Contribuição Internacional

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.

Due to the potent vasoconstrictor and mitogenic properties of endothelin-1, it has been implicated in the pathogenesis of cardiovascular and renal disease. Studies with endothelin receptor antagonists have elucidated possible beneficial effects of ET_A and/or ET_B antagonism in systemic hypertension, pulmonary hypertension, coronary artery disease, heart failure and renal disease. In addition, there have been studies suggesting possible beneficial effects of endothelin antagonism in organ transplantation, respiratory and gastrointestinal disease. The purpose of this review is to give an overview of the biology of endothelins, focusing on their role in cardiovascular disease, and discuss the recently accumulated body of knowledge on the effects and potential uses of endothelin receptor antagonists.

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

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Introduction

Vascular endothelial cells produce a number of important vasodilator and constrictor substances. Prostacyclin and nitric oxide are potent vasodilators secreted by vascular endothelium[1,2]. The isolation of endothelium-derived vasodilators initiated a search for counterbalancing constricting factors (or EDCFs). A long acting vasoconstrictor substance was isolated from porcine aortic endothelial cells in 1988, and called endothelin[3]. Endothelins are family of peptides, which

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comprises endothelin-1, endothelin-2 and endothelin-3, each containing 21 amino acids[4] (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 known[4,5].

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[4] (Figure 1). Endothelin-1 generation is increased by many stimuli, including vasoactive hormones, growth factors, hypoxia, shear stress, lipoproteins, free radicals, endotoxin and cyclosporin[6]. Production of endothelin-1 is inhibited by endothelium-derived nitric oxide, nitrovasodilators, natriuretic peptides, heparin and prostaglandins[6].

The major site of generation of endothelin-1 is in endothelial cells[4,5]. Endothelin-1 is also produced in the heart, kidney, CNS and posterior pituitary[6]. Endothelin-2 is produced in endothelial cells, heart and kidney[7,8]. Endothelin-3 is expressed in the endocrine, gastro-intestinal and central nervous systems, but not in endothelial cells[6].

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-1[3,9] (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 ECE[15], 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 distributions[11]. 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

