Recent Developments on Endothelin Antagonists as Immunomodulatory Drugs - from Infection to Transplantation Medicine

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Abstract: Endothelin, a potent endogenous vasoconstrictor and mitogen that acts through the ET_A and ET_B receptors, has been not only implicated in the regulation of cardiovascular homeostasis but also in inflammatory responses, including that induced by infection and solid organ transplantation. Changes in capillary perfusion and leukocyte recruitment are important features of inflammation. The concentrations of ET are elevated in many forms of inflammation and are especially high in sepsis. The rise in plasma levels of ET during early stages of inflammation may initially have some positive homeostatic effects that might help to maintain vascular tone and blood pressure. However, high levels of ET compromise the appropriate matching of flow to tissue needs and contribute to the pathophysiology of microcirculatory derangements. Attempts at regulating the effects of ET by the use of pharmacological antagonists are complicated by important interactions between the ET_A and ET_B receptors. This review highlights findings of recent studies and patents in this area showing that the ET system, apart from being a marker of vascular and tissue injury, is directly involved in the pathophysiology of these disease processes as an immunomodulatory mediator.

Keywords: Bacteria, disease, drug therapy, human, physiology, vasoconstriction, rejection, trypanosoma.

INTRODUCTION

In response to several infectious and non-infectious stimuli, monocytes and macrophages release a number of mediators including cytokines which are involved in the inflammatory response [1]. These cytokines lead to further expression of mediators and co-stimulatory molecules which feed back into the inflammatory cascade [1, 2]. Endothelin is among the mediators released during inflammatory activation and together with different proinflammatory molecules, such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α, may play a role in the cascade of events leading to tissue inflammation [3, 4]. Endothelin (ET) was initially characterized as a potent smooth-muscle spasmogen [5, 6] and is indeed a potent endogenous vasoconstrictor and mitogen [7]. Endothelin has been reported to have numerous biologic properties within the cardiovascular, respiratory, renal, endocrine, gastrointestinal, and neurologic systems [8-10] and is produced by several cell types, including endothelial cells, leukocytes, macrophages and monocytes [10-13].

ET-1, the predominant of three endothelin isoforms [14], is a product of endopeptidasic cleavage of prepro-ET-1 to big ET-1 which is later transformed by ET-converting enzyme (ECE)-1 and/or ECE-2 into its active form (Fig. **1**) [9, 15] Regulation of ET production primarily occurs at the transcriptional level which is achieved by controlling the activity of the promoter and the stability of messenger RNA [16]. In a variety of cells, ET expression is controlled by autocrine and paracrine mechanisms [17] and induced by physiochemical factors such as blood flow, pulsatile stretch, sheer stress, hypoxia, pH or by cytokines, hormones and vasoactive agents [9, 15, 18-21] which also change the biological responsiveness to ET [15, 22].

In mammals, two ET receptors have been identified: ET receptor A (ET_A) and B (ET_B), which are members of the G-protein-coupled superfamily [8, 9, 15]. ET_A receptors have selective affinity for ET-1 and ET-2, whereas ET_B receptors have similar affinity for all ET isoforms (Fig. **1**) [15, 23]. Both receptors stimulate phospholipase C which leads to increased formation of diaceylglycerol and inositol-1,4,5-triphosphate which activates protein kinase C pathway and increases intracellular Ca^{2+}, respectively [24]. ET_A receptors are the predominant subtype mediating vasoconstriction and cells in humans whereas ET_B receptors are mainly expressed in vascular endothelial cells, mediating vasodilatation via release of nitric oxide (NO) and dilator prostanoids and thereby inhibiting cell proliferation and inflammation [8, 9, 23].

In this review, we summarize the current understanding of the mechanisms and signal transduction pathways triggered by ET in inflammatory and immunomodulatory processes and discuss the findings of previous studies evaluating the use of selective and non-selective ET receptor antagonists.

