INHIBITION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AS A NOVEL APPROACH FOR CANCER THERAPY

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Abstract
Of the numerous growth factors and cytokines that have been shown to have angiogenic effects, vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), appears to be a key factor in pathological situations which involve neovascularization as well as enhanced vascular permeability. Our aim was to design a low molecular weight synthetic molecule that potently and selectively blocks the VEGF/VEGF receptor system after oral administration, suitable for the chronic therapy of VEGF-dependent pathological neovascularization. PTK787/ZK 222584 is a potent inhibitor of VEGF receptor tyrosine kinases, active in the submicromolar range. It also inhibits other class III kinases, like the PDGFR-β tyrosine kinase, c-Kit and c-Fms, but at higher concentrations. It is not active against kinases from other receptor families such as EGFR, FGFR-1, c-Met and Tie-2 or intracellular kinases like c-Src, c-Abl, PKC-alpha. PTK787/ZK 222584 inhibits VEGF-induced autophosphorylation of KDR, and endothelial cell proliferation, migration and survival in the nanomolar range in cell based assays. In concentrations up to 1 µM, PTK787/ZK 222584 does not have any cytotoxic or anti-proliferative effect on cells that do not express VEGF receptors. After oral dosing (50 mg/kg) to mice, plasma concentrations of PTK787/ZK 222584 remain above 1 µM for more than 8 h. PTK787/ZK 222584 induces dose-dependent inhibition of VEGF- and PDGF-induced angiogenesis in a growth factor implant model, as well as a tumor cell-driven angiogenesis model after once daily oral dosing (25-100 mg/kg). In the same dose range, it also inhibits the growth of several human carcinomas, grown subcutaneously in nude mice, as well as a murine renal carcinoma and its metastases in syngeneic, orthotopic models. Histological examination of tumors reveals inhibition of microvessel formation in the interior of the tumor. PTK787/ZK 222584 also significantly inhibits ascites formation induced by a human ovarian carcinoma grown in the peritoneum of nude mice as well as pleural effusion induced by a human lung adenocarcinoma in nude mice. PTK787/ZK 222584 is very well tolerated and does not impair wound healing. It also does not have any significant effects on circulating blood cells or bone marrow leukocytes as a single agent, or impair hematopoetic recovery following concomitant cytotoxic anti-cancer agent challenge. These studies indicate that compounds that inhibit the effects of VEGF, such as PTK787/ZK 222584, have the potential to provide a novel, effective and well-tolerated therapy for the treatment of solid tumors. These agents may also provide a new therapeutic approach for the treatment of other diseases where angiogenesis plays an important role.

Key words: cancer therapy, VEGF, VPF

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Angiogenesis, the formation of new vessels from an existing vascular network, is an essential event in a variety of physiological and pathological processes. Under physiological conditions, angiogenesis is restricted to processes such as embryogenesis, ovulation and wound healing. Angiogenesis also occurs in pathological processes such as inflammation, rheumatoid arthritis, psoriasis, ocular neovascularization, and tumor growth and the formation of metastases.

Of the numerous growth factors and cytokines that have been shown to have angiogenic effects, vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), appears to be a key factor in pathological situations which involve neovascularization as well as enhanced vascular permeability. VEGF receptors, Flt-1 (Fms-like tyrosine kinase, VEGF-R1) and KDR (kinase insert domain-containing receptor, VEGF-R2), are almost exclusively located on endothelial cells. Expression of these receptors is low in normal tissues and only upregulated during the development of these pathological states when neovascularization occurs. Both receptors have seven immunoglobulin-like domains in their extracellular region, a single transmembrane-spanning domain, and an intracellular split tyrosine kinase domain and belong to the same family of receptors as PDGFR, c-Kit, c-Fms, Flt-3 and Flt-4. Flt-1 binds VEGF-A and -D, whereas KDR binds VEGF-C and -D. Flt-1 is a potent mitogen of inflammatory cells such as granulocytes and monocytes, and mediates a mitogenic response. In contrast to Flt-1, KDR is strongly autophosphorylated upon VEGF-stimulation and mediates a mitogenic response. Gene knock-out experiments for VEGF receptors have highlighted the pivotal role of VEGF/VEGF-receptor system in the development of the embryonic vascular system. Various different approaches have been used to interfere with the VEGF/VEGF-receptor system in adult animals and thereby determine the role of VEGF receptors in the various pathological states. These approaches include VEGF-neutralizing antibodies, antibodies against the VEGF receptors, recombinant soluble VEGF receptor proteins, a tetra cyclic-regulated VEGF expression system, dominant negative mutants of the VEGF receptors and VEGF receptor tyrosine kinase inhibitors. Results from these approaches suggest that the VEGF/VEGF-receptor system is a novel and attractive therapeutic target for suppression of pathological neovascularization and, in particular, for inhibiting tumor growth.

