At the heart of tissue: endothelin system and end-organ damage

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ABSTRACT

ET (endothelin)-1 was first described as a potent vasoconstrictor. Since then, many other deleterious properties mediated via its two receptors, ETα and ETβ, have been described, such as inflammation, fibrosis and hyperplasia. These effects, combined with a wide tissue distribution of the ET system, its up-regulation in pathological situations and a local autocrine/paracrine activity due to a high tissue receptor binding, make the tissue ET system a key local player in end-organ damage. Furthermore, ET-1 interacts in tissues with other systems such as the RAAS (renin–angiotensin–aldosterone system) to exert its effects. In numerous genetically modified animal models, non-specific or organ-targeted ET-1 overexpression causes intense organ damage, especially hypertrophy and fibrosis, in the absence of haemodynamic changes, confirming a local activity of the ET system. ET receptor antagonists have been shown to prevent and sometimes reverse these tissue alterations in an organ-specific manner, leading to long-term benefits and an improvement in survival in different animal models. Potential for such benefits going beyond a pure haemodynamic effect have also been suggested by clinical trial results in which ET receptor antagonism decreased the occurrence of new digital ulcers in patients with systemic sclerosis and delayed the time to clinical worsening in patients with PAH (pulmonary arterial hypertension). The tissue ET system allows therapeutic interventions to provide organ selectivity and beneficial effects in diseases associated with tissue inflammation, hypertrophy or fibrosis.

INTRODUCTION

ET (endothelin)-1, the most abundant and best characterized of the ET family of peptides, was first identified as a potent vasoconstrictor secreted by vascular endothelial cells [1]. Since the initial description of ET-1 [2], it has become evident that the ET peptides, in addition to modulating vascular tone, are intimately involved in numerous pathophysiological processes and that they are expressed not only by endothelial cells, but by many other cell types. Indeed, ET-1 is a potent profibrotic agent, a co-mitogen, a pro-inflammatory and pro-angiogenic factor, and also increases ROS (reactive oxygen species) production.

Numerous lines of evidence indicate that ET-1 acts locally in an autocrine and paracrine fashion in physiological as well as pathological situations. Contribution of the ET system to pathology can be due to either an increase in tissue ET-1 production or an increase in tissue expression of its receptors. In the present review, we will focus on the function of the ET axis [preproET-1, big ET-1, ECE (ET-converting enzyme), ET-1 and ET receptors] as an autocrine/paracrine tissue system. The ET system expression pattern and ET-1-related key words: endothelin, end-organ damage, fibrosis, hypertrophy, inflammation, remodelling.

Abbreviations: AngII, angiotensin II; AP-1, activator protein-1; BP, blood pressure; CHF, chronic heart failure; CTGF, connective tissue growth factor; ECE, ET-converting enzyme; ET, endothelin; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; IPF, idiopathic pulmonary fibrosis; NEP, neutral endopeptidase; NF-κB, nuclear factor κB; PAH, pulmonary arterial hypertension; RAAS, renin–angiotensin–aldosterone system; SHR, spontaneously hypertensive rat; α-SMA, α-smooth muscle actin; TF, tissue factor; TGF-β, transforming growth factor-β; VCAM-1, vascular cell adhesion molecule-1.

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properties will be reviewed in normal and pathological situations; then, we will consider how local genetic modulation of the ET system affects tissue remodelling and discuss the interactions with other tissue hormonal systems in pathology. Finally, we will evaluate the long-term consequences of ET receptor blockade and the benefits that can be expected in clinical practice, especially regarding prevention of end-organ damage and impact on long-term outcomes.