VASCULAR EFFECTS OF ENDOTHELIN

Experimental studies using molecular and pharmacological inhibition of the ET system have demonstrated that ET-1 takes part in normal cardiovascular homeostasis [23, 25, 26]. Thus, ET-1 plays a major role in the functional and structural changes observed in arterial and pulmonary hypertension, glomerulosclerosis, atherosclerosis, mainly

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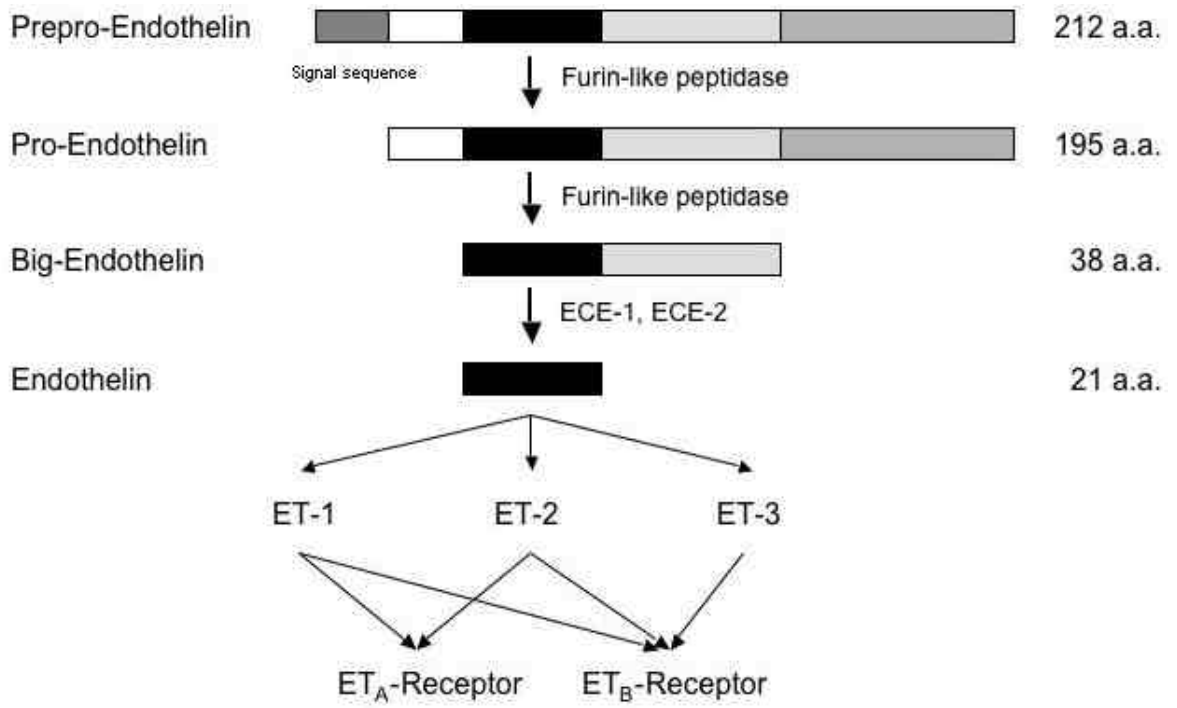

Fig. (1). Molecular components of the endothelin system. Processing of precursor peptides by furin-like peptidase results in formation of big-ET-1, big-ET-2 and big-ET-3. These 38-amino acids (a.a.) peptides are further processed by ECE-1 and ECE-2, chymases and non-ECE metalloprotease into vasoactive ET which activates tissue ET_A and/or ET_B receptors. a.a., amino acids; ECE, endothelin-converting enzyme; ET, endothelin.

through pressure-independent mechanisms [15]. Under experimentally induced pathological conditions (e.g. heart failure), the expression of ET-1 and its receptors in cardiomyocytes is increased, and treatment with ET receptor antagonists improves survival and cardiac function [15].

ET_A receptors are the predominant ET vasoconstrictor receptors in arteries. ET_A and ET_B receptors on smooth muscle mediate contraction, cell proliferation, and hypertrophy [27]. Vasoconstrictor ET_B receptors are present in the veins [28] and pulmonary vessels [29-31] in larger numbers than in arteries, although ET_A still predominate over ET_B receptors in these vessels. ET_B receptors are also localized on endothelial cells and act through the production of NO and prostacyclin to exert vasodilator and antiinflammatory effects [26]. Whether vasoconstriction or vasodilatation is the most important effect of ET-1 under normal conditions may depend on the vascular bed [32]. It has been reported that in coronary arteries there are few endothelial vasodilator ET_B receptors [33]. As a result, ET-1 acts on coronary vessels mainly as a vasoconstrictor. In other vascular beds, however, ET-1 may even function as a vasodilator under physiological conditions [34, 35].

ENDOTHELIN IN INFLAMMATORY AND IMMUNOMODULATORY PROCESSES

Increasing evidence supports a role for ET as a proinflammatory cytokine and fibrotic factor which is released in inflammatory and immune reactions [36, 37]. ET-1 acts as a proinflammatory peptide via vascular and non-vascular related mechanisms: ET-1 induces the release of proinflammatory cytokines [13, 36], provokes local ischemia [38] or alters the epithelial permeability allowing antigen translocation [39].

