiotensin II (Ang II), through AT_1-receptors (AT_1-R), modulates dopaminergic neurotransmission and plays a crucial role in inflammatory responses in brain vasculature and glia. Studies from our laboratory showed the involvement of AT_1-R on astrocyte reactivity and neuronal survival in the pre-limbic cortex after repeated exposure to Amph. Our aim for the present work was to extend the role of AT_1-R in alterations induced by repeated exposure to Amph. Astrocyte reactivity, neuronal survival and brain microvascular network were analyzed at the somatosensory cortex. The thermal nociception was evaluated as a physiological outcome of this brain area. Male Wistar rats (250-320g), at standard laboratory conditions, were administered with AT_1-R antagonist Candesartan/vehicle (3 mg/kg p.o., day 1-5) and Amph/saline (2.5 mg/kg i.p., day 6-10). On day 17, animals were sacrificed and the brains processed for immunohistochemistry against Von Willebrand factor and glial fibrillary acidic protein (G-FAP), and Nissl staining. Thermal nociception was evaluated using hot plate test on day 17 in another group of animals. Data were analyzed with two-way ANOVA followed by Bonferroni test. Our results indicate that Amph exposure induces an increase in: occupied area by vessels and their tortuosity, astrocyte reactivity and neuronal apoptosis. Moreover, Amph exposure decreased the paw lick threshold behavior. Pretreatment with candesartan prevented the described alterations induced by psychostimulant. The Amph-induced structural changes at somatosensorial cortex, involving astrocytes, vasculature and neurons, implies AT_1-R activation. The decreased thermal nociception and the structural changes could be considered as extended neuroadaptative responses to Amph.

INHIBITORY EFFECT OF MELATONIN ON MAST CELL ACTIVATION

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Melatonin is a chronobiotic hormone widely distributed in the body. It has a variety of extrapineal non chronobiotic functions such as neuromodulator, antiproliferative, antioxidant, immunomodulatory and oncostatic actions. Immunomodulatory effects are exerted on different immune cells. Mast cells have been involved in the pathogenesis of a number of immune and inflammatory disorders including contact dermatitis, allergic rhinitis, asthma, cancer, multiple sclerosis, rheumatoid arthritis, ulcerative colitis and peptic ulcer. However, the effect of melatonin on mast cell activation remains unknown. The aim of this study was to determine whether melatonin inhibits mast cell activation induced by mast cell secretagogues that act by different molecular mechanisms of action. Peritoneal mast cells from adult male rats were removed and then purified and activated with compound 48/80, calcium ionophore A23187, neurotensin or a phospholipase A_2 activator peptide (PLA_2AP). Morphological studies by light microscopy and confocal fluorescence microscopy were performed. The percentage of serotonin release was determined by HPLC as a marker of degranulation. Melatonin inhibited mast cell activation induced by the calcium ionophore A23187 (10 µg/ml A23187: 16.35±1.6% versus 20 µg/ml melatonin: 2.8±0.21%, $P<0.001$) and did not alter mast cell activation induced by compound 48/80, neurotensin or PLA_2AP. Granule morphological changes induced by the calcium ionophore A23187 were also inhibited by melatonin at non-cytotoxic doses, suggesting an interaction of the hormone with calcium-binding proteins, among other mechanisms. Melatonin is an attractive molecule, which could be useful for prevention and/or treatment of mast cell-mediated disorders.

PREMIO JOVEN INVESTIGADOR / YOUNG INVESTIGATOR AWARD

DESARROLLO DE ESTRATEGIAS FARMACÉUTICAS PARA LA REDUCCIÓN DE LA TOXICIDAD LEUCOCITARIA, INCREMENTO DE LA ACTIVIDAD ANTIBIOFILM Y MEJORA DE LAS PROPIEDADES FISICOQUÍMICAS DE CLORANFENICOL/ DEVELOPMENT OF PHARMACEUTICAL STRATEGIES FOR THE REDUCTION OF LEUCOCYTIC TOXICITY, INCREMENT OF ANTIBIOFILM AND IMPROVEMENT OF PHYSICOCHEMICAL PROPERTIES OF CHLORANPHENICOL

MEJORA DE LAS PROPIEDADES FISICOQUÍMICAS DE CLORANFENICOL MEDIANTE COMPLEJACIÓN CON CICLODEXTRINAS Y AMINOÁCIDOS/ IMPROVEMENT OF PHYSICOCHEMICAL PROPERTIES OF CHLORAMPHENICOL BY CYCLODEXTRINE AND AMINOACID COMPLEXATION

ARIANA ZOPPI

Las infecciones causadas por patógenos multirresistentes a antimicrobianos requiere la introducción de ingredientes farmacéuticos activos (IFA) nuevos para su tratamiento. Entre 1980 y 1984, la Food and Drug Administration aprobó 20 antimicrobianos (ATM) nuevos, sin embargo entre 2005 y 2009 sólo tres fueron aprobados. Esta disminución en la aprobación de nuevos ATM refleja la caída brusca de la productividad en el sector de desarrollo de ATM de la industria farmacéutica. También, la creciente resistencia en las bacterias exige que los nuevos agentes presenten diferentes mecanismos de acción, lo que aumenta aún más el desafío. De acuerdo a lo expuesto se torna más factible y promisoria la aplicación de la síntesis supramolecular para un mejor aprovechamiento de los ATM de uso aprobado como es el caso de CP. El desarrollo de sistemas supramoleculares es un campo muy activo, con retos pendientes que extienden la actividad tradicional dedicada a la preparación de nuevos IFA, hacia la obtención de materiales con características determinadas, que se puedan seleccionar en lo posible sobre la base de mejorar los inconvenientes que presenten ATM de importante relevancia terapéutica, tales como solubilidad, estabilidad, permeabilidad y toxicidad. La formación de complejos ofrece la oportunidad de modificar la composición de un ATM y sus propiedades fisicoquímicas, biofarmacéuticas, microbiológicas, toxicológicas y farmacotécnicas, sin alterar los enlaces covalentes preexistentes en este, lo que implica una mejora indiscutida en la relación costo-beneficio de la obtención de nuevos medicamentos ya que se ven acorta-