**TISSUE PRODUCTION OF ET-1: EVIDENCE FOR AN AUTOCRINE AND PARACRINE ROLE OF ET-1**

ET-1 is synthesized as an inactive 203-amino-acid precursor, preproET-1, which is cleaved to form a second inactive 39-amino-acid segment called ‘big’ ET-1. The last part of the process involves a chymotrypsin-like peptidase called ECE and leads to the production of the pharmacologically active 21-amino-acid peptide ET-1. PreproET-1 mRNA transcription is widespread in animal and human tissues, including the heart, lung and adrenals [3]. Analysis of the distribution of ECE-1 and its substrate big ET-1 reveals a similar tissue pattern, characterized by a high expression in the cardiovascular, endocrine and reproductive systems [4]. ECE-1 gene disruption leads to developmental abnormalities similar to those observed in ETα- and ETβ-receptor-deficient animals, although mature ET-1 can be found in these ECE-1−/− embryos, suggesting that other proteases can generate ET-1, but also that tissue-specific production of ET-1 influences development. NEP (neutral endopeptidase) 24.11 can be an alternative way to cleave big ET-1 into ET-1. As seen for ECE-1, analysis of NEP 24.11 distribution reveals a broad tissue expression [5]. Beside ECE-1 and NEP 24.11, another source of tissue ET-1 is chymase. In addition to being a major source of AngII (angiotensin II) in interstitial tissues, chymase has been shown to generate ET-1-(1–31) from big ET-1 [6]. ET-1-(1–31) also exerts biological effects and can be converted into ET-1-(1–21) via NEP 24.11 and ECE-1 [7]. Although NEP 24.11 and ECE-1 have been found to be expressed in a wide array of cells, chymase is present only in mast cells. With respect to mast cell distribution (e.g. skin, synovium and perivascular tissue), this mode of production would also allow a tissue-wide production of ET-1-(1–31) and ET-1-(1–21) [8]. Table 1 summarizes the distribution of the ET system in various tissues and indicates that both the source cells (producing ET-1) and target cells (expressing ET receptors) are localized in the same tissues.

Two key characteristics can explain the tissue tropism of the ET system: (i) the mode of synthesis and the secretion of ET-1 that is orientated towards the tissue; and (ii) the stoichiometric binding of ET-1 to its receptors.

When produced by endothelial cells, 80% of ET-1 is secreted basolaterally towards the vessel wall and hence the tissue, whereas only 20% is released apically into the bloodstream [9]. Upon stimulation by thrombin, endothelial ET-1 production is increased and remains orientated towards the tissue, suggesting a similar mode of secretion in pathology as well. This polarized

### Table 1 Tissue localization of the ET system beyond endothelial cells

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cell type</th>
<th>ET system component</th>
<th>Disease</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Lung</td>
<td>VSMCs</td>
<td>PreproET-1 mRNA</td>
<td>PAH</td>
<td>[43]</td>
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<td></td>
<td>Alveolar epithelium and pulmonary</td>
<td>PreproET-1, ETα and</td>
<td>Scleroderma-associated fibrotic lung disease</td>
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<td>interstitium</td>
<td>ETβ mRNA</td>
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<td>Heart</td>
<td>Cardiomyocyte</td>
<td>PreproET-1 and ECE-1</td>
<td>Failing heart</td>
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<td>Kidney</td>
<td>Renal cortex and medulla</td>
<td>PreproET-1, ETα and</td>
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<td>ETβ mRNA</td>
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<td>Adrenal cortex</td>
<td>Zona glomerulosa cells and</td>
<td>PreproET-1, ETα and</td>
<td>Aldosterone-producing adenoma</td>
<td>[71]</td>
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<td></td>
<td>aldosterone-producing adenoma cells</td>
<td>ETβ mRNA</td>
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<tr>
<td>Skin</td>
<td>Fibroblasts</td>
<td>ETα and ETβ mRNA</td>
<td>Scleroderma</td>
<td>[19]</td>
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<tr>
<td></td>
<td>Keratinocytes</td>
<td>preproET-1, ETα and</td>
<td>Scleroderma</td>
<td>[106]</td>
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<td></td>
<td></td>
<td>ETβ mRNA</td>
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<td>Atherosclerotic lesions</td>
<td>Coronary VSMCs and macrophages</td>
<td>PreproET-1 and ETβ</td>
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<td></td>
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<td>mRNA</td>
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<td>Stellate cells</td>
<td>PreproET-1, ETα and</td>
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<td></td>
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<td>ETβ mRNA</td>
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<td>Endometrium and myometrium</td>
<td>Stroma and epithelial cells</td>
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<td>[109]</td>
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<td></td>
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<td>preproET-1 mRNA</td>
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<tr>
<td>Central nervous system</td>
<td>Hypothalamus and neurons</td>
<td>PreproET-1 mRNA</td>
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<td>[111]</td>
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abuminal secretion is fundamental to the paracrine action of ET-1 since it can in this way directly activate pericytes, leading to a contractile phenotype [10].