Thus, ET-1 has been implicated in many diseases characterized by inflammation and/or fibrotic remodeling, such as atherosclerosis [40], ischemia–reperfusion injury [41], alveolitis [42], dermatitis [43], Crohn's disease and ulcerative colitis [38, 44, 45], systemic sclerosis [46, 47], dermatomyositis/polymyositis [48], systemic lupus erythematodes [49], rheumatoid arthritis [50], liver cirrhosis [51], and glomeruloslerosis [52, 53] (Table **1**).

Both ET_A and ET_B receptors appear to be involved in these vascular and non-vascular related processes [54-56]. In patients with Crohn's diesease and ulcerative colitis, the plasma levels of ET-1 are significantly higher than in healthy controls and the density of ET positive cells as well as the expression of ET_A and ET_B receptors is increased [8, 38]. ET-1 is involved in many cell signaling pathways that include Ca^{2+} mobilization and activation of proinflammatory cytokines, extracellular signal-regulated kinases (ERK)1/2, and cyclin D1 [10, 57]. ET-1 also increases vascular permeability [58] as well as time- and dose-dependent entrapment of circulating platelets [59].

Recently, the downstream intracellular signal transduction pathways of ET-1 have been in part identified. In this regard, a key role of mitogen-activated protein kinases (MAPK) has been suggested, which operate via phosphorylation cascades responsible for regulation of several substrates, mainly including transcription factors implicated in inflammation, development, cell proliferation and

Table 1. Sites of Endothelin and Endothelin Receptors Expression During Inflammatory and Immune Reactions

Tissue	Cell type	Source
Brain	Neurons	[7-8, 71, 96]
	Astrocytes	[7-8, 71]
	Microglia	[7-8, 71]
Peripheral nerve		[7-8]
Blood	Macrophages	[7-8, 11, 14, 161]
	Monocytes	[7-8, 12]
	Leukocytes	[7-8, 14, 57, 89, 92]
Vascular system	Endothelial cells	[6-8, 14-19, 39, 48, 63, 70, 76, 79, 83, 97, 142]
	Vascular smooth muscle cells	[7-8, 14, 74]
Heart	Cardiomyocytes	[7-8, 10, 14, 64, 78, 83, 85, 106, 136, 165-66, 179]
Liver	Kupfer cells	[7-8, 14, 149, 165]
	Hepatocytes	[7-8, 14, 50, 147, 163-65, 169]
Renal	Peritubular cells	[7-8, 14, 20, 51, 163-4]
	Mesangial cells	[7-8, 52]
Pancreas	Exocrine	[7-8, 14, 164]
	Endocrine	[7-8, 117]
Gastro-intestinal	Epithelial cells	[7-8, 14, 37, 38, 43, 44, 81, 139, 144]
Lung	Epithelial cells	[3, 7-8, 14, 41, 57, 102, 157, 165, 169]
	Endothelial cells	[3, 7-8, 14, 157]
	Vascular smooth muscle cells	[3, 7-8, 14, 102]
Prostate		[7-8, 14]

apoptosis [60, 61]. Three major MAPK subgroups are currently known, activated by dual phosphorylation on tyrosine and threonine residues and named c-Jun N-terminal kinases (JNK), p38, and ERK1/2, respectively [62]. In particular, ERK activation is frequently required for cell growth and differentiation induced by various stimuli, also including ET-1 [63, 64]. Important downstream targets of the MAPKs and ET-1 pathways are cell cycle regulatory molecules such as cyclins, cyclin-dependent kinases and cyclin-dependent kinase inhibitors [65]. The cyclins are a target of both ET-1 and the MAPK pathway [66].