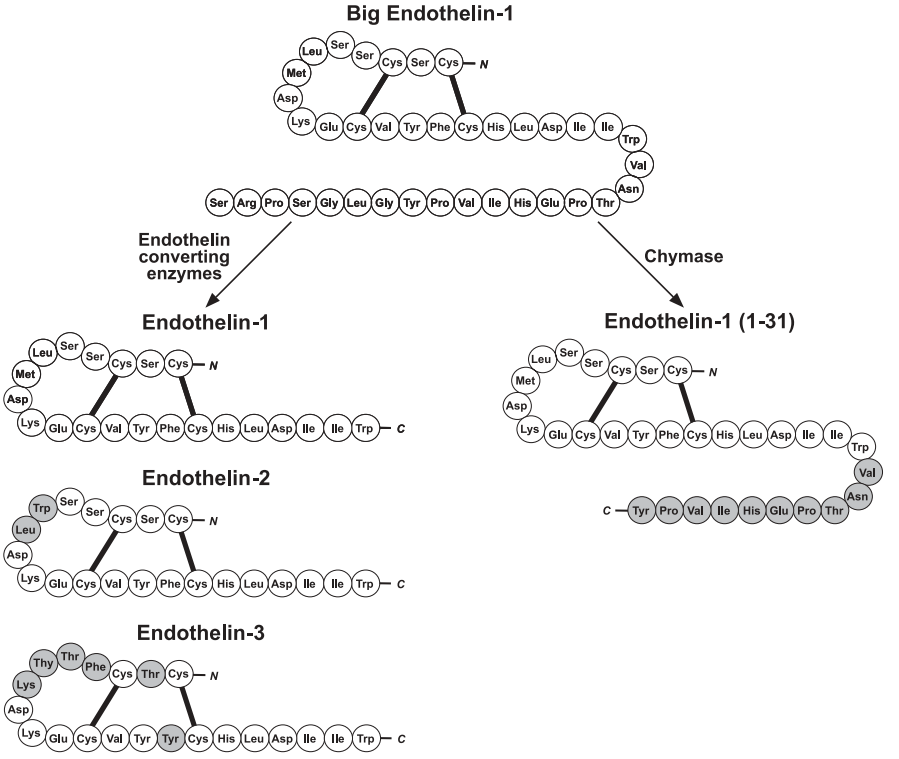

Figure 1 – The synthetic pathway for the generation of endothelin-1 and endothelin-1 (1-31). Amino acid sequences of the four members of the endothelin family. Each isoform contains two intra-chain disulphide bridges linking paired cysteine amino acid residues, thus producing an unusual semi-conical structure. Shaded circles indicate where amino acids differ from endothelin-1.

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[6]. ECE has also been found on alpha-actin filaments in smooth muscle cells[83]. 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[88] (Figure 1).

Intra-arterial infusion of big endothelin-1 produces dose-dependent forearm vasoconstriction in humans[10]. 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. Pre-treatment with a large dose of unlabelled endothelin-1 blocks pulmonary clearance of radiolabelled endothelin-1, supporting receptor mediated clearance[12]. Selective ET_B inhibition increases plasma endothelin-1 concentrations[14] and does not affect big endothelin-1 or C-terminal fragment concentrations[13,14], 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[16,177].

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[11]. 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[178,179]. Table 1 summarizes currently known ECE inhibitors.

Signal transduction

ET_A and ET_B are two distinct endothelin receptor types with different pharmacologic characteristics. The ET_A receptor affinity for endothelin-1 is much higher than for endothelin-3[17, 18]. ET_A receptors are located in vascular smooth muscle cells, but not in endothelial cells[19] (Figure 2). A number of peptide and non-peptide ET_A antagonists have been synthesised; the prototype being the cyclic pentapeptide BQ-123[20]. ET_B receptors are located on endothelial cells[21]. Endothelin-1 and endothelin-3 equally activate the ET_B receptor, which in turn leads to vasodilation via production of nitric oxide and prostaglandins. ET-1 (1-31) has also been demonstrated to cause vascular smooth muscle constriction via ET_A receptor stimulation[91] and to induce NO production in endothelial cells via ET_B receptors[92]. Some ET_B receptors are located in vascular smooth muscle[22], where they may mediate vasoconstriction[23] (Figure 2). Sarafotoxin S6c and endothelin-3 are selective peptide agonists and BQ-788 is a selective peptide antagonist at the ET_B receptor[18,24].

The number of endothelin receptors is regulated by various factors. Angiotensin II and phorbol esters downregulate endothelin receptors[25] whereas ischaemia and cyclosporin increase the number of endothelin receptors[26,27]. Table 1 summarizes currently known selective ET_A, ET_B and nonselective ET_A receptor antagonists.

Intracellular events

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

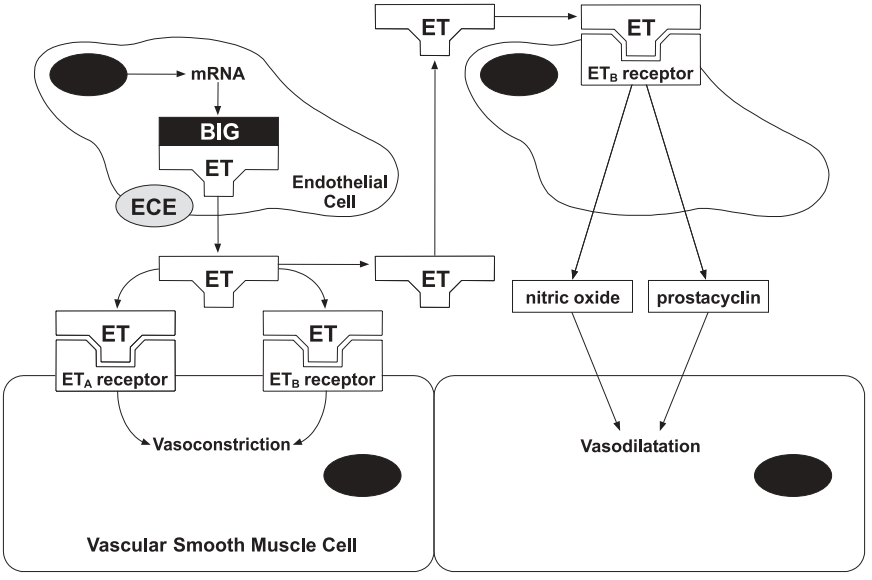

Figure 2 – Vascular actions of endothelin-1 (ET).
Big ET = Big endothelin-1; ECE = endothelin converting enzyme.