Not only endothelial cells, but many other resident cells such as smooth muscle cells or fibroblasts can produce ET-1 within the tissue. In the heart, ET-1 acts as a paracrine hypertrophic factor and its production by fibroblasts induces myocyte hypertrophy via binding to ET_\text{A} receptors [11]. In vivo experiments in dogs confirmed a paracrine role of tissue-generated ET-1, as infusion of its inactive precursor, big ET-1, induced vasoconstriction without any significant increase in plasma ET-1 [12]. These findings indicate that, after uptake or diffusion at the endothelial level, big ET-1 is cleaved \textit{in situ} into ET-1 that will act within the media to induce smooth muscle cell contraction. Similarly, in healthy human subjects, the ECE inhibitor phosphoramidon caused vasodilation when infused alone, suggesting that endogenous ET-1, mainly produced by endothelial cells, is processed towards the media to induce smooth muscle cell contraction [13]. Taken together, these observations confirm that enzymatic processing of ET-1 from its precursor, ET-1 secretion and then binding to its receptors is orientated towards the vasculature and hence the tissue.

In addition to a tissue-oriented secretion, the paracrine activity of ET-1 can be explained by its binding properties on ET receptors. Biological effects of ET-1 are mediated by two G-protein-coupled receptors ET_\text{A} and ET_\text{B}. The receptors are distributed on a wide variety of cells, including endothelial cells, epithelial cells and macrophages (ET_\text{B}), vascular smooth muscle cells, cardiomyocytes, stellate cells, adrenal cells, renal cortex and medulla, fibroblasts and endometrium (ET_\text{A} and ET_\text{B}). In addition to a high tissue receptor density, estimated to be 10–100 nM in the vascular wall [14], it is striking to observe that ET receptors display a very high affinity for ET-1 as shown by a small \( K_d \) (dissociation constant) value between ET_\text{A} receptor and ET-1, estimated to be 20 pM in membrane preparations [15]. Such characteristics, i.e. receptor densities much higher than the \( K_d \) values, are typical of a stoichiometric binding condition. Therefore non-stop secretion of ET-1 by endothelial cells will be constantly buffered by the surrounding tissue receptors, which will keep the free (unbound) concentrations at low levels, whereas tissue-bound ET-1 concentrations will remain high. Stoichiometric binding conditions will favour an autocrine and powerful action of ET-1, as secreted ET-1 will be avidly trapped by surrounding cells because of its particularly high affinity for its receptors, and low concentrations of ET-1 will be sufficient to activate its receptors (Figure 1).

The combination of polarized secretion and high tissue receptor binding will affect ET-1 plasma concentrations, since tissue ET receptors act as a buffer system by trapping secreted ET-1. Therefore the fact that plasma ET-1 concentrations are below expected active...
concentrations can be explained by the fact that free tissue ET-1 concentrations are low [14]. Once tissue receptors are saturated, a spillover may occur and excess ET-1 will enter the circulation, where it can exert systemic effects. As a consequence, plasma ET-1 concentrations would not reflect tissue ET-1 activity, but rather a late stage of activation of the tissue ET system. Indeed, it is striking to see that, in patients with mild-to-moderate systemic hypertension, vascular and not plasma ET-1 concentrations are selectively increased [16].

These characteristics should be taken into consideration when studying the ET system in both preclinical and clinical studies. Hence injection of exogenous ET-1 might not mimic a pure increase in endogenous ET-1 production, but will result in a biphasic action involving first an activation of endothelial ET\(_A\) receptors followed by an ET\(_A\)-mediated vasoconstriction. In the same animals, big ET-1 does not elicit the initial transient depressor effect but, through a vascular EC-dependent process, directly triggers vasoconstriction [17]. Therefore, in order to reproduce the pathophysiological activity of the ET system and especially the mode of secretion of ET-1, other approaches, such as big ET-1 infusion, perivascular administration or tissue-targeted prepro-ET-1 gene overexpression, should be preferred [12,18].