EVIDENCE THAT ENDOTHELIN TRIGGERS MEDIATORS OF INFLAMMATION AND CELL ADHESION MOLECULES

ET-1 is known to stimulate neutrophils to release elastase [67, 68] and to stimulate monocytes to produce various cytokines, including IL-1 [36, 69], IL-6 [70, 71], IL-8 [72], IL-10 [73], TNF-α [74], tumor growth factor (TGF)-β [42], granulocyte-macrophage colony-stimulating factor (GCSF) [75], monocyte chemoattractant protein (MCP)-1 [76, 77] and nuclear factor (NF)-κB [13, 78] promoting the inflammatory cascade (Table **2**). Overexpression of ET-1 stimulates expression of TNF-α, interferon (IFN)-γ, IL-1 and IL-6 [79]. Conversely, TNF-α and IL-1 modulate the expression of ET-1 in the inflammatory cascade [80, 81], which may further amplify its deleterious effects. Akin to ET-1, many of these mediators increase expression of cell adhesion molecules [82, 83], which induce the adhesion of circulating leucocytes to endothelial cells, an initial step in the events leading to cellular infiltrate in an inflamed tissue. Lopez-Farre *et al.* [84] demonstrated that ET-1 enhances neutrophil accumulation, suggesting a direct link between ET-1 and the inflammatory process. In cultured endothelial cells, ET-1 promotes neutrophil aggregation [85] and stimulates surface expression of CD11b/CD1 on human neutrophils augmenting their adhesion to endothelial cells [84] and myocytes [86]. Activated endothelial cells are an important source of cytokines, adhesion molecules and growth factors enhancing inflammatory cells to migrate and accumulate in the extravascular tissue [87, 88]. In addition to the neutrophil-activating properties of ET-1 it also acts as a chemoattractant for monocytes [89], activator of mast cells [90-92] and inductor of significant eosinophil migration mediated by an increase in the local levels of eotaxin and IL-5 [93].

Table 2. Stimulatory Effects of Endothelin During Inflammatory and Immunomodulatory Reactions

Target/cell type	Stimulatory effects	Source
Neutrophils, endothelial cells	Elastase	[66, 67]
Pulmonary mast cells	Eotaxin	[91]
Monocytes	E-Selectine	[95]
Macrophages	GCSF	[74]
Monocytes	ICAM-1	[95]
Monocytes	IFN-γ	[78]
Monocytes, endothelial cells	IL-1	[24, 68, 78]
Pulmonary mast cells	IL-5	[91]
Monocytes, endothelial cells	IL-6	[69, 70, 78]
Endothelial cells	IL-8	[71]
Endothelial cells	IL-10	[72]
Fibroblasts	MCP-1	[75, 76]
Macrophages, endothelial cells	NF-κB	[12, 77]
-	TGF-β	[41]
Neutrophils, monocytes	TNF-α	[73, 78]
Endothelial cells	Angiogenesis	[7-8, 99]
Neutrophils, monocytes, macrophages	Cell adhesion	[7-8, 83-85]
Monocytes	Chemotaxis	[88-91]

Macrophage, monocytes and polymorphonuclear leukocytes also secrete ET-1 [12, 13, 94]. In addition to its ability to prime leukocytes for chemotaxis [95], ET-1 stimulates also the expression of adhesion molecules which are key players in the leukocyte-endothelial cell interaction [96]. Hayasaki *et al*. [86] documented that ET-1 induces intracellular adhesion molecule (ICAM)-1 expression on cultured cardiomyocytes and endothelial cells. Similarly, Zouki *et al*. [97] reported that ET-1 increased the expression of E-selectin and ICAM-1 on cultured human endothelial cells in a concentration-dependent fashion. The production of MCP-1, a potent chemoattractant for monocytes, can be also induced by ET-1 through ET_A receptor activation in endothelial cells. This effect is augmented by the proinflammatory cytokines TNF-α and IL-1 [98].

Previous studies demonstrated that ET-1 and ET-3, acting through the ET_B receptor, have dose-dependent stimulatory, proliferative and migratory effects on endothelial cells [99]. While ET-1 and ET-2 are reported to be equipotent in promoting DNA synthesis, ET-3 is less active [100]. During the formation of new blood vessels, endothelial cells are stimulated to migrate, proliferate and invade surrounding tissue to form capillaries [101]. ET-1, similar to VEGF, induces these angiogenic effects and, in concert with VEGF, displayes a potent additive effect on the different stages of the angiogenic process. In this scenario, ET-1 signaling is mediated mainly by the ET_B receptor [102]. Recent studies emphasize a key inflammatory role of the endothelial cells, either by overexpression of inflammatory mediators or by stimulating formation of new blood vessels, in the disease process leading to the systemic organ involvement [103]. ET released after activation and/or damage of endothelial cells, might play an important role inflammatory processes and vasculopathy in autoimmune diseases [48]. Increased plasma ET levels have been found in various autoimmune diseases such as systemic lupus erythematodes [49], systemic sclerosis [47] or rheumatoid arthritis [50]. Moreover, raised ET serum levels have been implicated in the pathophysiology of both fibrotic and vascular manifestations of systemic sclerosis [47].