subsequent hydrolysis of phosphatidyl inositol and generation of membrane-bound diacylglycerol and cytosolic inositol trisphosphate[28,29]. Inositol trisphosphate causes release of [Ca^{2+}] from intracellular stores and opening of membrane Ca^{2+} channels[30]. Diacylglycerol increases sensitivity of the contractile apparatus to elevation in intracellular Ca^{2+} by activating protein kinase 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 *in vitro*[3]. Endothelin-1, 2 and 3 induce transient vasodilatation due to nitric oxide and prostacycline release before the development of sustained vasoconstriction[36,85]. Vasoconstriction to endothelin-1 is mediated by vascular smooth muscle cell ET_A and ET_B receptors. Endothelial cell ET_B receptors mediate vasodilatation through production of endothelium-derived vasodilators (Figure 2). Vasoconstriction to ET_B receptor agonists varies with species, vessel type and vessel size[22].

Bolus injections of endothelins in animals cause a blood pressure increase which persists for at least 60 min[3]. The coronary and renal vascular beds exhibit maximal vasoconstriction to systemic administration endothelin-1 in animals[34]. Bolus injections of endothelin usually also cause transient hypotension which is most marked for endothelin-3[4], and is mediated by endothelial ET_B receptors. This initial hypotensive response does not occur if endothelin concentrations rise more slowly,

Table 1 – Currently known anti-endothelin agents

ET_A receptor antagonists	$ET_{A/B}$ receptor antagonists	ET_B receptor antagonists	ECE inhibitors
A-127722 (non-peptide)	A-182086 (non-peptide)	A-192621 (non-peptide)	B-90063 (non-peptide)
ABT-627 (non-peptide)	CGS 27830 (non-peptide)	A-308165 (non-peptide)	CGS 26393 (non-peptide)
BMS 182874 (non-peptide)	CP 170687 (non-peptide)	BQ-788 (peptide)	CGS 26303 (non-peptide)
BQ-123 (peptide)	J-104132 (non-peptide)	BQ-017 (peptide)	CGS 35066 (non-peptide)
BQ-153 (peptide)	L-751281 (non-peptide)	IRL 1038 (peptide)	Phosphoramidon (peptide)
BQ-162 (peptide)	L-754142 (non-peptide)	IRL 2500 (peptide)	PP-36 (peptide)
BQ-485 (peptide)	LU 224332 (non-peptide)	PD-161721 (non-peptide)	SM-19712 (non-peptide)
BQ-518 (peptide)	LU 302872 (non-peptide)	RES 701-1 (peptide)	TMC-66 (non-peptide)
BQ-610 (peptide)	PD 142893 (peptide)	RO 468443 (non-peptide)	
EMD-122946 (non-peptide)	PD 145065 (peptide)		
FR 139317 (peptide)	PD 160672 (non-peptide)		
IPI-725 (peptide)	RO-470203 (**bosentan**) (non-peptide)		
L-744453 (non-peptide)	RO 462005 (non-peptide)		
LU 127043 (non-peptide)	RO 470203 (non-peptide)		
LU 135252 (non-peptide)	RO 485695 (non-peptide)		
PABSA (non-peptide)	RO61-0612 (tezosentan) (non-peptide)		
PD 147953 (peptide)	SB 209670 (non-peptide)		
PD 151242 (peptide)	SB 217242 (non-peptide)		
PD 155080 (non-peptide)	TAK-044 (peptide)		
PD 156707 (non-peptide)			
RO 611790 (non-peptide)			
SB-247083 (non-peptide)			
Sitaxsentan sodium (non-peptide)			
TA-0201 (non-peptide)			
TBC 11251 (non-peptide)			
TTA-386 (peptide)			
WS-7338B (peptide)			
ZD-1611 (non-peptide)			
Aspirin (non-peptide)			

ET = endothelin; ECE = endothelin converting enzyme.

which is likely to occur under physiological conditions[37].