**ET-1-MEDIATED EFFECTS: BEYOND VASOCONSTRICTION**

The ability of ET-1 to induce vasoconstriction was the first property described [2] but, since then, ET-1, via its two receptors ET\(_A\) and ET\(_B\), has been shown to induce tissue remodelling. These structural changes can be attributed to the strong binding of ET-1 to its receptors, and its ability to trigger fibrosis, inflammation, hypertrophy and apoptosis via specific signalling pathways, such as protein phosphorylation and transcription factor activation. As a profibrotic agent, ET-1 is a key modulator of extracellular matrix composition. In fibroblasts, ET-1 increases collagen production (I/III) via both ET\(_A\) and ET\(_B\) receptors, decreases MMP-1 (matrix metalloprotease-1) collagenase activity [19] and participates in the epithelial–mesenchymal transition [20]. In various cell types, ET-1 is also a pro-inflammatory mediator that activates NF-κB (nuclear factor κB) [21], leading to increased IL (interleukin)-6 production [22]. Hypertrophic activity of ET-1 involves several long-term signalling pathways involving phosphorylation, e.g. NHE-1 (Na\(^+\)/H\(^+\) exchanger-1) activation [23] via MAPK (mitogen-activated protein kinase) [24], p38 [25] and activation of transcription factors such as NF-κB [26]. Furthermore, ET-1, upon stimulation of its receptors, can exert its mitogenic activity via transactivation of EGFR (epidermal growth factor receptor)-dependent signalling pathways [27].

Finally, ET-1 is a potent survival factor that protects cells from apoptosis, as shown in fibroblasts [28], vascular smooth muscle cells [29] and cardiomyocytes [30].

**ROLE OF THE TISSUE ET SYSTEM IN PATHOLOGY**

Despite the pleiotropic effects of ET-1, it is striking to note that blockade of its receptors has so little effect on physiology, in contrast with what is observed in pathological models and human diseases [31]. This discrepancy can be explained by the fact that both tissue ET-1 and its receptors are up-regulated in pathological states. We will also see that tissue activation of the ET system correlates well with disease severity, be it in the vasculature, heart, lung or kidney.

Rat models of systemic hypertension, such as DOCA (deoxycorticosterone acetate)-salt, SHRSP [stroke-prone SHRs (spontaneously hypertensive rats)], Dahl salt-sensitive or AngII-infused rats, exhibit vascular increases in ET-1 in association with vascular hypertrophy and endothelial dysfunction. In these rats, ET receptor antagonists decrease vascular inflammation and remodelling, and improve endothelial function. Conversely, SHRs or one-clip hypertensive rats, which do not exhibit increased tissue ET-1, are not responsive to ET receptor antagonists [32]. In hypertensive patients, vascular but not plasma concentrations of immunoreactive ET-1 are increased [16,33,34]. In addition to this tissue increase in ET-1, functional studies suggest that the ET receptor distribution is modified. Although, in healthy subjects, selective ET\(_A\) receptor antagonists induced greater vasodilation than did dual antagonists [35], suggesting a vasodilatory role of endothelial ET\(_B\) receptors, the opposite was observed in hypertensive patients: dual antagonism was superior to selective ET\(_A\) receptor antagonism in terms of maximal efficacy on forearm blood flow, indicating a predominant vasoconstricting activity of the ET\(_B\) receptors located on smooth muscle cells [36]. Therefore the change in the pattern of expression in the ET receptors observed in pathological conditions induces a shift in the activity of ET-1, leading to severe detrimental effects.

As in systemic hypertension, modifications of the tissue ET system are critical in cardiac hypertrophy and CHF (chronic heart failure). In a rat model of cardiac hypertrophy, ventricular ET-1 levels increased markedly following pressure overload, correlating with the level of cardiac hypertrophy, whereas plasma ET-1 levels increased transiently following surgery. Local ventricle is the source of the ET-1 in vivo as preproET-1 mRNA and the density of ET-1-binding sites (both ET\(_A\) and ET\(_B\) receptors) are increased in cardiac myocytes from hypertrophied hearts and in CHF models [37,38]. Similarly, in patients with CHF, tissue ET-1 levels as well
as ET\textsubscript{A} receptors are increased in the failing heart [39,40]. Therefore a global activation of the tissue ET system can be observed in CHF.

The ET system is known to be up-regulated in IPF (idiopathic pulmonary fibrosis). In the bleomycin rat model, this activation is characterized by an up-regulation of ET\textsubscript{A} receptor expression on pulmonary fibroblasts and an increase in lung ET\textsubscript{B} receptor immunoreactivity due to invading monocytes [41]. In patients with IPF, strong diffuse expression of ECE-1 located in airway epithelium, proliferating type II pneumocytes, and in endothelial and inflammatory cells has been reported. ECE-1 immunostaining co-localized with big ET-1 and ET-1 immunostaining, and correlated with disease activity [42].