ET-1 exerts mitogenic activity on smooth muscle cells, myocytes, and fibroblasts [15, 104, 105]. In epithelial cells, ET-1 is known to induce gene expression and release of fibronectin [106] which is an important extracellular matrix component, as well as being chemoattractic factor for fibroblasts [107]. There is increasing evidence that ET-1 can function as a profibrotic cytokine by stimulating fibroblast chemotaxis and proliferation [108-110] and procollagen production [111, 112]. ET-1 may act in concert with several other profibrogenic molecules, including TGF-β [113], platelet-derived growth factor (PDGF) [114], epidermal growth factor (EGF) [115], fibroblast growth factor (FGF) [116], insulin-like growth factor (IGF)-1 [117, 118], IL-11 [119] and insulin [64, 120, 121] to potentiate cellular transformation or replication. In particular, the synergistic interactions involving these mediators are crucial for implementing the tissue repair response leading to fibroblast proliferation, myofibroblast differentiation and collagen synthesis [117]. Within this context, a pivotal role is mediated by MAPKs that participate in the cross-talk between ET-1 and the other fibrogenic factors at several levels. Furthermore, rat cardiac fibroblasts have been shown to synthesize ET-1 [122], which induces collagen synthesis via both ET_A and ET_B receptors [123] as well as induces matrix metalloproteinase-2 expression [124].

The role of ET-1 in the pathogenesis of infectious diseases has only recently received attention. For example, ET-1 has been implicated in the etiology of vascular compromise and multi-organ dysfunction in the setting of septic shock [125, 126] and neuronal injury due to *Streptococcus pneumonia* meningitis [127]. Investigators have reported that circulating ET levels increase significantly in septic individuals which may be a beneficial effect in maintaining the blood pressure and organ perfusion during the early phase of septic shock [22, 87] and which correlates with mortality [128, 129]. Recent evidence also suggests that ET-1 plays a significant role in vascular dysfunction and organ failure associated with sepsis and septic shock [126]. It has been shown hat endotoxin administration results in upregulation of the ET system [130, 131] causing monocytes to produce proinflammatory cytokines such as TNF-α [36]. Endotoxin stimulates the production of TNF-α in monocytes and macrophages [13, 132]. Indeed, several reports have illustrated that antagonism of ET in septic animals improves metabolic acidosis as well as coronary, renal, splanchnic, pulmonary and intestinal perfusion [133-135]. In particular,

release of ET-1 is stimulated by endotoxin [136, 137]. Moreover, patients with *Rickettsia conorii*-induced vasculitis exhibit increased plasma levels of ET-1 [138]. *Rickettsia* share some important similarities with *Trypanosoma cruzi* (Chagas' disease) as both can reside within the endothelial cell cytoplasm and cause perturbations of the host cell [139]. ET is expressed during *Trypanosoma cruzi* infection in mice [65], and high plasma levels of immunoreactive ET were found in patients with severe chagasic cardiomyopathy [140].

EFFECTS OF ENDOTHELIN RECEPTOR BLOCKADE ON INFLAMMATORY AND IMMUNE PROCESSES

Cell Adhesion and Migration

Activated leukocyte adhesion is the initial and rate-limiting step in the development of inflammatory cellular infiltrate. Both ET_A and ET_B receptors are involved in the ET-induced activation of leukocyte adhesion [86, 97, 141]. Based on these observations, selective and non-selective ET receptor antagonists might be an effective treatment as immunomodulatory agents blocking the recruitment of leukocytes by reduction of leukocyte adhesion and preventing the ET-1 dependent progression of the inflammatory cascade.

Anthoni *et al*. [142] reported that bosentan, a non-selective ET_A/ET_B receptor antagonist, prevents the adhesion of leukocytes in colonic submucosal venules and reduces inflammation in a mouse model of inflammatory bowel disease. A comparable effect was also observed with the non-selective ET_A/ET_B receptor antagonist LU420627. Prophylactic oral administration of bosentan reduces inflammation and myeloperoxidase activity in colonic tissue in trinitrobenzene sulphonate-induced colitis in rats [143, 144]. The mechanisms of bosentan-induced reduction of leukocyte firm adherence remain speculative, but may depend on the blockade of ET-1 induced up-regulation of cell adhesion molecules on the endothelium and leukocytes [84, 86, 97, 145-147]. In particular, the finding that anti-inflammatory effects of ET-1 blockade are mediated by vascular cell adhesion molecule (VCAM)-1 in isolated endothelial cells suggests that these cell adhesion molecules may play a role in the attenuation of sticking and the elevation of rolling velocity [146]. This is supported by the observation that treatment with an anti-VCAM-1 oligonucleotide exerts a more potent protective effect on leucocyte endothelial adhesion when compared to bosentan [148], which reduces the infection-associated increase in nitrate/nitrite (NOx) serum concentration [149]