Brachial artery infusion of endothelin-1 in humans causes a slow dose-dependent decrease in forearm blood flow[38,39], which is sustained for 2 hours after discontinuation of endothelin-1[38]. In addition, endothelin-1 causes slow-onset, sustained constriction of cutaneous veins *in vivo*[38,40]. Identical doses of endothelin cause greater vasoconstriction when infused over a longer period of time[23]. Endothelin-1 increases blood pressure in human subjects by 5-10% when given intravenously at doses of ~1 pmol/kg/min over 60 min[41,35]. 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[35,42,43].

Cardiac

Endothelin-1 has positive chronotropic and inotropic effects *in vitro*[32]. Intracoronary administration of endothelin-1 causes coronary vasoconstriction, resulting in myocardial ischaemia and lethal ventricular arrhythmias[33]. In animals, low doses of endothelin have a positive inotropic effect *in vivo*, whereas higher doses have negative inotropic effect[34], 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[35].

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[45]. This suggests that the endothelins stimulate nitric oxide production by vascular endothelial cells[46] (Figure 2). Cyclo-oxygenase inhibitors potentiate the constrictor effects of endothelin-1[45], suggesting that endothelin-1 also increases prostacyclin production by endothelial cells[46] (Figure 2). In addition, adrenomedullin generation by endothelial cells is increased by endothelin-1[44]. 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[47,48,49,50]. This hypotensive effect of anti-endothelin therapy is not observed in short term studies (i.e. < 10 min)[51,52,53]. 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

Table 2 – Major physiologic actions of endothelin-1 relevant to cardiovascular disease

Organs and organ systems	Effects of Endothelin-1
Systemic vascular bed	Causes vasoconstriction through vascular smooth muscle cell ET_A and ET_B receptors. Causes vasodilatation through ET_B receptors located on endothelial cells. Mitogenic effect on vascular smooth muscle cells.
Pulmonary vascular bed	Causes vasoconstriction through vascular smooth muscle cell ET_A and ET_B receptors.
Heart	Positive chronotropic and inotropic effects *in vitro*. Decreases cardiac output *in vivo*, due to increased afterload and a baroreceptor mediated decrease in heart rate. Mitogenic effect on cardiac myocytes and coronary vascular smooth muscle cells.
Kidneys	Constriction of afferent and efferent arterioles, decrease in renal plasma flow and glomerular filtration rate through ET_A receptors. Preventing tubular reabsorbtion of sodium and water through ET_B receptors. Mitogenic effect on human mesangial cells.
Endocrine	Stimulates ACE and aldosterone release.

ET = endothelin; ACE = angiotensin converting enzyme.

animals[54]. 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[10]. Local administration of peptide ET_A receptor antagonist BQ-123, or peptide ET_A and ET_B receptor antagonist TAK-044 cause slow onset forearm vasodilatation[10,55]. In humans, systemic administration of TAK-044 causes systemic vasodilatation and decreases arterial pressure by 10-20%[55]. These findings suggest that endogenous endothelin-1 has an ET_A receptor mediated physiological vasoconstrictor effect important for blood pressure maintenance. The non-peptide $ET_{A/B}$ antagonist bosentan also decreases blood pressure in normotensive humans[56].