In other lung diseases, activation of the tissue ET system can also be observed, as in the lungs of patients with PAH (pulmonary arterial hypertension), where ET-1 co-localized with pulmonary arteries that had medial thickening and intimal fibrosis [43]. In other forms of PAH, such as scleroderma-associated lung disease and PAH related to CTEPH (chronic thromboembolic PAH), an increase in tissue ET-1 and in the ET\textsubscript{A}/ET\textsubscript{B} receptor ratio in the interstitium and vasculature has been reported [44,45]. It is of interest to note that, together with the increased expression of ET-1 and its receptors, the change observed in the ET\textsubscript{A}/ET\textsubscript{B} receptor ratio reflects a selective up-regulation of ET\textsubscript{B} receptors [45,46].

Several models of renal disease indicate a variety of changes in the expression pattern of the ET system, as evidenced in isolated kidney from rats with hypertension, diabetes mellitus or hypercholesterolaemia. In all models, endothelial ET\textsubscript{B} receptor expression in the kidney was decreased when compared with that in vascular smooth muscle cells. This switch was associated with a decrease in NO production [47]. A tissue increase in ET-1 was observed in rat models of polycystic disease [48], mimicking the observation made in patients with an autosomal-dominant form of this disease, in whom immunoreactive ET-1 was increased at the level of the epithelial, mesangial and vascular smooth muscle cells [49]. In rats subjected to renal mass reduction, a time-dependent increase in proteinuria paralleled the up-regulation of intrarenal ET-1 mRNA expression [50]. Such a correlation between functional changes and tissue activity was also found in patients with IgA nephropathy, where the highest levels of ET\textsubscript{B} receptor and preproET-1 gene expression were found among patients presenting with higher-grade proteinuria [51].

In conclusion, in addition to an up-regulation of tissue ET secretion observed in pathological states, a topological shift in receptor expression is also observed, characterized by a decrease in epithelial/endothelial ET\textsubscript{B} receptors and an up-regulation of tissue ET\textsubscript{A} and ET\textsubscript{B} receptors, favouring the deleterious long-term effects of ET-1 (e.g. remodelling and fibrosis).

### GENETIC MODULATION OF TISSUE ET SYSTEM

As mentioned previously, tissue-specific genetic modulation of the expression of the ET system is a meaningful way to highlight the deleterious effects of ET-1 and its receptors on end-organ damage.

In transgenic mice overexpressing human ET-1, especially in the brain, heart, lung and kidney, a tissue-specific profibrotic phenotype was characterized by increased production of cardiac collagen type III and laminin, and the development of renal interstitial fibrosis associated with glomerulosclerosis [52]. Endothelium-restricted ET-1 overexpression led to endothelial dysfunction, vascular hypertrophy and inflammation with activation of transcription factors such as AP-1 (activator protein-1) and NF-κB [53]. Lung-specific ET-1 overexpression triggered tissue fibrosis and chronic inflammation as shown by the recruitment of CD4-positive inflammatory cells [54]. ET-1 also induced inflammation in the heart, where selective cardiomyocyte overexpression of ET-1 caused macrophage infiltration, NF-κB translocation, and increased IL-1, IL-6, TNF-α (tumour necrosis factor-α) and IFN-γ (interferon-γ), as well as dilated cardiomyopathy leading to premature death [55]. In these different transgenic models, no development of systemic hypertension or PAH was observed, ruling out pressure-dependent mechanisms for the tissue findings and confirming the paracrine mode of action of ET-1.

Although activation of the ET system leads to tissue fibrosis and inflammation, genetic modifications aiming at switching off this system have revealed functional and structural beneficial effects. Neuron-specific preproET-1-knockout mice are resistant to acute and neuropathic pain [56]. Cardiomyocyte-specific disruption of preproET-1 leads to increased susceptibility to apoptosis [30], confirming in vitro findings suggesting that ET-1 acts as a survival factor and is critical in tissue remodelling. Indeed, these animals are resistant to hypertrophy induced by thyroid hormones [57], indicating that ET-1 is a crucial downstream effector for the hypertrophic response to thyroid stimulation, pointing to an interplay with hormonal systems.