Previous studies using cardiac myocytes have shown that the selective ET_B receptor antagonist BQ-788 inhibits neutrophil-endothelial cell adhesion and ICAM-1 expression on endothelial cells [86], whereas the selective ET_A receptor antagonist S-0139 promotes neutrophil-cardiac myocytes adhesion and ICAM-1 production. This may be related to the differences in ET_A and ET_B receptor density on various cell types. For example, ET_A represents 90% of the receptors on the surface of cardiac myocytes, while the ET_B receptor is the dominant receptor on endothelial cells and macrophages which also predominantly express the ET_B receptor [150, 151]. However, different groups have reported varying results. Zouki *et al*. [97] reported that the ET_A-selective antagonist FR139317 markedly attenuated the ET-1 stimulated neutrophil adherence, suggesting a role of the ET_A receptor in neutrophil-endothelial attachment. This discrepancy of the results may be due to the use of different antagonists. It also remains possible that short- *versus* long-term exposure of endothelial cell to ET-1 may affect endothelial adhesiveness differently. The finding that the ET_A receptor-selective antagonist BQ610, but not the ET_B receptor-selective antagonist BQ788, decreased ET-1 induced MCP-1 expression implicates a role for the ET_A receptor in ET-1-mediated inflammation [98]. Recently, it has been shown that aryl-alkane-sulfonamides and their derivatives may ameliorate ET-1-mediated inflammation [152, 153] as well as an ET_A selective N-(5-isoxazolyl) benzene-sulfonamide receptor antagonist that can be modified in the 4-position with aryl and substituted aryl groups to generate a ET_B selective receptor antagonist [154, 155].

The mechanisms responsible for increased production of TNF-α by ET-1 and inhibition of TNF-α production by ET-1 receptor antagonism remain unclear. It has been suggested that ET-1 may play a potential proinflammatory role in pathological conditions [156]. However, there have recently been published a number of studies investigating this question. Quehenberger *et al*. [157], for example, demonstrated that the human ET-1 gene has NF-κB binding site and reported that transcription of the ET-1 gene is controlled by NF-κB. This group clearly demonstrated that binding of advanced glycation end products (AGE's) on the surface of erythrocytes, which occurs during oxidative stress, results in a NF-κB-dependent ET-1 induction *in vitro* [157]. It is likely that inhibition of TNF-α production by ET receptor antagonism is mediated by the NF-κB pathway. The effects of NF-κB in inflammation are well known, e.g., induction of synthesis of several proinflammatory cytokines including IL-1 and IL-6 [158]. Browatzki *et al*. [159] recently demonstrated that ET_A receptor activation induces IL-6 synthesis via NF-κB activation in human vascular smooth muscle cells, extending recent observations that ET-1 is a potent stimulus for activation of NF-κB in human hepatic stellate cells [160]. These mechanisms may be responsible for the synthesis of most of proinflammatory cytokines including the autocrine activation of ET-1 synthesis.

Allergic Inflammation

ET-1 has been reported to induce eosinophil migration mediated by an increase in the local levels of eotaxin and IL-5 [93, 161] This observation explains previous reports showing the accumulation of eosinophils rather than neurophils, as well we an increase in immunoreactive ET in the broncho-alveolar fluid in experimental airway inflammation [162-164], and in a murine model of allergic pleurisy [165, 166]. Treatment of these animals with a selective ET receptor antagonist significantly decreased the production of proinflammatory mediators [42] and eosinophilia [161].

In this context recent patents propose to use a novel class of indole-based ECE inhibitors to diminish the production of

ET-1 to inhibit bronchoconstriction and pulmonary vasoconstriction in newborn mammals [167, 168].

Atherosclerosis and Fibrosis

Inflammation of the vessel wall is a characteristic feature of atherosclerosis. ET-1 contributes to the pathogenesis of atherosclerosis and its chronic complications [40]. Browatzki *et al*. [78] investigated the effect of ET-1 on the proinflammatory transcription factor NF-κB in human monocytes which are the major source of inflammatory mediators in atheroma and are located in rupture-prone plaque areas. In these monocytes, ET-1 caused NF-κB activation which was blocked by inhibition of the I-κB-α-degrading proteasome complex as well as the ET_A receptor antagonist BQ123 but not by the ET_B receptor antagonist BQ788. ET-1 stimulated expression of the proinflammatory molecule CD40 but not of the cytokine IL-6 in a NF-κB-dependent manner. The data demonstrate the ability of ET-1 to differently activate inflammatory pathways in human monocytes [78].

