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In human medicine there have been many studies reporting the importance of evaluating serum ET-1 or big ET-1 in various diseases as a clinical marker, especially related to pulmonary hypertension and several tumors. In veterinary medicine, however, few studies have been performed on the clinical importance of serum ET-1 or big ET-1. In this study we explored the feasibility of using the serum big ET-1 as a clinical marker in dogs with various cardiopulmonary and neoplastic diseases. Pulmonary hypertension was diagnosed in dogs by echocardiography based on the velocity of tricuspid valve regurgitation. Serum big ET-1 and NT-pro BNP concentrations in these dogs were measured by ELISA and compared with those of healthy dogs. Serum big ET-1 concentration in dogs with various neoplastic diseases was also assessed and compared. Our results showed a significant increase of serum big ET-1 in dogs with pulmonary hypertension, lung tumor and hemangiosarcoma when compared to normal dogs. No significant difference was observed in NT-pro BNP concentration between healthy and pulmonary hypertension dogs. Although further studies are necessary, these findings point to the potential of serum big ET-1 as a clinical marker in canine pulmonary hypertension and for some tumor types.

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An unexpected pulmonary hypertensive crisis: Eying the culprit

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A 56-year-old man developed sudden dyspnoea after resection of choroidal melanoma in his left eyeball. Worsening hypoxia required intensive treatment, including percutaneous cardiopulmonary support. On contrast-enhanced computed tomography there was no evidence of either thrombi in the pulmonary arteries or obvious lung diseases. A Swan–Ganz catheter showed increased mean pulmonary arterial pressure and no elevation of pulmonary capillary wedge pressure. These findings were consistent with a diagnosis of pulmonary arterial hypertension. Because reports have described a significant relationship between melanoma and endothelin (ET)-1, we hypothesized that a substantial amount of ET-1 had been released from malignant melanoma cells during resection, thus triggering the pulmonary hypertensive crisis in our patient. The patient fully recovered after intensive treatment and administration of the endothelin receptor antagonist bosentan. The success of bosentan treatment, along with an extremely high level of ET-1 on pathologic analysis, confirmed our hypothesis regarding an increase in plasma ET-1 level — 9.60 pg/mL (normal range < 2.3 pg/mL).

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Pharmacokinetics of SPI-1620 in a Phase I, open label, ascending dose study of the safety, tolerability, pharmacokinetics and pharmacodynamics of the endothelin B receptor agonist, SPI-1620, in recurrent or progressive carcinoma

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Objective: The primary objective of the Phase I study was to assess the safety and tolerability of SPI-1620 administered to patients with recurrent or progressive carcinoma who had failed all standard therapy. Secondary objectives were to assess PK and PD profiles of SPI-1620, and to identify the optimum dose of SPI-1620 to be used in future Phase II studies. The pharmacokinetic properties of SPI-1620 will be presented. Methods: Eligible patients received SPI-1620 by intravenous infusion over 1 min in an accelerated dose escalation scheme. SPI-1620 doses ranged from 0.5 μg/m^2 to 15.1 μg/m^2. Serial blood samples were collected from each patient prior to infusion (0 min) and at pre-specified intervals from the start of the infusion. Human plasma samples were analyzed by a validated HPLC–MS/MS method. Descriptive PK parameters were determined by standard model independent methods based on the concentration–time data of each subject. Results & conclusion: The highest concentration of SPI-1620 was achieved by the end of infusion. SPI-1620 C max increased proportionally as a function of SPI-1620 dose while the AUC (0–T) increased in a more than dose proportional manner. The SPI-1620 T 1/2 was short and ranged from 4.38 min to 8.29 min. SPI-1620 had a low systemic clearance and small VD (approximately equal to the intravascular volume).

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Endothelin-1-induced β-arrestin signalosome is linked to chemoresistance, EMT and stem-cell like properties in ovarian cancer cells

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The epithelial–mesenchymal transition (EMT) is known to play a crucial role in the aggressiveness of epithelial ovarian cancer (EOC), contributing to chemoresistance and cancer stem cell populations. In this tumor, the endothelin (ET)-1/endothelin A receptor (ETAR) axis, by regulating EMT and invasion, endows EOC cells with an increased chemoresistance. Here we examined whether β-arrestin-1 (β-arr1) can act as a nuclear hub orchestrating nuclear signaling in ETAR-driven EMT and chemoresistance. A significant higher expression of β-arr1 and ET-1/ETAR and the stronger presence of β-arr1 in the nuclear compartment upon ETAR activation are present in chemoresistant cells, compared to sensitive cells. In the nuclei, β-arr1 robustly interacts with β-catenin to form a nuclear complex localized on the ET-1 promoter region, leading to transcription of ET-1, demonstrating that β-arr1 drives the positive inter-regulation of ET-1 itself. This autocrine circuit is involved in β-arr1-driven appearance of EMT features and acquisition of stem-cell like properties. Moreover, at functional level, chemoresistant cells, with high nuclear β-arr1, display higher invasive potential and increased resistance to chemotherapeutic drugs. These effects were inhibited by ET-1 receptor blockade with macitentan, or by β-arr1 nuclear mutant.

