Role of endothelin in cardiovascular disease

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Abstract

The endothelins are a family of peptides secreted by vascular endothelium that possess potent and sustained vasoconstrictor properties. Endothelin-1 also has inotropic actions, influences salt and water homeostasis, alters central and peripheral sympathetic activity, stimulates the renin-angiotensin-aldosterone system, and is involved in cell growth and inflammation. There are 2 major endothelin receptors – ET$_A$ and ET$_B$ receptors, which typically have opposite effects. Stimulation of smooth muscle ET$_A$ receptors by endothelin-1 contributes to basal vascular tone and blood pressure. Stimulation of endothelial ET$_B$ receptors leads to the release of vasodilator substances and opposes ET$_A$ receptor mediated vasoconstriction.

Keywords: Endothelium; Endothelin; Endothelin antagonists; Hypertension; Chronic heart failure.

Introduction

Vascular endothelial cells produce a number of important vasodilator and constrictor substances. Prostacyclin and nitric oxide are potent vasodilators secreted by vascular endothelium. The isolation of endothelium-derived vasodilators initiated a search for counterbalancing constricting factors (or EDCF). A long acting vasoconstrictor substance was isolated from porcine aortic endothelial cells in 1988, and called endothelin. Endothelins are family of peptides, which...
comprises endothelin-1, endothelin-2 and endothelin-3, each containing 21 amino acids (Figure 1). In addition, human chymase selectively cleaves big endothelins at the Tyr31-Gly32 bond and produces novel 31-amino acid-length endothelins, ETs (1-31)88. Endothelin-1 is the predominant isoform expressed in vasculature and the most potent vasoconstrictor currently known4,5.

Endothelin-1 is a potent vasoconstrictor and has inotropic, chemotactic and mitogenic properties. In addition, endothelin-1 influences salt and water homeostasis through its effects on the renin-angiotensin-aldosterone, vasopressin and atrial natriuretic peptide and stimulates sympathetic nervous system. The overall action of endothelin is to increase blood pressure and vascular tone. Therefore, endothelin antagonists may play an important role in the treatment of cardiac, vascular and renal diseases associated with regional or systemic vasoconstriction and cell proliferation, such as essential hypertension, pulmonary hypertension, chronic heart failure and chronic renal failure. In this article we review the biology of the endothelins and the accumulated evidence from preclinical and clinical studies on the potential role of endothelin antagonists in the treatment of a variety of human disorders.

### Endothelin production

#### Regulation and sites of generation

Each member of the endothelin family is represented by a separate gene that encodes a specific precursor for the mature isoform (Figure 1). Endothelin-1 generation is increased by many stimuli, including vasoactive hormones, growth factors, hypoxia, shear stress, lipoproteins, free radicals, endotoxin and cyclosporin6. Production of endothelin-1 is inhibited by endothelium-derived nitric oxide, nitrovasodilators, natriuretic peptides, heparin and prostaglandins6.

The major site of generation of endothelin-1 is in endothelial cells4,5. Endothelin-1 is also produced in the heart, kidney, CNS and posterior pituitary6. Endothelin-2 is produced in endothelial cells, heart and kidney7,8. Endothelin-3 is expressed in the endocrine, gastro-intestinal and central nervous systems, but not in endothelial cells6.

### Biosynthesis, clearance and significance of plasma endothelin levels

The initial product of the human endothelin-1 gene is a 212 amino acid peptide called preproendothelin-1, which is converted to proendothelin-1 after removal of a short secretory sequence. Proendothelin-1 is then cleaved by furin to generate a biologically inactive 38 amino acid precursor, big endothelin-13,9 (Figure 1). The formation of mature endothelin-1 requires cleavage of big endothelin-1 by one of several endothelin converting enzymes (ECE’s), unrelated to angiotensin converting enzyme. ECE-1 is the physiologically active ECE15, which is relatively selective for big endothelin-1. There are two splice variants, ECE-1a and ECE-1b, that have functionally distinct roles and tissue distributions11. ECE-1a is expressed in the Golgi network of endothelin-producing cells, such as endothelial cells, and cleaves big endothelin-1 to form endothelin-1. ECE-1b is localized at the plasma...
membrane where it cleaves extracellular big endothelin-1. Both ECE-1 and ECE-2 are inhibited by phosphoramidon, but not by selective neutral endopeptidase or ACE inhibitors. ECE has also been found on alpha-actin filaments in smooth muscle cells. More recently, it was discovered that chymase from human mast cells can selectively cleave big endothelins at the Tyr31-Gly32 bond to produce novel trachea-constricting 31-amino acid-length endothelins, ETs (1-31), without any further degradation products (Figure 1).

Intra-arterial infusion of big endothelin-1 produces dose-dependent forearm vasoconstriction in humans. ECE inhibition by phosphoramidon completely blocks vasoconstriction to big endothelin-1. Thus, it is likely that vasoconstriction to big endothelin-1 reflects vascular conversion to the mature peptide by a phosphoramidon-sensitive ECE, located in endothelial and smooth muscle cells.

A significant part of endothelin-1 clearance occurs through receptor binding and internalisation. Pretreatment with a large dose of unlabelled endothelin-1 blocks pulmonary clearance of radiolabelled endothelin-1, supporting receptor mediated clearance. Selective ETB inhibition increases plasma endothelin-1 concentrations and does not affect big endothelin-1 or C-terminal fragment concentrations, confirming that the increase is mediated by displacement of endothelin-1 from receptors. Neutral endopeptidase also plays a role in enzymatic degradation of the endothelins.