Recently, Ammarguellat *et al*. [169, 170] reported that myocardial inflammation and fibrosis in hearts of deoxycorticosterone acetate hypertensive rats were, at least in part, mediated by ET-1. In this model, the selective ET_A receptor antagonist A-127722 prevented cardiac fibrosis in hearts by normalizing the levels of procollagen I and III and TGF-β. A-127722 also abrogated the activation of the inflammatory mediator NF-κB and the expression of platelet-endothelial cell adhesion molecule (PECAM)-1 and VCAM-1 [169, 170]. Hocher *et al*. [171] also demonstrated that ET-1 plays an important role in the development of cardiac fibrosis in a two-kidney, one-clip rat model of renovascular hypertension. In this model, the use of the ET_B-selective antagonist IRL1038, but not the ET_A-selective antagonist BQ-123, attenuated cardiac fibrosis.

While benzo-1,3-dioxolyl- and ET benzofuranyl-substituted pyrrolidine derivatives could be useful for the treatment of congestive heart failure [172-175], recent patents reveal that pyrrolidine and piperidine derivates act as selective ET_A and ET_B receptor antagonists and that novel alkanesulfonamides may have beneficial effects in atherosclerosis [176, 177].

Transplantation

In organ transplantation, several studies confirmed the involvement of ET-1 in ischemia-reperfusion injury in heart, lung, liver, and kidney allografts [178, 179]. Treatment with the selective ET_A receptor antagonist BSF208075 has also protective effects on the microcirculation after ischemia/reperfusion. Uhlmann *et al*. [180, 181] were able to show that BSF208075 not only affects the expression of vasoactive genes, but also decrease gene expression of proinflammatory cytokines such as TNF-α, IL-1 and IL-6 *in vitro*. Ischemia/reperfusion injury promotes a microcirculation-associated inflammatory response, involving release of deleterious mediators, such as reactive oxygen species, TNF-α, IL-1, the upregulation of leucocytic and endothelial adhesion molecules such as selectins, β-integrins, intracellular adhesion molecule-1, and the interaction of platelets and leucocyte with the microvascular endothelium [180-182]. Based on these results selective antagonism of ET_A receptors may have anti-inflammatory potential through suppression of the mRNA of the genes of proinflammatory cytokines such as TNF-α, IL-1 and IL-6. Several other studies demonstrated also a pathogenetic involvement of ET-1 in ischemia/reperfusion injury of heart, lung, liver and kidney allografts [178, 183].

Elevated levels of ET-1 have been associated with inflammation and immune responses [15, 184, 185] as well as with transplant-associated diseases [179]. High ET-1 expression in early posttransplant biopsy specimens was related to poor long-term allograft function following renal transplantation [186], and combined endothelial and myocardial protection could be achieved by ET antagonism enhanceing transplant allograft preservation [187]. Multiple chemokines, cytokines, and proinflammatory mediators are involved in the immune response following transplantation [188]. ET-1 accumulates during cold storage of allografts and can be detected in the effluent preservation solution [189]. In addition, ET-1 is very likely to play a pivotal role in the development of chronic allograft dysfunction [190] which represents one of the major causes of late allograft loss [191]. Increased expression of components of the ET system has been reported in areas of neointimal proliferation, a hallmark of chronic allograft vasculopathy [187, 189].

There is growing evidence that hemodynamic effects of calcineurin inhibitors (CNI) may be caused by an altered expression of ET-1 [37, 192]. Enhanced levels of ET-1 lead to constriction of smooth muscle cells and cell proliferation, as could be shown in cell culture experiments [193, 194] The plasma levels of big ET-1 are dependent on CNI plasma levels 1 year after successful heart transplantation in patients [195]. Previous studies using ET_A receptor antagonism have used concomitant immunosuppressive therapy [15]; thus, the potential immunomodulatory actions of ET-1 and the actual contribution of ET-1 to chronic rejection were masked because of the immunosuppressive agents. In a recent study in the absence of immunosuppression, we found that treatment with the ET_A receptor antagonist darusentan following cardiac transplantation in the absence of immunosuppression changed circulating levels of IL-1 and TNF-α tissue and gene expression of different interleukins in a organ-specific fashion independent of graft atherosclerosis [190]. These findings are compatible with a direct immunomodulatory role for ET_A receptors during allograft rejection [190, 196]. Braun *et al*. [197] reported that ET_A receptor blockade with LU135252 prevented long-term deleterious changes on renal allograft function and morphology independently from systemic blood pressure and did not lower proteinuria, strongly arguing against any effect on glomerular hemodynamics and upregulation of renal ET-1 synthesis in a rat model of renal allograft rejection in which a role for the ET_A receptor has been demonstrated [198].