Local administration of ET_A receptor antagonist causes greater vasodilatation than local $ET_{A/B}$ antagonism in humans[10,55]. Local forearm ET_B receptor antagonism by BQ-788 causes sustained vasoconstriction in humans, which opposes the vasodilator action of BQ-123[57]. ET_B receptor antagonists can cause vasoconstriction by blockade of tonic endothelial ET_B receptor mediated stimulation of nitric oxide and prostacyclin generation (Figure 2). However, ET_B receptor antagonists also block clearance receptors, thereby increasing endothelin-1 concentrations[14,61]. The pressor effects of ET_B receptor antagonism are present even when nitric oxide generation is inhibited, and these effects can be blocked by ET_A antagonism, suggesting an effect on clearance of endothelin-1[62]. Renal ET_B receptors cause natriuresis by preventing tubular reabsorption of sodium[58,59,60].

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_A and ET_B mediated effects. Therefore, the cardiovascular effects of endogenous endothelin-1 generation may change in disease states if the number or function of ET_A and ET_B receptors are altered. For example, endothelial dysfunction with loss of nitric oxide activity would be expected to attenuate ET_B mediated vasodilatation and promote ET_A mediated vasoconstriction.

Renal effects

Endothelin-1 causes equal constriction of afferent and efferent arterioles *in vitro*[67], and decreases renal plasma flow and glomerular filtration rate (GFR)[68,69,70]. ET_A receptors mediate renal vasoconstriction in humans[71,87]. In humans, $ET_{A/B}$ receptor antagonism with TAK-044 increases renal plasma flow by ~ 20%, without any effect on GFR[181], 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[72]. 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[86]. Endothelin-1 also blocks water reabsorbtion in the collecting duct by inhibiting the effects of ADH[73]. In volume depleted rats, endothelin-1 production in the renal tubule decreases and endothelin receptor number in glomeruli and tubules increases[74]. These tubular effects occur with ET_B receptor agonists and are not blocked by BQ-123, suggesting that they are mediated by ET_B receptors[75,76,86]. Deletion of renal ET_B receptors in ET_B knockout rats causes salt-sensitivity and hypertension that is not reversible with ET_A antagonism[180]. In summary, endothelin-1 has two direct renal actions, causing renal vasoconstriction (ET_A) and tubular sodium and water loss (ET_B), 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*[63]. Endothelin-1 (1-21) has a potent mitogenic effect on vascular smooth muscle cells[63], cardiac myocytes[64] and glomerular mesangial cells[65]. Endothelin-1 (1-31) also stimulates proliferation of porcine and human coronary artery smooth muscle cells[89,90] and human mesangial cells[93]. The endothelins are also potent stimulators of monocyte production of cytokines such as tumour necrosis factor, interleukins (1, 6 and 8) and granulocyte-macrophage colony-stimulating factor[66]. Endothelin-1 enhances neutrophil adhesion to human coronary artery endothelial cells, which is mediated by ET_A receptors[84].

Endocrine effects

Endothelin-1 stimulates ACE activity in cultured endothelial cells[77] and stimulates the tissue renin-angiotensin system in the rat isolated mesenteric bed[78]. In addition, endothelin-1 stimulates release of aldosterone from isolated cortical zona glomerulosa cells[79], and adrenaline from medullary chromaffin cells[80]. 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