Concentrations of endothelin-1 in blood are lower than those that cause vascular contraction in vitro or in vivo. Cultured endothelial cells secrete substantially more endothelin-1 towards the adjacent vascular smooth muscle than into the lumen.

Therefore, endothelin is thought to be a locally acting paracrine substance rather than a circulating endocrine hormone. Nevertheless, venous plasma concentrations of endothelin-1 have been used as a marker for synthesis of the peptide by vascular endothelium. Measurement of big endothelins and the C-terminal fragment formed when they are cleaved by ECE substantially assists interpretation of plasma endothelin levels. Table 1 summarizes currently known ECE inhibitors.

### Signal transduction

ETA and ETB are two distinct endothelin receptor types with different pharmacologic characteristics. The ETA receptor affinity for endothelin-1 is much higher than for endothelin-3. ETA receptors are located in vascular smooth muscle cells, but not in endothelial cells (Figure 2). A number of peptide and non-peptide ETA antagonists have been synthesised; the prototype being the cyclic pentapeptide BQ-123. ETB receptors are located on endothelial cells. Endothelin-1 and endothelin-3 equally activate the ETB receptor, which in turn leads to vasodilatation via production of nitric oxide and prostaglandins. ET-1 (1-31) has also been demonstrated to cause vascular smooth muscle constriction via ETA receptor stimulation and to induce NO production in endothelial cells via ETB receptors. Some ETB receptors are located in vascular smooth muscle, where they may mediate vasoconstriction (Figure 2). Sarafotoxin S6c and endothelin-3 are selective peptide agonists and BQ-788 is a selective peptide antagonist at the ETB receptor.

The number of endothelin receptors is regulated by various factors. Angiotensin II and phorbol esters downregulate endothelin receptors whereas ischaemia and cyclosporin increase the number of endothelin receptors. Table 1 summarizes currently known selective ETA, ETB and nonselective ETA receptor antagonists.

### Intracellular events

The main intracellular pathway after activation of ETA or ETB receptors includes a G-protein dependent activation of phospholipase C and

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**Figure 2** – Vascular actions of endothelin-1 (ET).

Big ET = Big endothelin-1; ECE = endothelin converting enzyme.
subsequent hydrolysis of phosphatidyl inositol and generation of membrane-bound diacylglycerol and cytosolic inositol trisphosphate\textsuperscript{28,29}. Inositol trisphosphate causes release of $[\text{Ca}^{2+}]$ from intracellular stores and opening of membrane $\text{Ca}^{2+}$ channels\textsuperscript{30}. Diacylglycerol increases sensitivity of the contractile apparatus to elevation in intracellular $\text{Ca}^{2+}$ by activating protein kinase $\text{C}^{31}$. Diacylglycerol also affects nuclear signaling with possible effects on cell growth regulation.

**Actions** (major physiologic actions of endothelin-1 relevant to cardiovascular disease are summarized in Table 2).

**Vascular**

Endothelin-1 is the most potent vasoconstrictor agent of conduit arteries \textit{in vitro}\textsuperscript{3}. Endothelin-1, 2 and 3 induce transient vasodilatation due to nitric oxide and prostacycline release before the development of sustained vasoconstriction\textsuperscript{30,35}. Vasoconstriction to endothelin-1 is mediated by vascular smooth muscle cell $\text{ET}_A$ and $\text{ET}_B$ receptors. Endothelial cell $\text{ET}_B$ receptors mediate vasodilatation through production of endothelium-derived vasodilators (Figure 2). Vasoconstriction to $\text{ET}_B$ receptor agonists varies with species, vessel type and vessel size\textsuperscript{32}.

Bolus injections of endothelins in animals cause a blood pressure increase which persists for at least 60 min\textsuperscript{3}. The coronary and renal vascular beds exhibit maximal vasoconstriction to systemic administration endothelin-1 in animals\textsuperscript{34}. Bolus injections of endothelin usually also cause transient hypotension which is most marked for endothelin-3, and is mediated by endothelial $\text{ET}_B$ receptors. This initial hypotensive response does not occur if endothelin concentrations rise more slowly.

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<tr>
<th><strong>ET\textsubscript{A} receptor antagonists</strong></th>
<th><strong>ET\textsubscript{AB} receptor antagonists</strong></th>
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<tr>
<td>A-127722 (non-peptide)</td>
<td>A-182086 (non-peptide)</td>
<td>A-192621 (non-peptide)</td>
<td>B-90063 (non-peptide)</td>
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<td>ABT-627 (non-peptide)</td>
<td>CGS 27830 (non-peptide)</td>
<td>A-308165 (non-peptide)</td>
<td>CGS 26393 (non-peptide)</td>
</tr>
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<td>BMS 182874 (non-peptide)</td>
<td>CP 170687 (non-peptide)</td>
<td>BQ-788 (peptide)</td>
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<td>BQ-123 (peptide)</td>
<td>J-104132 (non-peptide)</td>
<td>BQ-017 (peptide)</td>
<td>CGS 35066 (non-peptide)</td>
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<td>BQ-153 (peptide)</td>
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<td>IRL 1038 (peptide)</td>
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<td>PD-161721 (non-peptide)</td>
<td>SM-19712 (non-peptide)</td>
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<td>RES 701-1 (peptide)</td>
<td>TMC-66 (non-peptide)</td>
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<td>RO 468443 (non-peptide)</td>
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<td>FR 139317 (peptide)</td>
<td>PD 160672 (non-peptide)</td>
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<td>IPI-725 (peptide)</td>
<td>RO-470203 \textbf{(bosentan)} \textsuperscript{(non-peptide)}</td>
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<td>RO 462005 (non-peptide)</td>
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<tr>
<td>LU 127043 (non-peptide)</td>
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<td>PABSA (non-peptide)</td>
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<td>TA-0201 (non-peptide)</td>
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<td>TBC 11251 (non-peptide)</td>
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ET = endothelin; ECE = endothelin converting enzyme.
which is likely to occur under physiological conditions.