### ET-1 INTERACTS WITH OTHER TISSUE SYSTEMS

ET-1 closely interacts with local paracrine systems in order to mediate its long-term effects on organ remodelling. In this section, we will review the interrelationships between the ET system and (i) the RAAS (renin–angiotensin–aldosterone system) and (ii) TGF-β (transforming growth factor-β) signalling.
Lüscher’s group first showed that AngII was able to increase ET-1 production in vascular smooth muscle cells [58]. This was confirmed in vivo, where AngII infusion increased tissue ET-1 and induced vascular hypertrophy, whereas administration of the selective ET<sub>A</sub> receptor antagonist darusentan prevented the vascular changes in geometry [59]. At the cardiac level, ET-1 and AngII can act synergistically as mediators of mechanical-stress-induced cardiomyocyte hypertrophy [60,61]. Blockade of the ET system counteracts AngII-related fibrotic and hypertrophic effects, such as leucine incorporation, CTGF (connective tissue growth factor) production and intracellular pH increase after mechanical stretch, a known hypertrophic pathway in cardiomyocytes [62,63]. In vivo experiments confirmed that dual ET<sub>A</sub>/ET<sub>B</sub> receptor antagonism indeed prevented AngII-induced end-organ damage in a BP (blood pressure)-independent manner [64] and also was able to improve survival on top of ACE (angiotensin-converting enzyme) inhibition in rats with CHF [65].

Aldosterone is another hormone that is known to be produced locally within the heart [66] and to participate in cardiac remodelling and fibrosis [67,68]. There is pre-clinical and clinical evidence that aldosterone and ET-1 can interact, as hyperaldosteronism is associated with increased ET-1 production [69] and treatment with the dual ET<sub>A</sub>/ET<sub>B</sub> receptor antagonist bosentan (Tracleer®; Actelion Pharmaceuticals Limited) decreases plasma aldosterone concentrations in patients with CHF [70]. Besides a direct effect on aldosterone production by the adrenal gland, which expresses both ET<sub>A</sub> and ET<sub>B</sub> receptors [71], this interaction appears to occur at the tissue level as shown in a rat model of liquorice-induced hypertension in which vascular ET-1 content was increased. In this model, tissue ET-1 content was normalized by mineralocorticoid receptor antagonists, resulting in improved endothelial function [72]. Conversely, ET<sub>A</sub> receptor blockade prevented vascular hypertrophy, production of ROS [73], cardiac fibrosis [74] and renal inflammation [75] in aldosterone-infused rats. Therefore ET-1 appears to be a key mediator of aldosterone-mediated deleterious effects.

TGF-β is a potent tissue fibrotic agent that induces preproET-1 gene expression in many cell types, such as vascular smooth muscle cells [76], mesangial cells [77] and cardiac cells [11]. This increase in gene expression involves two DNA elements of the preproET-1 gene: an AP-1 site for constitutive and induced expression, and a regulatory sequence that constitutes a specific binding site for Smad transcription factors [78,79]. TGF-β-induced fibrosis appears to be ET-1-dependent, since TGF-β-induced production of α-SMA (α-smooth muscle actin), a protein that promotes extracellular matrix contraction and remodelling, can be blocked by bosentan [78]. In human fibroblasts, gene expression analysis revealed that dual ET<sub>A</sub>/ET<sub>B</sub> receptor blockade prevented the induction by TGF-β of 109 transcripts involved in tissue remodelling [80], including procontractile genes (tropomyosin 1 and α-SMA), adhesive genes (CTGF) and matrix-accumulation-promoting genes [type I collagen and TIMP-3 (tissue inhibitor of metalloproteinases-3)]. Conversely, TGF-β has been shown to be a mediator of ET-1-induced epithelial–mesenchymal transition, a key step in the pathogenesis of IPF and other forms of pulmonary fibrosis [81]. In a rat model of chronic allograft nephropathy characterized by a sclerotic process, including the up-regulation of ET-1 and a TGF-β-dependent increase in deposition of extracellular matrix protein (e.g. fibronectin), treatment of allograft-recipient rats with bosentan prevented the up-regulation of fibronectin and TGF-β [82]. Taken together, these results suggest a close interplay between the two systems, and provide better understanding of the clinical observations showing that ET-1 and TGF-β are up-regulated in parallel in several diseases, such as in the lung of patients with IPF or scleroderma and in dermal fibroblasts from patients with systemic sclerosis [83,84].

Although the interplay between these systems is clearly highlighted by pharmacological blockade of the ET receptors, the effects of the RAAS and TGF-β on ET-1 system activation warrants further investigation (e.g. the impact on ET receptor expression and ECE activity).