Table 3 – Potential clinical indications for selected endothelin antagonists

Clinical indications	Animal models	Clinical studies
Systemic hypertension	**Bosentan.** Antihypertensive effect in angiotensin II-induced, perinephritic, DOCA-salt, and SHRSP hypertension[94,97,105,108]. **LU135252.** Antihypertensive effect in Dahl salt-sensitive hypertension[109]. **Tezosentan.** Antihypertensive effect in SHR[188].	**Bosentan.** Antihypertensive effect in essential hypertension, this effect similar to treatment with 20 mg of enalapril[96].
Pulmonary hypertension	**Bosentan.** Attenuates hypoxia-reoxygenation-induced pulmonary hypertension, decreases leukocyte-mediated injury and improves pulmonary function[132].	**Bosentan.** Improves exercise capacity, improves pulmonary hemodynamics, reduces Borg dyspnea index, and improves WHO functional class in primary or secondary pulmonary hypertension[134,183].
Coronary artery disease	**Bosentan.** Antiatherosclerotic effect[111,112,116]. Suppresses intrapericardial endothelin-1-induced ventricular arrhythmias[163]. **LU135252.** Reduces the number of cyclic coronary flow reductions in a model of variant angina[168]. **ABT-627.** Preserves coronary endothelial function in experimental hypercholesterolemia[173].	**Bosentan.** Increases coronary diameter in angiographically documented stable coronary artery disease[166].
Renal failure	**Tezosentan.** Prevents acute renal failure due to experimental rhabdomyolysis[188].	**ABT-627.** Prevents the decrease in renal perfusion and glomerular filtration rate caused by ET-1 infusion[190].
Chronic heart failure	**Bosentan.** Improves hemodynamics, prevents the progression of left ventricular dysfunction, attenuates left ventricular remodeling and improves long-term survival[138,146,153]. **LU135252.** Restores cardiac output dose-dependently, decreases blood pressure and heart rate, and limits left ventricular remodeling[139].	**Bosentan.** Improves hemodynamics, clinical status and favorably alters the progression of heart failure[137,151,176]. **LU135252.** Improves hemodynamics, attenuates the impairment of conduit vessel endothelial function[187,189]. **Tezosentan.** Improves hemodynamics[185].

DOCA = deoxycorticosterone acetate; SHR = spontaneously hypertensive rats; SHRSP = stroke-prone spontaneously hypertensive rats; WHO = World Health Organization.

receptor blockade by TAK-044 resulted in a decrease in arterial pressure, ET-1 contents and mRNA expression level in the kidney, heart and brain of SHRSPs[103]. In contrast, rats with renovascular hypertension (Goldblatt) are relatively insensitive to endothelin receptor blockade[110]. In adrenocorticotrophic hormone-induced hypertension in rats, combined ET_A and ET_B receptor blockade by bosentan had no effect on blood pressure[101]. The latter finding may be attributed to blocking the natriuretic effects of endothelin-1 via ET_B receptor blockade by bosentan. SHR have exaggerated vasoconstrictor responses of coronary arteries to endothelin-1 mediated via both ET_A and ET_B receptors[95]. Despite the known positive inotropic effect of endothelin-1, bosentan reduces arterial pressure in dogs with perinephritic hypertension and in DOCA-salt hypertensive rats by decreasing total peripheral resistance without affecting myocardial contractility[97,99].

In humans with essential hypertension, the ET_A and ET_B receptor antagonist TAK-044 caused greater forearm vasodilatation compared to normotensive controls, perhaps because of decreased ET_B-mediated tonic NO release from endothelial cells in essential hypertension[100]. Combined blockade of ET_A and ET_B receptors by BQ-123 and BQ-788 results in a greater forearm vasodilatation compared to selective ET_A receptor blockade by BQ-123 in patients with essential hypertension[104]. Interestingly, ET_B receptor blockade by BQ-788 produced forearm vasodilatation in hypertensive patients as opposed to vasoconstriction in normotensive controls[104]. This can be attributed to relative upregulation of vasoconstrictory smooth muscle ET_B receptors and downregulation of ET_B receptors located on endothelial cells in essential hypertension, as well as to hypertensive endothelial dysfunction.

In humans, four-week treatment with bosentan at a dose of 1000 mg twice daily decreases 24 hr ambulatory diastolic blood pressure in patients with essential hypertension by ~10 mmHg, this effect similar to treatment with 20 mg of enalapril. This effect of bosentan was not accompanied by activation of the sympathetic nervous or renin-angiotensin systems[96].

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[94]. 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[102]. In addition to its blood pressure lowering effect, combined ET_A and ET_B receptor blockade by TAK-044 in SHRSPs resulted in a decrease in blood urea nitrogen, creatinine concentrations, plasma aldosterone, heart and kidney weight[103]. Combined $ET_{A/B}$ receptor blockade in SHRSP completely prevents cerebral arteriolar hypertrophy, despite only partial decrease in arterial pressure[105].

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 ET_A receptor stimulation[128]. Endothelin-1 levels are increased in air embolization-induced pulmonary hypertension in sheep[131] and hypoxia-reoxygenation-induced pulmonary hypertension in piglets[132], which suggests that endothelin-1 is involved in the pathogenesis of these forms of pulmonary hypertension.