Brachial artery infusion of endothelin-1 in humans causes a slow dose-dependent decrease in forearm blood flow, which is sustained for 2 hours after discontinuation of endothelin-1. In addition, endothelin-1 causes slow-onset, sustained constriction of cutaneous veins. Identical doses of endothelin cause greater vasoconstriction when infused over a longer period of time. Endothelin-1 increases blood pressure in human subjects by 5-10% when given intravenously at doses of ~1 pmol/kg/min over 60 min. As in animals, the haemodynamic effects develop slowly and are sustained for more than 1 hour. Systemic administration of endothelin-1 also causes renal and splanchnic vasoconstriction in humans.

**Cardiac**

Endothelin-1 has positive chronotropic and inotropic effects *in vitro*. Intracoronary administration of endothelin-1 causes coronary vasoconstriction, resulting in myocardial ischaemia and lethal ventricular arrhythmias. In animals, low doses of endothelin have a positive inotropic effect *in vivo*, whereas higher doses have negative inotropic effects, possibly due to myocardial ischaemia from coronary vasoconstriction and high afterload. Systemic administration of endothelin-1 in humans decreases cardiac output, probably through increased afterload and a baroreceptor mediated decrease in heart rate.

**Interactions with other endothelial mediators**

Nitric oxide synthase inhibitors attenuate the transient initial vasodilatation to endothelin administration and potentiate the constrictor and pressor effects of endothelin-1. This suggests that the endothelins stimulate nitric oxide production by vascular endothelial cells. Cyclo-oxygenase inhibitors potentiate the constrictor effects of endothelin-1, suggesting that endothelin-1 also increases prostacyclin production by endothelial cells. (Figure 2). In addition, adrenomedullin generation by endothelial cells is increased by endothelin-1. The endothelial effects of endothelin-1 to increase production of vasodilator substances are mediated by the ET\_B receptor, which thus acts to physiologically antagonize ET\_A receptor mediated vasoconstriction.

**Role of endothelin-1 in maintenance of vascular tone and blood pressure**

Inhibition of ECE or ET\_A receptors has a slow-onset hypotensive effect in normotensive animals. This hypotensive effect of anti-endothelin therapy is not observed in short term studies (i.e. < 10 min). Slow onset vasodilatation to anti-endothelin agents is consistent with the sustained vasoconstriction to endothelin-1 and also with the slow effect of endothelin receptor antagonists to reverse the pressor effects of endothelin-1 in

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<td>Pulmonary vascular bed</td>
<td>Causes vasoconstriction through vascular smooth muscle cell ET_A and ET_B receptors.</td>
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<td>Heart</td>
<td>Positive chronotropic and inotropic effects <em>in vitro</em>. Decreases cardiac output <em>in vivo</em>, due to increased afterload and a baroreceptor mediated decrease in heart rate. Mitogenic effect on cardiac myocytes and coronary vascular smooth muscle cells.</td>
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<td>Endocrine</td>
<td>Stimulates ACE and aldosterone release.</td>
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ET = endothelin; ACE = angiotensin converting enzyme.
animals. Intra-arterial administration of the ECE and neutral endopeptidase inhibitor phosphoramidon causes slow onset forearm vasodilatation in humans, whereas selective neutral endopeptidase inhibition alone does not cause vasodilatation. Local administration of peptide ET receptor antagonist BQ-123, or peptide ET and ET receptor antagonist TAK-044 cause slow onset forearm vasodilatation. In humans, systemic administration of TAK-044 causes systemic vasodilatation and decreases arterial pressure by 10-20%. These findings suggest that endogenous endothelin-1 has an ET receptor mediated physiological vasoconstrictor effect important for blood pressure maintenance. The non-peptide ET antagonist bosentan also decreases blood pressure in normotensive humans.

Local administration of ET receptor antagonist causes greater vasodilatation than local ET antagonist in humans. Local forearm ET receptor antagonist by BQ-788 causes sustained vasoconstriction in humans, which opposes the vasodilator action of BQ-123. ET receptor antagonists can cause vasoconstriction by blockade of tonic endothelial ET receptor mediated stimulation of nitric oxide and prostacyclin generation. However, ET receptor antagonists also block clearance receptors, thereby increasing endothelin-1 concentrations. The pressor effects of ET receptor antagonism are present even when nitric oxide generation is inhibited, and these effects can be blocked by ET antagonist, suggesting an effect on clearance of endothelin-1. Renal ET receptors cause natriuresis by preventing tubular reabsorption of sodium.