Our aim was to design low molecular weight synthetic molecules that potently and selectively block the VEGF/VEGF receptor system after oral administration, suitable for the chronic therapy of VEGF-dependent pathological neovascularization. A series of 1-anilino-(4-pyridylmethyl)-phthalazines have been synthesized that are inhibitors of the VEGF-receptor tyrosine kinase and have favourable pharmacokinetic properties following oral administration to animals. One of these compounds, PTK787/ZK222584, has been shown to be a potent inhibitor of VEGF receptor tyrosine kinases and to have favourable pharmacokinetic properties following oral administration to animals. PTK787/ZK222584 also inhibits the VEGF-receptor tyrosine kinase inhibitor must enter cells in order to inhibit the kinase inhibitor. Using enzymatic assays with recombinant GST-fused kinase domains and synthetic substrates, PTK787/ZK222584 has been shown to be a potent inhibitor of VEGF receptor tyrosine kinases, active in the submicromolar range (Figure 2). PTK787/ZK222584 also inhibits other class III kinases, like the PDGFR-B tyrosine kinase, c-Kit and c-Fms, but at higher concentrations. However, it is not active against kinases from other receptor families such as EGFR, FGFR-1, c-Met and Tie-2 or intracellular kinases like c-Src, c-Abl, PKC-alpha. Since a kinase inhibitor must enter cells in order to inhibit the kinase.
nase domain of the receptor, the effects of PTK787 / ZK 222584 were tested in cell-based receptor autophosphorylation assays using human umbilical vein endothelial cells (HUVEC) that naturally express KDR, or KDR-transfected CHO cells. PTK787 / ZK 222584 inhibits VEGF-induced autophosphorylation of KDR in both cell systems. Potential anti-proliferative effects of PTK787 / ZK 222584 unrelated to VEGF inhibition, were tested using cells that do not express the VEGF receptors. In concentrations up to 1 µM, PTK787 / ZK 222584 does not have any cytotoxic or anti-proliferative effect on these cells. In contrast, PTK787 / ZK 222584 selectively inhibits VEGF-mediated functions of endothelial cells, which express the KDR receptor. Inhibition of VEGF-mediated proliferation, migration and survival is observed in the nanomolar range. PTK787 / ZK 222584 inhibits angiogenesis in an in vitro assay; the formation of capillary sprouts from pieces of blood vessel cultured in a fibrin gel (Figure 3).

Since our aim was to develop a compound that would inhibit VEGF-induced angiogenesis after oral administration, we tested whether PTK787 / ZK 222584 is absorbed after oral administration in mice. After oral dosing (50 mg/kg) to mice, plasma concentrations of PTK787 / ZK 222584 remain above 1 µM for more than 8 h. The compound also has excellent oral bioavailability in rats, dogs and humans. To determine whether PTK787 / ZK 222584...
inhibits VEGF mediated angiogenesis in vivo, we tested the effects of PTK787 / ZK 222584 on the angiogenic response induced by VEGF in a growth factor implant model in mice. To test the specificity of the response, the effects on PDGF-induced angiogenesis were also tested. Consistent with its in vitro inhibitory profile, PTK787 / ZK 222584 induces dose-dependent inhibition of VEGF- and PDGF-induced angiogenesis in this growth factor implant model, with greater potency against VEGF (Figure 4). To determine whether PTK787 / ZK 222584 inhibits an angiogenic response mediated by tumor cell in vivo, we tested the effects of PTK787 / ZK 222584 on the angiogenic response induced by epithelial carcinoma A431 encapsulated in alginate beads and implanted subcutaneously on the dorsal flank of nude mice. This study revealed that PTK787 / ZK 222584 also inhibits tumor cell-driven angiogenesis. In the same dose range (25-100 mg/kg), it also inhibits the growth of several human carcinomas, grown subcutaneously in nude mice, as well as a murine renal carcinoma and its metastases in syngeneic, orthotopic models (Figure 5). Histological examination of tumors reveals inhibition of microvessel formation in the interior of the tumor. Consistent with its potential to block the effects of VEGF on vascular permeability, PTK787 / ZK 222584 also significantly inhibits ascites
formation induced by a human ovarian carcinoma grown in the peritoneum of nude mice as well as pleural effusion induced by a human lung adenocarcinoma in nude mice.

PTK787 / ZK 222584 was very well tolerated in all the animal models in which it was tested. Surprisingly, although it inhibits the effects of 2 angiogenic growth factors (VEGF and PDGF) it did not impair wound healing in any of the animal models involving surgery or in a dermal incisional wound in the rat. Despite its inhibition of c-Kit, the receptor for stem cell factor, PTK787 / ZK 222584 does not have any significant effects on circulating blood cells or bone marrow leukocytes as a single agent, or impair hematopoietic recovery following concomitant cytotoxic anti-cancer agent challenge.

These studies indicate that compounds that inhibit the effects of VEGF, such as PTK787 / ZK 222584, have the potential to provide a novel, effective and well-tolerated therapy for the treatment of solid tumors. These agents may also provide a new therapeutic approach for the treatment of other diseases where angiogenesis plays an important role, such as diabetic retinopathy, macular degeneration and rheumatoid arthritis. Phase I trials with PTK787 / ZK 222584 in patients with advanced cancers have confirmed its excellent tolerability and kinetic properties after oral administration.

References

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The painful reality is that there is no holy grail, no magic cure-all bullet, and no quick fix (for cancer). And the paradox is that all the complexity, now that the fog is lifting, has a coherent pattern and makes a great deal of sense. And, as often when seen retrospectively, it is difficult to imagine how it could have been otherwise. Demystifying the disease is to travel over a new and more realistic landscape. It's not the easiest of journeys but it's the only ticket worth having.

La dolorosa realidad es que no hay ningún santo grial, ninguna bala mágica, y ningún arreglo (para el cáncer). Y lo paradójico es que toda esta complejidad, ahora que se levantó la bruma, tiene un patrón coherente y tiene mucho sentido. Y, como se ve tan a menudo retrospectivamente, es difícil imaginar cómo podría haber sido de otra manera. Desmystificando la enfermedad es emprender un camino nuevo y más realista. No es el más fácil de los viajes pero es el único boleto que vale la pena tener.

Mel Greaves

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