In monocrotaline-induced pulmonary hypertension in rats, the ET_A antagonist TA-0201 is as effective as an oral prostacyclin analog in the prevention of progression of pulmonary hypertension and right ventricular hypertrophy[129]. Both ET_A receptor antagonism by FR139317 and $ET_{A/B}$ receptor antagonism by TAK-044 significantly attenuated the increase in pulmonary artery pressure during air embolization in sheep[131]. In piglets, bosentan treatment attenuated hypoxia-reoxygenation-induced pulmonary hypertension, decreased leukocyte-mediated injury and improved pulmonary function[132]. Pretreatment with ET_A receptor antagonist PD156707 blocks rebound pulmonary hypertension observed on acute withdrawal of inhaled NO in lambs[182]. Sitaxsentan sodium, an oral selective ET_A receptor antagonist, dose-dependently attenuated chronic hypoxia-induced and monocrotaline-induced pulmonary hypertension, right heart hypertrophy and pulmonary vascular remodeling in rats[133]. 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[135]. Therefore, anti-endothelin treatment might be of interest in the treatment of patients with various forms of pulmonary hypertension.

Administration of the ET_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[130]. 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[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[183]. The frequency and characteristics of adverse events did not differ between bosentan and placebo groups in this trial[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_A receptors[113,114]. In cultured mouse VSMC, ECE inhibition by phosphoramidon and ET_A receptor blockade by BQ-123 inhibits DNA synthesis induced by oligosaccharides of hyaluronic acid[115]. ET_A receptor blockade by BQ-123 inhibits endothelin-1-induced proliferation of human coronary smooth muscle cells[114]. $ET_{A/B}$ receptor antagonism by bosentan inhibited neointimal development in collared carotid arteries of rabbits, a known model of atherosclerosis[111]. Bosentan significantly attenuated the development of graft atherosclerosis in rat cardiac allografts[112]. ET_A receptor blockade in hyperlipidemic hamsters inhibits formation of early atherosclerotic lesions by decreasing the number and size of macrophage-foam cells[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[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[155]. Even if administered simultaneously with coronary reperfusion, $ET_{A/B}$ antagonism by TAK-044 improved cardiac hemodynamics, myocardial energy metabolism and decreased CPK release after 35 minutes of ischemia in rat hearts[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[157]. TAK-044 significantly reduced ischemic cellular injury in ischemia/reperfused rat hearts[158]. Both selective ET_A antagonism by BQ-123 and selective ET_B antagonism by BQ-788 improved myocardial contractility and endothelium-dependent vasodilatation in a heterotopically transplanted rat heart reperfused after ischemia[159,160]. BQ-123 significantly increased myocardial blood flow during early reperfusion[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[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[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_A receptor antagonists in human internal mammary arteries *in vitro*[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[164]. Mixed $ET_{A/B}$ antagonism by TAK-044 significantly reduced reperfusion ventricular tachycardia in ischemia/reperfused rat hearts[158]. Endogenous pulmonary big endothelin produces arrhythmogenic effects that are aggravated in Watanabe heritable hyperlipidemia in rabbits[162]. Mixed $ET_{A/B}$ antagonism by bosentan significantly suppresses intrapericardial endothelin-1-induced ventricular arrhythmias in animals[163].

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[172]. Acute intracoronary administration of ET_A antagonist FR-139317 or $ET_{A/B}$ receptor antagonist bosentan did not affect vasodilatation to acetylcholine in experimental hypercholesterolemia in pigs[174]. However, chronic $ET_{A/B}$ antagonism by RO 48-5695 and ET_A receptor antagonism by ABT-627 preserves coronary endothelial function in experimental hypercholesterolemia in pigs[173]. Forearm vasodilator response to ET_A antagonist BQ-123 is increased in hypercholesterolemic patients, whereas vasoconstriction to exogenous endothelin-1 is unchanged[175].