In this context a recent patent reports that tripeptide derivatives [199, 200], as well as pyrimidine sulfamides derivatives and benzo-fused heterocycles could show significant benefit in the treatment of ischemia and proliferative disorders associated with ET-1 [176, 201].

Infection

Ono *et al*. [202] have reported that myocardial and ET-1 plasma levels increased in mice with encephalomyocarditis

virus-induced myocarditis. The observation that ET-1 levels were higher in the myocardium than those in plasma suggests that the heart is major source of endogenous ET-1 in myocarditis. The non-selective ET_A/ ET_B receptor antagonist bosentan improved survival in these mice and reduced cellular infiltration and myocardial necrosis [202]. In addition, Pandey *et al*. [203] reported that another non-selective ET_A/ET_B receptor antagonist, SB217242, improved cardiac contractility and relaxation in these mice as well. ET-1 contributes to the pathogenesis of murine viral myocarditis and treatment with the non-selec-tive ET_A/ET_B receptor antagonists bosentan or SB217242 significantly improved myocarditis [202, 203]. In another study, Albertini *et al*. [128] reported that ET_A receptor blockade in rats completely prevented endotoxin-induced mortality and attenuated serum indices of myocardial, renal, liver and lung injury. Previous studies on experimental septic shock models evaluated the effects of ET_A receptor antagonists on injury and dysfunction of a single organ and showed that ET-1 has strong cardiodepressant effects in sepsis [135, 204].

We have recently reported that ET-1 contributes to the pathogenesis of chagasic cardiomyopathy *(Trypanosoma cruzi)* showing that interventions inhibiting the synthesis of ET-1 and/or neutral endopeptidase might have either deleterious or protective effects on myocardial structure and function in chagasic cardiomyopathy [137, 149, 184, 205]. *Trypanosoma cruzi* infection induces ET-1 release from endothelial cells [206], and the released ET-1 contracts vascular smooth muscle via activation of ET_A receptors [207]. The mechanism by which ET-1 controls *Trypanosoma cruzi* infection are not investigated yet, however, ET-1 acting mainly on ET_A receptors can activate macrophages to release cytokines such as TNF-α [55]. As TNF-α-induced NO production by macrophages plays an important role in the acute phase of the infection [208], it is possible that the ability of ET-1 to release TNF-α may account for the effects observed. In this regard, we have shown that inflammatory mediators such as platelet-activating factors and chemokines, which like ET-1 act on G-protein-coupled receptors, activate macrophages to produce NO and kill *Trypanosoma cruzi* [209].

CURRENT AND FUTURE DEVELOPMENTS

Endothelin (ET), a naturally occurring polypeptide with a broad range of activities including vasospastic, proinflammatory and profibrotic properties, has been implicated in inflammatory and immunomodulatory processes. These are characterized by vascular damage, inflammatory infiltrates and progressive fibrosis of the skin and internal organs. Plasma concentrations of ET-1 may be elevated in certain inflammatory reactions and may favor the inflammatory cascade by facilitating the recruitment of leukocytes, the expression of cell adhesion molecules and cytokines. ET-1 also induces fibrosis by stimulating fibrotic factors, collagen synthesis, and fibroblast proliferation. Although there is growing evidence to suggest that ET-1 may act as a proinflammatory, profibrotic, and mitogenic factor, it is not clear whether activation of the ET system under these conditions is adaptive or pathogenic. While the relative importance of the receptors mediating these effects remains uncertain, both ET_A and ET_B receptors appear to be involved. In this context, the ET represents a novel target for immunomodulation. Recent studies with selective ET_A, ET_B or non-selective ET_A/ET_B receptor antagonists indicate that ET-receptors blockade, indeed, holds the potential to markedly improve consequences of local or systemic inflammatory processes improving symptoms and prognosis of the underlying disease. Clinical trials – in part under way [210, 211, 212] are now required to test this hypothesis in patients.

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