In summary, the overall physiological effect of endothelin-1 is to increase blood pressure. The cardiovascular effect of endogenous endothelin-1 depends on the balance between ET and ET mediated effects. Therefore, the cardiovascular effects of endogenous endothelin-1 generation may change in disease states if the number or function of ET and ET receptors are altered. For example, endothelial dysfunction with loss of nitric oxide activity would be expected to attenuate ET mediated vasodilatation and promote ET mediated vasoconstriction.

Renal effects

Endothelin-1 causes equal constriction of afferent and efferent arterioles in vitro, and decreases renal plasma flow and glomerular filtration rate (GFR) by 20%, without any effect on GFR, suggesting that the efferent arteriole is the predominant site of action of endogenous endothelin-1. Endothelin-1 blocks sodium reabsorption by tubular Na+/K+-ATPase inhibition in the proximal tubule and collecting duct. More recently it was demonstrated that endothelin-1 decreases chloride flux in the thick ascending limb of the loop of Henle, thus contributing to the natriuretic effect. Endothelin-1 also blocks water reabsorption in the collecting duct by inhibiting the effects of ADH. In volume depleted rats, endothelin-1 production in the renal tubule decreases and endothelin receptor number in glomeruli and tubules increases. These tubular effects occur with ET receptor agonists and are not blocked by BQ-123, suggesting that they are mediated by ET receptors. Deletion of renal ET receptors in ET knockout rats causes salt-sensitivity and hypertension that is not reversible with ETA antagonism. In summary, endothelin-1 has two direct renal actions, causing renal vasoconstriction (ET) and tubular sodium and water loss (ET), these actions probably reflecting separate sites of production in renal blood vessels and tubules.

Effects on cell growth and inflammation

Endothelin-1 increases mRNA expression for the growth promoting proto-oncogenes c-fos and c-myc. Endothelin-1 (1-21) has a potent mitogenic effect on vascular smooth muscle cells, cardiac myocytes and glomerular mesangial cells. Endothelin-1 (1-31) also stimulates proliferation of porcine and human coronary artery smooth muscle cells, and granulocyte-macrophage colony-stimulating factor. Endothelin-1 enhances neutrophil adhesion to human coronary artery endothelial cells, which is mediated by ET receptors.

Endocrine effects

Endothelin-1 stimulates ACE activity in cultured endothelial cells and stimulates the tissue renin-angiotensin system in the rat isolated mesenteric bed. In addition, endothelin-1 stimulates release of aldosterone from isolated cortical zona glomerulosa cells, and adrenaline from medullary chromaffin cells. Angiotensin II increases endothelin-1 tissue levels and ECE activity in vivo, and the haemodynamic and proliferative effects of angiotensin II.
can be blocked by ET$_A$ receptor antagonism$^{81,82}$. These findings suggest that a positive feedback loop linking angiotensin II and endothelin-1 may exist in disease states such as heart failure. Antagonism of the endothelin system may help in patients with persistent RAAS activation despite maximally tolerated ACE inhibition or angiotensin receptor blockade.

**Anti-endothelin agents in essential hypertension** (potential clinical indications for selected endothelin antagonists are summarized in Table 3).

Endothelin acts as a mediator in the pathogenesis of hypertension and its complications because of its actions to increase vascular tone, activate the sympathetic nervous and renin-angiotensin-aldosterone systems and increase mitogenesis. In animal studies, anti-endothelin therapy seems to have different blood-pressure-lowering effect in different models of hypertension. Models of salt-sensitive hypertension (DOCA-salt and Dahl rats) and malignant hypertension (stroke-prone spontaneously hypertensive rats [SHRSPs]) are especially sensitive to the antihypertensive effect of endothelin receptor blockade$^{105,106,107,108,109}$. PABSA, a potent long-acting oral ET$_A$ receptor antagonist with weak ET$_B$ antagonist activity, reduced blood pressure in DOCA-salt hypertensive rats, spontaneously hypertensive rats (SHRs) and SHRSPs$^{98}$. Combined ET$_A$ and ET$_B$