dos ciertos plazos de obtención, especialmente aquellos relacionados con el descubrimiento y la toxicología. CP presenta baja solubilidad en soluciones acuosas por lo cual la formación de complejos multicomponentes con ciclodextrinas utilizando compuestos con actividad antioxidante como tercer componente (cisteína, glicina y N-acetilcisteína) resultó una estrategia útil para sortear este inconveniente.

ESTRATEGIAS FARMACÉUTICAS PARA PREVENIR EL ESTRÉS OXIDATIVO INDUCIDO POR CLORANFENICOL EN LEUCOCITOS/PHARMACEUTICAL STRATEGIES TO PREVENT OXIDATIVE STRESS INDUCED BY CHLORAMPHENICOL IN LEUCOCYTES

VIRGINIA AIASSA

Es ampliamente conocido que CP está relacionado con la producción de anemia aplásica en personas sensibles o en casos de sobredosis. La depresión de la médula ósea (MO) es el efecto adverso más serio del cloranfenicol. Existen dos tipos de depresión de la MO: una que no depende de la dosis administrada ni del tiempo de uso (anemia aplásica irreversible) y otro dependiente de la dosis y de las concentraciones plasmáticas que revierte espontáneamente al suspender la medicación. Estos efectos secundarios graves e incluso fatales limitan el uso de este fármaco.

La toxicidad de diversos fármacos está relacionada con un aumento de la producción de especies reactivas de oxígeno (ROS) con la consecuente producción de estrés oxidativo. Por lo tanto, el desarrollo de ensayos de toxicidad para evaluar alteraciones metabólicas previas a la anemia aplásica podría ser útil para reducir este grave riesgo. Estudios previos han demostrado que las ROS y la producción de nitrito, junto con la alteración de las enzimas antioxidantes, pueden explicar la leucotoxicidad de CP. De hecho, se sabe que las células contienen algunos sistemas antioxidantes para protegerse de la lesión inducida por el aumento de ROS intracelular. En consecuencia, resultó interesante determinar el efecto de los antioxidantes glicina, cisteína y N-acetilcisteína (utilizados como tercer componente en los sistemas supramoleculares) sobre el estrés oxidativo causado por CP en leucocitos humanos. Nuestros resultados confirmaron la producción de estrés oxidativo en leucocitos inducido por CP, mientras que, cuando se ensayaron los sistemas supramoleculares multicomponentes, la producción de ROS fue significativamente menor incluso que en las muestras no tratadas indicando de este modo las ventajas de estas formulaciones de múltiples componentes que contienen glicina, cisteína o N-acetilcisteína en la reducción de los efectos nocivos de CP.

AUMENTO DE LA ACTIVIDAD ANTIBIOFILM DEL SISTEMA MULTICOMPONENTE CLORANFENICOL: β-CICLODEXTRINA: N-ACETILCISTEÍNA/ INCREASE OF ANTIBIOFILM ACTIVITY OF THE MULTICOMPONENT SYSTEM CHLORANPHENICOL: BCYCLODEXTRINE: N-ACETYLCISTEINE

DIAMELA M. ROCCA

Un biofilm es una comunidad microbiana caracterizada por células unidas irreversiblemente a un sustrato biótico o abiótico, embebidas en una matriz polimérica extracelular producida por ellas mismas y que exhiben un fenotipo alterado con respecto al índice de crecimiento y trascripción de genes. Los biofilms son formas de vida adaptadas para sobrevivir en medios hostiles, por lo tanto su resistencia a agentes antimicrobianos y a las defensas del huésped es entre cien a mil veces superior a la de su contraparte planctónica (células en estado libre). Dado que los biofilms están involucrados en más del 80% de las infecciones bacterianas, existe una creciente necesidad de prevenir su formación ya que aumentan aún más la posibilidad de resistencia a los agentes antimicrobianos. La erradicación de biofilms preformados requiere de estrategias especiales, dado que la matriz reduce la interacción del antimicrobiano con la bacteria, y consecuentemente, aumenta el número de fallas terapéuticas. N-acetilcisteína es una droga no antibiótica que presenta acción dispersiva sobre la matriz del biofilm, lo cual hace a las bacterias que lo forman más susceptibles a los agentes antimicrobianos y además, posee un excelente perfil de seguridad siendo ampliamente utilizada en la práctica médica por vía inhalatoria, oral e intravenosa por lo que resulta un tercer componente apropiado para ser utilizado en una formulación farmacéutica de CP.

En nuestro trabajo, la formación del complejo multicomponente cloranfenicol:β-ciclodextrina: N-acetilcisteína mostró tener un efecto significativo en la reducción de la actividad metabólica y biomasa de biofilms de *Staphylococcus aureus* y *Staphylococcus* coagulasa negativo, incluso resultó más efectivo que CP a una concentración igual a 10 veces su concentración inhibitoria mínima (CIM).