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 a-1-adrenoreceptor agonist phenylephrine[170]. 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[170]. ET_A receptor antagonism by LU 135252 significantly reduces the number of cyclic coronary flow reductions in an animal model of variant angina[168].

Intravenous administration of a mixed $ET_{A/B}$ antagonist bosentan to patients with angiographically documented stable CAD increased coronary diameter, particularly in vessels with no or mild angiographic changes[166]. 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)[165]. 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[167].

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[123, 124]. Glomerular expression of ET_A and ET_B receptor mRNA is increased in experimental glomerulosclerosis[125]. 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[117]. Endothelin-1 induces growth of cultured human mesangial cells by stimulating their ET_B receptors[120]. As was shown in mice, 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[121].

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[118]. Importantly, the decrease in mortality was higher with bosentan, and only bosentan treatment decreased albuminuria and focal renal necrosis[118]. This is a very important finding given that human proteinuric renal disease tends to progress faster with more severe proteinuria[127], and that in patients with chronic renal failure, plasma concentrations of endothelin-1 are increased, up to 4-

fold in those on haemodialysis[126]. However, some investigators have been unable to demonstrate any benefit of ET_A receptor blockade or $ET_{A/B}$ receptor blockade in animal models of progressive renal injury[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[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[138]. The gene expression of endothelin-1 precursor and ECE is up regulated 4 and 3-fold, respectively, in the failing human heart[145]. The cardiac production of endothelin-1 is markedly increased in cardiomyopathic hamsters with CHF, and chronic ET_A receptor antagonism by TA-0201 improves cardiac hemodynamics and survival in these animals[142].

Long-term ET_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[136,144]. In low cardiac output heart failure in dogs, bosentan decreased aortic pressure and increased stroke volume[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_A receptor antagonism by BQ 123 impairs cardiac contractility in isolated animal hearts perfused at constant flow[141], ET_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[139]. In dogs with moderate CHF induced by intracoronary microembolizations, long-term therapy with the $ET_{A/B}$ receptor blocker bosentan prevents the progression of left ventricular dysfunction and attenuates left ventricular remodeling[146]]. However, mixed $ET_{A/B}$ receptor antagonism by TAK-044 did not alter hemodynamics or vascular remodeling in rabbits with sustained volume overload[147]. In pigs with rapid atrial pacing and CHF, combined AT(1) receptor blockade by valsartan and $ET_{A/B}$ receptor blockade by bosentan resulted in a greater improvement in left ventricular function than with valsartan alone[148]. In dogs with CHF, both the selective ET_A receptor antagonist FR 139317 and the $ET_{A/B}$ receptor antagonist TAK-044 decreased cardiac pressures, increased cardiac output and sodium excretion[140]. However, only TAK-044 decreased plasma aldosterone levels, which may prove to be an additional advantage of $ET_{A/B}$ receptor antagonism in the treatment of CHF[140]. Long-term survival of coronary ligated CHF animals increases from 43% to 85% after ET_A blockade with BQ-123, but only from 47% to 65% after $ET_{A/B}$ blockade with bosentan[152,153]. Possible deleterious effects of ET_B receptor blockade may explain the higher survival benefit with selective ET_A receptor antagonism by BQ-123.

Plasma endothelin levels are elevated in patients with CHF[149] and correlate closely with the degree of haemodynamic and functional impairment[150]. In patients with symptomatic stable heart failure, acute (4-6 h) infusion of intravenous non-peptide $ET_{A/B}$ 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[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[185]. Chronic oral administration of the $ET_{A/B}$ antagonist bosentan reduces systemic and pulmonary vascular resistances by 24% and 20% and does confer additional hemodynamic benefits in CHF patients receiving ACE inhibitors[151]. One third of patients on bosentan demonstrated improvement in clinical status compared to 0% on placebo[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[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[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[143]. Chronic ET_A blockade with LU 135252 attenuates the impairment of conduit vessel endo[187].

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_B receptor blockade or mixed $ET_{B/B}$ 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 $ET_{A/B}$ blockade or selective ET_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_A or ET_B receptor antagonism *versus* mixed $ET_{A/B}$ receptor antagonism in human diseases.

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