**ET RECEPTOR ANTAGONISM PREVENTS END-ORGAN DAMAGE AND IMPROVES SURVIVAL IN VIVO**

Having reviewed the tissue-wide distribution of the ET system and its involvement in various pathological processes, we will now address the impact of chronic ET receptor antagonists in different pathologies associated with severe tissue remodelling (see Table 2).

In models of hepatic fibrosis and of chronic renal failure, ET receptor antagonism not only prevents the development of the disease, but it can also induce a regression of the fibrotic process. In a rat model of established hepatic fibrosis, dual ET<sub>A</sub>/ET<sub>B</sub> receptor blockade both prevented cellular production of type I collagen and fibronectin and reduced stellate cell activation and matrix production [20]. In preventive studies, use of bosentan in rats harbouring both human renin and angiotensinogen genes blunted activation of tissue NF-κB and AP-1 and of their downstream-regulated genes ICAM-1 (intercellular adhesion molecule-1), VCAM-1 (vascular cell adhesion molecule-1) and TF (tissue factor), independently of BP-lowering activity [64]. An improvement in renal function was obtained in diabetic rats by chronic administration of the dual ET<sub>A</sub>/ET<sub>B</sub> receptor antagonist macitentan, which prevented the development of renal lesions and hence decreased proteinuria [85]. Beyond end-organ damage, positive survival outcomes have been observed.
with ET receptor antagonists: the anti-inflammatory effects observed in high-renin rats were associated not only with decreased albuminuria and renal injury, but also with a lower mortality when compared with the effects of the non-selective vasodilator hydralazine [64]. In an NO-deficient mouse model of nephropathy, administration of bosentan after the development of fibrotic lesions normalized collagen type I expression and renal vascular fibrosis, resulting in a reduction in mortality independently of BP-lowering effects [86]. Similarly, bosentan, administered 7 days after surgery in rats with remnant kidney, decreased proteinuria and significantly improved survival [87,88].

Although clinical head-to-head comparisons between selective ET<sub>A</sub> and dual ET<sub>A</sub>/ET<sub>B</sub> receptor antagonists are missing, contribution of both ET<sub>A</sub> and ET<sub>B</sub> receptors in the long-term detrimental effects of ET-1 are suggested by long-term studies showing that only dual and not selective ET<sub>A</sub> receptor antagonists improve survival in other models of cardiovascular disease such as PAH [85,89,90] and CHF [65,91,92].

Therefore, by decreasing tissue inflammation and fibrotic processes, dual ET<sub>A</sub>/ET<sub>B</sub> receptor blockade has the potential to improve organ function and hence survival.

**CLINICAL EVIDENCE**

Clinical data obtained with ET receptor antagonists (Table 3) suggest the potential for beneficial long-term outcomes. In patients with PAH (Classes II to IV), ET receptor antagonists bosentan and ambrisentan slow disease progression, as shown by their ability to delay time to clinical worsening in double-blind placebo-controlled trials [93–95]. Comparison of actual survival with predicted survival curves based on historical data from patients with idiopathic PAH showed that bosentan-treated patients had a high survival rate, with Kaplan–Meier survival estimates of 96% at 12 months and 89% at 24 months, whereas predicted survival was 69 and 57% respectively [96]. Clinical data suggest that bosentan also improves survival in other forms of PAH, in particular Eisenmenger disease [97], scleroderma [98], and in children [99]. Clinical evidence for a role of ET-1 in connective tissue diseases has been reported for scleroderma patients, in whom ET-1 plasma

Table 2 Protective effects of ET receptor antagonists on end-organ damage in pre-clinical models