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<td><strong>Bosentan.</strong> Antihypertensive effect in essential hypertension, this effect similar to treatment with 20 mg of enalapril$^{96}$.</td>
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<td><strong>Pulmonary hypertension</strong></td>
<td><strong>Bosentan.</strong> Attenuates hypoxia-reoxygenation-induced pulmonary hypertension, decreases leukocyte-mediated injury and improves pulmonary function$^{132}$.</td>
<td><strong>Bosentan.</strong> Improves exercise capacity, improves pulmonary hemodynamics, reduces Borg dyspnea index, and improves WHO functional class in primary or secondary pulmonary hypertension$^{134,183}$.</td>
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<td><strong>Coronary artery disease</strong></td>
<td><strong>Bosentan.</strong> Antiatherosclerotic effect$^{111,112,116}$. Suppresses intrapericardial endothelin-1-induced ventricular arrhythmias$^{163}$. <strong>LU135252.</strong> Reduces the number of cyclic coronary flow reductions in a model of variant angina$^{168}$. <strong>ABT-627.</strong> Preserves coronary endothelial function in experimental hypercholesterolemia$^{173}$.</td>
<td><strong>Bosentan.</strong> Increases coronary diameter in angiographically documented stable coronary artery disease$^{166}$.</td>
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<td><strong>Renal failure</strong></td>
<td><strong>Tezosentan.</strong> Prevents acute renal failure due to experimental rhabdomyolysis$^{188}$.</td>
<td><strong>ABT-627.</strong> Prevents the decrease in renal perfusion and glomerular filtration rate caused by ET-1 infusion$^{190}$.</td>
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<td><strong>Chronic heart failure</strong></td>
<td><strong>Bosentan.</strong> Improves hemodynamics, prevents the progression of left ventricular dysfunction, attenuates left ventricular remodeling and improves long-term survival$^{138,146,153}$. <strong>LU135252.</strong> Restores cardiac output dose-dependently, decreases blood pressure and heart rate, and limits left ventricular remodeling$^{139}$.</td>
<td><strong>Bosentan.</strong> Improves hemodynamics, clinical status and favorably alters the progression of heart failure$^{137,151,176}$. <strong>LU135252.</strong> Improves hemodynamics, attenuates the impairment of conduit vessel endothelial function$^{187,189}$. <strong>Tezosentan.</strong> Improves hemodynamics$^{185}$.</td>
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DOCA = deoxycorticosterone acetate; SHR = spontaneously hypertensive rats; SHRSP = stroke-prone spontaneously hypertensive rats; WHO = World Health Organization.
Anti-endothelin agents and complications of hypertension

The effects of endothelin-1 on renal function, cardiac and vascular growth may indicate their potential in preventing the complications of hypertension. Bosentan treatment entirely prevented the effects of a 10-day angiotensin II infusion in rats, such as hypertension, cardiovascular hypertrophy, reduction in renal blood flow and albuminuria. The reductions in blood pressure and cardiovascular hypertrophy by bosentan were similar to the effects of losartan in this model of hypertension, which suggests possible modulation of local action of angiotensin-II by endothelin. In addition to its blood pressure lowering effect, combined ETA and ETB receptor blockade by TAK-044 in SHRSPs resulted in a decrease in blood urea nitrogen, creatinine concentrations, plasma aldosterone, heart and kidney weight. Combined ETAB receptor blockade in SHRSP completely prevents cerebral arteriolar hypertrophy, despite only partial decrease in arterial pressure.

Anti-endothelin agents and pulmonary hypertension

The vasoconstrictor and mitogenic effects of endothelin-1 make it a likely participant in the pathophysiology of pulmonary hypertension, because pulmonary hypertension is characterized by endothelial injury, vascular smooth muscle proliferation and vasoconstriction of pulmonary resistance vessels. In porcine hypoxic pulmonary hypertension, endothelin contributes to pulmonary vasoconstriction through ETA receptor stimulation. Endothelin-1 levels are increased in air embolization-induced pulmonary hypertension in sheep and hypoxia-reoxygenation-induced pulmonary hypertension in piglets, which suggests that endothelin-1 is involved in the pathogenesis of these forms of pulmonary hypertension.

In monocrotaline-induced pulmonary hypertension in rats, the ETA antagonist TA-0201 is as effective as an oral prostacyclin analog in the prevention of progression of pulmonary hypertension and right ventricular hypertrophy. Both ETA receptor antagonism by FR139317 and ETAB receptor antagonism by TAK-044 significantly attenuated the increase in pulmonary artery pressure during air embolization in sheep. In piglets, bosentan treatment attenuated hypoxia-reoxygenation-induced pulmonary hypertension, decreased leukocyte-mediated injury and improved pulmonary function. Pretreatment with ETA receptor antagonist PD156707 blocks rebound pulmonary hypertension observed on acute withdrawal of inhaled NO in lambs. Sitaxsentan sodium, an oral selective ETA receptor antagonist, dose-dependently attenuated chronic hypoxia-induced and monocrotaline-induced pulmonary hypertension, right heart hypertrophy and pulmonary vascular remodeling in rats. Patients with primary and secondary pulmonary hypertension exhibit increased pulmonary vascular endothelin-1 mRNA expression, with the degree of expression proportional.
to pulmonary vascular resistance\textsuperscript{135}. Therefore, anti-endothelin treatment might be of interest in the treatment of patients with various forms of pulmonary hypertension.

Administration of the ET\textsubscript{A} receptor antagonist BQ-123 in 3 infants with postoperative pulmonary hypertension following corrective surgery for congenital heart disease resulted in improvement in pulmonary hemodynamics, however associated with a reduction in systemic blood pressure and ventilation-perfusion mismatch\textsuperscript{30}. Bosentan treatment in patients with primary pulmonary hypertension and pulmonary hypertension due to limited scleroderma resulted in favorable pulmonary hemodynamic changes but caused systemic hypotension\textsuperscript{134}. As it was demonstrated in a randomised, placebo-controlled clinical trial, 12-week Bosentan treatment in patients with severe primary or secondary pulmonary hypertension improved exercise capacity, increased cardiac index, decreased pulmonary vascular resistance, reduced Borg dyspnea index, and improved WHO functional class\textsuperscript{183}. The frequency and characteristics of adverse events did not differ between bosentan and placebo groups in this trial\textsuperscript{183}. Bosentan was approved by the FDA for treatment of pulmonary hypertension in November 2001.