Moreover, in vivo, silencing of β-arr1 or macitentan treatment inhibited metastasis in sensitive and resistant EOC xenografts, providing evidence that blockade of ETAR/β-arr1-driven EMT can overcome chemoresistance and inhibit tumor progression. Collectively, our findings provide insights into how ETAR controls EMT transcriptional responses and tumor initiating trait, deciphering a novel function for β-arr1 for nuclear compartmentalization of ETAR signalling influencing the mechanism of acquired resistance, EMT and stem-cell like features.

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(Pro)renin receptor in breast cancer and its possible pathophysiological role in cancer cell proliferation

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The endothelin system is an important paracrine or autocrine system for cancer cell proliferation. Endothelin-1 and endothelin receptors are expressed in various types of cancers including breast cancer. (Pro)renin receptor ((P)RR) is a specific receptor for renin and prorenin. Receptor-bound prorenin becomes enzymatically active in converting angiotensinogen to angiotensin I, and binding then activates phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2), independent of angiotensin II generation. Furthermore, (P)RR is associated with vacuolar-type H+-ATPase (V-ATPase), which may be related to cell proliferation. The aim of the present study is to clarify the pathophysiological role of (P)RR in breast cancer. We investigated (P)RR expression in 69 clinical cases of breast carcinoma by immunohistochemistry and its correlation with clinicopathological parameters. Effects of (P)RR on cell proliferation and ERK1/2 phosphorylation were examined in cultured human breast carcinoma cells. Immunohistochemistry showed that (P)RR immunoreactivity was detected in carcinoma cells of breast carcinoma tissues, and was correlated with Ki-67 expression. The (P)RR specific small interference RNA or bafilomycin A1 (an inhibitor of V-ATPase activity) inhibited cell growth of breast carcinoma cell lines (MCF-7 and SK-BR-3). Prorenin stimulated the phosphorylation of ERK1/2 in MCF-7 cells. Treatment of MCF-7 cells with endothelin-1 had no significant effects on (P)RR expression levels. The present study has raised the possibility that, in addition to the endothelin system, (P)RR is involved in the pathophysiology of breast cancer by stimulating the proliferation of breast carcinoma cells via the association of V-ATPase and/or phosphorylation of ERK1/2.

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Poly-gamma-glutamic acid attenuates angiogenesis and inflammation in experimental colitis

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Poly-gamma-glutamic acid (PGA), naturally secreted from various strains of *Bacillus*, has anti-inflammatory activity. In inflammatory bowel disease (IBD), inflammation is promoted and sustained by angiogenesis; however, the role played by PGA in this condition is unclear. Therefore, we evaluated PGA effects on angiogenesis and inflammation in a dextran sulfate sodium (DSS)-induced mouse colitis model. Experimental colitis was induced in male C57BL/6 mice by administering 3% DSS. Disease activity index (DAI), histopathological scores, microvascular density, myeloperoxidase activity, and VEGF-A and VEGFR2 expression were compared among control mice, DSS-treated mice, and mice receiving 3% DSS along with PGA at 50 mg/kg body weight per day, or 3% DSS with PGA at 200 mg/kg body weight per day. We found that PGA significantly attenuated weight loss, DAI, and colon shortening. PGA also significantly reduced histopathological evidence of injury. Moreover, PGA significantly attenuated DSS-induced blood vessel densities. Furthermore, PGA attenuated DSS-induced expression of VEGF-A and its receptor, VEGFR2. In addition, PGA treatment led to reduced recruitment of leukocytes to the inflamed colon. Therefore, our results indicate that PGA has potential application in conditions marked by inflammatory-driven angiogenesis and mucosal inflammation.

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Identification of bladder endothelin-1 receptors and binding characteristics of bosentan and ambrisentan

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Endothelin (ET)-1 induces prolonged contractile responses in isolated bladder muscle strips. ET-like immunoreactivity was identified in detrusor muscles, epithelium and vascular endothelium. Selective ETA receptor antagonists have ameliorating effects on urinary dysfunctions. The current study aimed to identify bladder ET-1 receptors using radioligand binding assay and characterize receptor binding of clinically used ET-1 receptor antagonists. ET-1 receptors were measured in rat bladder using [125I]ET-1, and binding parameters of dissociation constant (Kd), and the maximal number of binding sites (Bmax) for [125I]ET-1 were estimated. The inhibition of specific [125I]ET-1 binding was measured in the presence of ET-1 and its receptor antagonists. Specific [125I]ET-1 binding in rat bladder was saturable and of high affinity, which characterized selective labeling of bladder ET-1 receptors. ET-1, bosentan, ambrisentan, and CI-1020 inhibited specific [125I] ET-1 binding in a concentration-dependent manner at nanomolar ranges of IC50. Nonlinear least squares regression analysis revealed the presence of high- and low-affinity ET-1 receptor sites for ambrisentan and CI-1020. Bosentan significantly increased Kd for bladder [125I]ET-1 binding without affecting Bmax, while ambrisentan increased Kd with a concomitant reduction in Bmax. Thus, bosentan seems to bind bladder ET-1 receptor in a competitive and reversible manner while ambrisentan may bind to bladder ET-1 receptors, partially in a non-competitive manner in addition to a competitive manner. Oral administration of bosentan caused a dose-dependent decrease in Bmax for bladder [125I]ET-1 binding, suggesting significant binding of bladder ET-1 receptors in vivo. These results indicate that pharmacologically relevant ET-1 receptors exist in rat bladder and they may become a promising target for the development of novel therapeutic agents for bladder dysfunction.

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