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Compound</th>
<th>End points</th>
<th>References</th>
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<td>Hepatic fibrosis (rat)</td>
<td>Bosentan (dual ET&lt;sub&gt;A&lt;/sub&gt;/ET&lt;sub&gt;B&lt;/sub&gt;)</td>
<td>Prevention of type I collagen and fibronectin increase and stellate cell activation.</td>
<td>[20]</td>
</tr>
<tr>
<td>High renin (dTGR rat)</td>
<td>Bosentan</td>
<td>Prevention of NF-κB, AP-1, ICAM-1, VCAM-1 and TF increase; decrease in proteinuria and improvement in survival.</td>
<td>[64]</td>
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<tr>
<td>Type I diabetes (streptozotocin rat)</td>
<td>Macitentan (dual ET&lt;sub&gt;A&lt;/sub&gt;/ET&lt;sub&gt;B&lt;/sub&gt;)</td>
<td>Prevention of glomerular and renal vascular lesions; decrease in proteinuria and improvement in survival.</td>
<td>[85]</td>
</tr>
<tr>
<td>Hypertension (L-NAME mouse)</td>
<td>Bosentan</td>
<td>Reversal of renal deposition of type I collagen and vascular fibrosis; improvement in survival.</td>
<td>[86]</td>
</tr>
<tr>
<td>Remnant kidney (rat)</td>
<td>Bosentan</td>
<td>Reversal of proteinuria and improvement of survival.</td>
<td>[88]</td>
</tr>
<tr>
<td>CHF (rat)</td>
<td>Bosentan</td>
<td>Decrease in both preload and afterload; increase in cardiac output, prevention of left ventricular hypertrophy, left ventricular dilatation and cardiac fibrosis, and improvement in survival.</td>
<td>[65,92]</td>
</tr>
<tr>
<td>CHF (rat)</td>
<td>Darusentan (selective ET&lt;sub&gt;B&lt;/sub&gt;)</td>
<td>Increase in left ventricle fractional shortening and cardiac index; reduction in left ventricular collagen density; no effect on survival.</td>
<td>[91]</td>
</tr>
<tr>
<td>PAH (monocrotaline rat)</td>
<td>Bosentan, macitentan and BSF420627 (dual ET&lt;sub&gt;A&lt;/sub&gt;/ET&lt;sub&gt;B&lt;/sub&gt;)</td>
<td>Prevention of both increase in pulmonary pressure and right ventricle hypertrophy; improvement in survival.</td>
<td>[85,89,90]</td>
</tr>
<tr>
<td></td>
<td>Darusentan</td>
<td>Prevention of both increase in pulmonary pressure and right ventricle hypertrophy; no effect on survival.</td>
<td>[90]</td>
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</tbody>
</table>

Table 3 Pharmacological characteristics of marketed ET receptor antagonists

<table>
<thead>
<tr>
<th>Drug</th>
<th>ET receptor selectivity</th>
<th>Indication(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bosentan (TRACLEER&lt;sup&gt;®&lt;/sup&gt;)</td>
<td>Dual ET&lt;sub&gt;A&lt;/sub&gt;/ET&lt;sub&gt;B&lt;/sub&gt;</td>
<td>Treatment of PAH; reduction in new digital ulcer number in patients with systemic sclerosis</td>
</tr>
<tr>
<td>Ambrisentan (LEITARIS&lt;sup&gt;®&lt;/sup&gt;)</td>
<td>ET&lt;sub&gt;A&lt;/sub&gt;-selective</td>
<td>Treatment of PAH</td>
</tr>
<tr>
<td>Sitaxentan (THELIN&lt;sup&gt;®&lt;/sup&gt;)</td>
<td>ET&lt;sub&gt;B&lt;/sub&gt;-selective</td>
<td>Treatment of PAH</td>
</tr>
</tbody>
</table>
concentrations as well as dermal and pulmonary ET-1 expression are increased [44,83,100]. In vitro, ET-1 application reproduces the pattern of scleroderma in normal lung fibroblasts, whereas bosentan application normalizes the phenotype of scleroderma fibroblasts [101]. The role of tissue ET-1 in the progression of fibrotic disease has been confirmed in patients with systemic sclerosis, in whom bosentan decreased the occurrence of new digital ulcers [102]. Long-term effects on disease progression and survival have been suggested in a recent clinical trial [103]. In this 48-week open-label study in patients with PAH related to various connective tissue diseases, bosentan treatment was associated with an improvement in, or stability of, clinical status.

CONCLUSIONS

Activation of the tissue ET system triggers a broad spectrum of structural changes associated with inflammation, hypertrophy and fibrosis. These deleterious outcomes result from an activation of both ETA and ETB receptors. Beyond vascular effects, the ET system is a key modulator of multiple organ structures and function and a driver of disease progression. Clinical trials already suggest that ET receptor antagonism is able to have an impact on long-term outcome and delay time to clinical worsening in PAH. Therefore the ET system is a promising target for pharmacological blockade as a disease-modifying agent in pathologies associated with tissue remodelling.

REFERENCES

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