**Anti-endothelin agents and atherosclerosis**

There is substantial evidence that mitogenic effects of endothelin-1 contribute to the development of atherosclerosis. Endothelin-1 markedly potentiates human vascular smooth muscle cell (VSMC) proliferation to platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF), acting mainly via ET\textsubscript{A} receptors\textsuperscript{113,114}. In cultured mouse VSMC, ECE inhibition by phosphoramidon and ET\textsubscript{A} receptor blockade by BQ-123 inhibits DNA synthesis induced by oligosaccharides of hyaluronic acid\textsuperscript{115}. ET\textsubscript{A} receptor blockade by BQ-123 inhibits endothelin-1-induced proliferation of human coronary smooth muscle cells\textsuperscript{114}. ET\textsubscript{A/B} receptor antagonist by bosentan inhibited neointimal development in collared carotid arteries of rabbits, a known model of atherosclerosis\textsuperscript{111}. Bosentan significantly attenuated the development of graft atherosclerosis in rat cardiac allografts\textsuperscript{112}. ET\textsubscript{A} receptor blockade in hyperlipidemic hamsters inhibits formation of early atherosclerotic lesions by decreasing the number and size of macrophage-foam cells\textsuperscript{116}. Therefore, in addition to its antihypertensive effects, anti-endothelin therapy may be anti-atherosclerotic.

**Anti-endothelin agents and coronary artery disease**

**Ischemia/reperfusion injury**

In rabbits subjected to coronary occlusion and reperfusion, ECE inhibition by SM-19712 reduced infarct size, the serum activity of creatinine phosphokinase (CPK) and the increase in serum endothelin-1\textsuperscript{154}. Reperfusion of hearts treated with BQ-123 showed a 30\% increase in the proportion of competent capillaries after 15‘ of ischemia and 300\% increase in the proportion of competent capillaries and a dramatic decrease in necrosis after 60‘ of ischemia compared to untreated hearts\textsuperscript{155}. Even if administered simultaneously with coronary reperfusion, ET\textsubscript{A/B} antagonism by TAK-044 improved cardiac hemodynamics, myocardial energy metabolism and decreased CPK release after 35 minutes of ischemia in rat hearts\textsuperscript{156}. Pretreatment of rabbit hearts with TAK-044 and the ACE inhibitor temocaprilat demonstrated a significant potentiation of the positive effects on myocardial energy metabolism of each agent alone during ischemia and reperfusion\textsuperscript{157}. TAK-044 significantly reduced ischemic cellular injury in ischemia/reperfused rat hearts\textsuperscript{158}. Both selective ET\textsubscript{A} antagonism by BQ-123 and selective ET\textsubscript{B} antagonism by BQ-788 improved myocardial contractility and endothelium-dependent vasodilatation in a heterotopically transplanted rat heart reperfused after ischemia\textsuperscript{159,160}. BQ-123 significantly increased myocardial blood flow during early reperfusion\textsuperscript{160}. Other investigators have reported that BQ-123 abolished post-ischemic increase in coronary flow rate in isolated rat hearts after ischemia and reperfusion and that bosentan significantly impaired the recovery of systolic function during reperfusion\textsuperscript{161}, although the majority of investigators report favorable actions of anti-endothelin agents in ischemia/reperfusion models.

Clinically, plasma endothelin levels predict 1-year mortality in patients after acute myocardial infarction\textsuperscript{171}. Aspirin is known to reduce reinfarction and mortality in patients who suffered an MI. Interestingly, millimolar concentrations of aspirin and sodium salicylate, its major blood metabolite, act as allosteric selective ET\textsubscript{A} receptor antagonists in human internal mammary arteries \textit{in vitro}\textsuperscript{169}. However, these concentrations of salicylates are not achieved in patients receiving 300 mg of aspirin once daily.

**Antiarrhythmic effects of endothelin antagonists**

Endothelin antagonists may have a potential as antiarrhythmic agents.
Selective ET$_A$ receptor antagonism by BQ-123 or selective ET$_B$ antagonism by PD161721 both reduced ischemia-induced ventricular arrhythmias in rats. Mixed ET$_{AB}$ antagonism by TAK-044 significantly reduced reperfusion ventricular tachycardia in ischemia/reperfused rat hearts. Endogenous pulmonary big endothelin produces arrhythmogenic effects that are aggravated in Watanabe heritable hyperlipidemia in rabbits. Mixed ET$_{AB}$ antagonism by bosentan significantly suppresses intraepicardial endothelin-1-induced ventricular arrhythmias in animals.

Endothelial dysfunction

Endothelial dysfunction is a predictor of the development of atherosclerosis and coronary artery disease. Hypercholesterolemia is known to lead to endothelial dysfunction. In vitro, the ECE inhibitor phosphoramidon improved bradykinin-induced vasodilatation of isolated porcine coronary artery. Acute intracoronary administration of ET$_A$ antagonist FR-139317 or ET$_{AB}$ receptor antagonist bosentan did not affect vasodilatation to acetylcholine in experimental hypercholesterolemia in pigs. However, chronic ET$_{AB}$ antagonism by RO 48-5695 and ET$_A$ receptor antagonism by ABT-627 preserves coronary endothelial function in experimental hypercholesterolemia in pigs. Forearm vasodilator response to ET$_A$ antagonist BQ-123 is increased in hypercholesterolemic patients, whereas vasoconstriction to exogenous endothelin-1 is unchanged.

Stable coronary artery disease

The potent vasoconstrictor and mitogenic actions of endothelin make it a possible participant in the pathophysiology of coronary artery disease. Coronary vasoconstriction in dogs persists 120 minutes after intracoronary administration of the a1-adrenoreceptor agonist phenylephrine. Pretreatment with ECE inhibitor phosphoramidon or the ET$_A$ receptor antagonist FR-139317 abolishes this vasoconstriction, which is consistent with the hypothesis that ET-1 is released during alpha1-adrenergic activation and causes sustained coronary vasoconstriction. ET$_A$ receptor antagonism by LU 135252 significantly reduces the number of cyclic coronary flow reductions in an animal model of variant angina.

Intravenous administration of a mixed ET$_{AB}$ antagonist bosentan to patients with angiographically documented stable CAD increased coronary diameter, particularly in vessels with no or mild angiographic changes. Selective ET$_A$ receptor antagonism by BQ-123 in patients with stable angina pectoris prevents distal coronary vasoconstriction occurring after percutaneous transluminal coronary angioplasty (PTCA). However, these data must be interpreted with caution, because intracoronary administration of BQ-123 can also prevent the normal reduction of myocardial ischemia on repeated balloon inflations during PTCA, which may be explained by a “steal” effect through coronary collaterals.

Anti-endothelin agents and chronic renal failure

Established chronic renal failure tends to progress to end-stage renal failure requiring dialysis. There is evidence that endothelin-1 plays an important role in progression of chronic renal failure. In animal models of progressive chronic renal failure induced by partial nephrectomy, there are increases in renal preproendothelin-1 mRNA, cortical tissue immunoreactive endothelin-1 and urinary endothelin excretion. Renal endothelin-1 generation positively correlated with glomerulosclerosis and urinary protein excretion in such animals. Glomerular expression of ET$_A$ and ET$_B$ receptor mRNA is increased in experimental glomerulosclerosis. Endothelin apparently participates in induction and progression of sclerotic renal changes, leading to progression to end-stage renal disease. ET$_A$ receptor stimulation by endothelin-1 (1-31) stimulates proliferation of cultured rat zona glomerulosa cells. Endothelin-1 induces growth of cultured human mesangial cells. Endothelin participates in the pathophysiology of renal vascular fibrosis by activating the collagen I gene. Treatment of mice with L-NAME-induced hypertension with bosentan decreased mortality, normalized expression of collagen I gene augmented by hypertension, and led to the regression of renal vascular fibrosis.

Therefore, anti-endothelin therapy might be a promising new direction in the prevention of progression of chronic renal failure in addition to the known benefits of RAAS inhibition. Four-week treatment with both hydralazine and bosentan decreased arterial pressure and cardiac hypertrophy in rats harboring both human renin and angiotensinogen genes.

Importantly, the decrease in mortality was higher with bosentan, and only bosentan treatment decreased albuminuria and focal renal necrosis. This is a very important finding given that human proteinuric renal disease tends to progress faster with more severe proteinuria, and that in patients with chronic renal failure, plasma concentrations of endothelin-1 are increased, up to 4-
fold in those on haemodialysis\textsuperscript{126}. However, some investigators have been unable to demonstrate any benefit of ET\textsubscript{A} receptor blockade or ET\textsubscript{A/B} receptor blockade in animal models of progressive renal injury\textsuperscript{119}.

Endothelin is also involved in the development of ischemic acute renal failure (ARF). In a model of ischemic ARF in rats, administration of SM-19712, a potent ECE inhibitor, caused a dose-dependent attenuation of the renal histopathological changes and ischemia/reperfusion-induced renal dysfunction\textsuperscript{122}.

### Anti-endothelin agents and heart failure

Endothelin plays an important role in the pathophysiology of chronic heart failure (CHF). Baseline plasma endothelin levels progressively increase with the development of CHF in dogs with rapid right ventricular pacing\textsuperscript{138}. The gene expression of endothelin-1 precursor and ECE is up regulated 4 and 3-fold, respectively, in the failing human heart\textsuperscript{145}. The cardiac production of endothelin-1 is markedly increased in cardiomyopathic hamsters with CHF, and chronic ET\textsubscript{A} receptor antagonism by TA-0201 improves cardiac hemodynamics and survival in these animals\textsuperscript{142}.

Long-term ET\textsubscript{A} receptor antagonism with BQ-123 in rats with CHF improves survival and alterations in the expression of various cardiac genes and inhibits the change from alpha-myosin heavy chain (MHC) to beta-MHC, which is regarded as a molecular marker for CHF\textsuperscript{136,144}. In low cardiac output heart failure in dogs, bosentan decreased aortic pressure and increased stroke volume\textsuperscript{138}. Despite the possible role of endogenous endothelin in the maintenance of cardiac contractility, anti-endothelin therapy in heart failure increases cardiac output, presumably by favorably altering loading characteristics. Although ECE inhibition by phosphoramidon and selective ET\textsubscript{A} receptor antagonism by BQ 123 impairs cardiac contractility in isolated animal hearts perfused at constant flow\textsuperscript{141}, ET\textsubscript{A} receptor antagonism by LU 135252 restores cardiac output dose-dependently, decreases blood pressure and heart rate, and limits left ventricular remodeling in rats with coronary ligation and developing heart failure\textsuperscript{139}. In dogs with moderate CHF induced by intracoronary microembolizations, long-term therapy with the ET\textsubscript{A/B} receptor blocker bosentan prevents the progression of left ventricular dysfunction and attenuates left ventricular remodeling\textsuperscript{146}. However, mixed ET\textsubscript{A/B} receptor antagonism by TAK-044 did not alter hemodynamics or vascular remodeling in rabbits with sustained volume overload\textsuperscript{147}. In pigs with rapid atrial pacing and CHF, combined AT(1) receptor blockade by valsartan and ET\textsubscript{A/B} receptor blockade by bosentan resulted in a greater improvement in left ventricular function than with valsartan alone\textsuperscript{148}. In dogs with CHF, both the selective ET\textsubscript{A} receptor antagonist FR 139317 and the ET\textsubscript{A/B} receptor antagonist TAK-044 decreased cardiac pressures, increased cardiac output and sodium excretion\textsuperscript{140}. However, only TAK-044 decreased plasma aldosterone levels, which may prove to be an additional advantage of ET\textsubscript{A/B} receptor antagonism in the treatment of CHF\textsuperscript{140}. Long-term survival of coronary ligated CHF animals increases from 43% to 85% after ET\textsubscript{A} blockade with BQ-123, but only from 47% to 65% after ET\textsubscript{A/B} blockade with bosentan\textsuperscript{152,153}. Possible deleterious effects of ET\textsubscript{B} receptor blockade may explain the higher survival benefit with selective ET\textsubscript{A} receptor antagonism by BQ-123.

Plasma endothelin levels are elevated in patients with CHF\textsuperscript{149} and correlate closely with the degree of haemodynamic and functional impairment\textsuperscript{150}. In patients with symptomatic stable heart failure, acute (4-6 h) infusion of intravenous non-peptide ET\textsubscript{A/B} receptor blocker tezosentan significantly increased cardiac index and decreased pulmonary and systemic vascular resistances without changes in heart rate or hemodynamic rebound after discontinuation of the drug\textsuperscript{184,186}. Prolonged (48 h) tezosentan infusion in patients with advanced heart failure was well tolerated and improved cardiac index, pulmonary capillary wedge pressure, and diastolic and systolic function\textsuperscript{185}. Chronic oral administration of the ET\textsubscript{A/B} antagonist bosentan reduces systemic and pulmonary vascular resistances by 24% and 20% and does confer additional hemodynamic benefits in CHF patients receiving ACE inhibitors\textsuperscript{151}. One third of patients on bosentan demonstrated improvement in clinical status compared to 0% on placebo\textsuperscript{151}. Two-week treatment with bosentan 1 g/day in addition to diuretics, digoxin and ACE inhibitors was evaluated in patients with symptomatic NYHA class III heart failure. This short-term treatment decreased peripheral and pulmonary vascular resistances, decreased systemic, pulmonary and right atrial pressures, and increased cardiac output\textsuperscript{137}. The REACH-1 trial demonstrated that initiation of bosentan therapy in patients with CHF leads to increased risk of worsening heart failure, whereas long-term bosentan therapy may improve symptoms and favorably alter the progression of heart failure\textsuperscript{176}. In addition, there is evidence that
Endothelin-1 may have a negative inotropic effect in humans with dilated cardiomyopathy, suggesting that endothelin antagonism may directly improve myocardial contractility in this condition. Chronic ET\textsubscript{A} blockade with LU 135252 attenuates the impairment of conduit vessel endothelium.

Therefore, endothelin receptor antagonists apparently are promising new agents in the treatment of heart failure with possible added benefits to RAAS antagonism in terms of morbidity and mortality. Further research is necessary to determine whether selective ET\textsubscript{B} receptor blockade or mixed ET\textsubscript{A/B} receptor blockade has greater benefits in patients with CHF.

**Conclusions**

Endothelin-1 is a peptide secreted mostly by vascular endothelial cells that possesses potent vasoconstrictor and mitogenic properties. Endothelin-1 is involved in salt and water homeostasis and stimulates the sympathetic and renin-angiotensin-aldosterone system. These actions make endothelin a potentially important mediator in hypertension and its complications, including coronary artery disease, heart failure and renal disease.

Endothelin appears to act as a mediator in the pathogenesis of hypertension and its complications. Further clinical trials are needed to examine whether combined ET\textsubscript{A/B} blockade or selective ET\textsubscript{A} blockade are superior in the treatment of hypertension. The effects of endothelin-1 on renal function, cardiac and vascular growth indicate the potential of anti-endothelin therapy in preventing the complications of hypertension, such as vascular remodeling, left ventricular hypertrophy, hypertensive kidney damage and atherosclerosis. Endothelin also appears to be involved in the pathophysiology of heart failure. Long-term anti-endothelin therapy may improve symptoms and favorably alter the progression of heart failure.

Established chronic renal failure tends to progress to end-stage renal failure requiring dialysis. Endothelin appears to participate in induction and progression of sclerotic renal changes, leading to progression to end-stage renal disease. Anti-endothelin therapy might offer additional benefits in the prevention of progression of chronic renal failure in addition to the known benefits of RAAS inhibition.

Further studies are necessary to determine the role of anti-endothelin therapy in the treatment of cardiovascular diseases and determine the different roles of selective ET\textsubscript{A} or ET\textsubscript{B} receptor antagonism versus mixed ET\textsubscript{A/B} receptor antagonism in human diseases.

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