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Review

The endothelin system in pulmonary arterial hypertension

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Abstract

Endothelin-1 (ET-1), a peptide produced primarily by vascular endothelial cells, was discovered in 1980 and it was characterized as a powerful vasoconstrictor and mitogen for smooth muscle. ET-1 binds to two types of receptors, ET_A and ET_B: ET_A-receptors are found in smooth muscle cells, whereas ET_B-receptors are localized on both endothelial cells and in smooth muscle cells. Activation of ET_A- and ET_B-receptors on smooth muscle cells mediates the vasoconstrictive and mitogenic effects of ET-1. Stimulation of endothelial ET_B-receptors promotes ET-1 clearance and activation of NO and prostacyclin release. Pulmonary arterial hypertension (PAH) is a severe condition characterized by a progressive increase in pulmonary vascular resistance leading to right ventricular failure and death. An activation of the ET-1 system has been demonstrated in both plasma and lung tissues of PAH patients as well as in animal models of PAH. The most efficient way to antagonize the ET-1 system is the use of ET-1 receptor antagonists that can block either ET_A- or ET_A- and ET_B-receptors. These drugs are effective in animal models of PAH and have been tested in multiple clinical trials in patients with PAH. Bosentan, an orally active, dual ET-1 receptor antagonist has been shown to improve symptoms, exercise capacity, hemodynamics, echocardiographic parameters and the outcome of patients with severe PAH, and it has been approved for clinical use in many countries. The selective ET_A-receptor antagonist sitaxentan has improved exercise capacity and hemodynamics of PAH patients in two preliminary studies. The main side effect of ET-1 antagonists is the increase of liver enzymes likely due to an accumulation of bile salts cytotoxic to hepatocytes. Additional trials with these drugs are currently ongoing. In conclusion, the hypothesis that the ET-1 system over-activation can be successfully antagonised in patients with PAH has been clearly demonstrated.

Keywords: Pulmonary circulation; Endothelins; Endothelial function; Endothelial receptors; Vasoconstriction/dilation

1. Introduction

A potent contracting factor was isolated in pulmonary and systemic endothelial cells in the mid-1980 [1] and it was eventually characterized in 1988 as a 21-amino acid peptide named endothelin (ET) [2]. ET was soon defined as the most potent and long-lasting endogenous vasoconstrictive substance yet discovered [2–4]. Since then the ET system has been found to be involved in multiple physiologic functions related to the nervous, renal, cardiovascular, respiratory, gastrointestinal and endocrine systems [4–7]. In addition, the ET system seems to be implicated in many disease states including carcinogenesis, bronchoconstriction, fibrosis, heart failure and pulmonary hypertension [5–7].

This review outlines the pathophysiologic role of the ET system in pulmonary arterial hypertension (PAH) a condition in which the ET receptor antagonism has gained a definite role in the treatment of the affected patients.

2. The endothelin system

The endothelin system (Fig. 1) is constituted by ET genes, preproET peptides, two activating peptidases, three 21-amino acid peptide ligand isoforms and two G-protein-coupled receptors.

2.1. Endothelin genes, peptidases and endothelin isoforms

ET is produced predominantly by endothelial cells but it is also produced by leukocytes, macrophages, smooth muscle cells, cardiomyocytes and mesangial cells [7]. The

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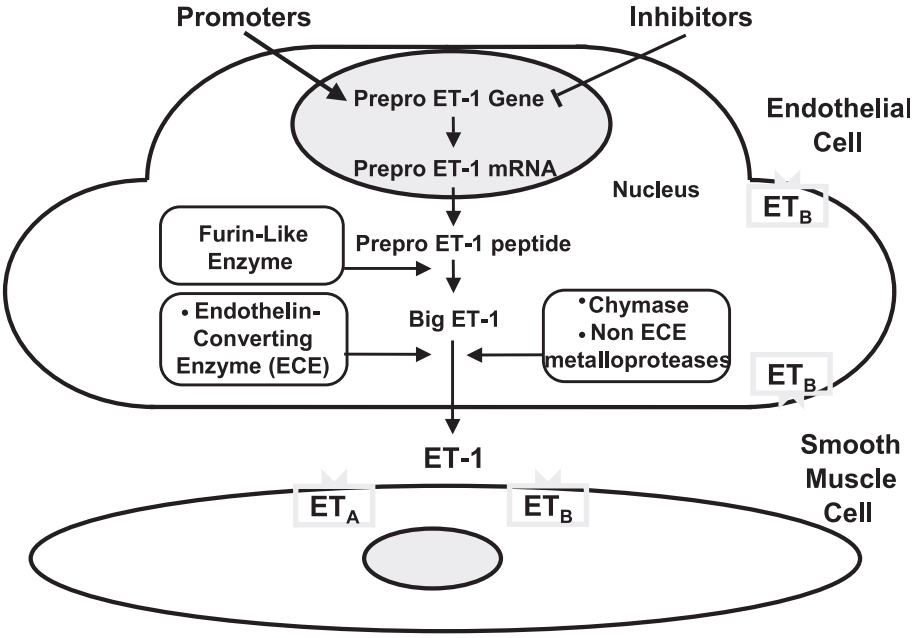

Fig. 1. Schematic representation of the vascular endothelin system. ET-1 = endothelin-1, ET_A = endothelin-1 receptor A, ET_B = endothelin-1 receptor B.

endothelial cells release at least 75% of ET abluminally toward the muscular media suggesting a paracrine/autocrine role [8]. Analysis of the human ET gene revealed the existence of two other ET-like peptide genes [9]. These peptides were named ET-2 and ET-3 in addition to endothelin-1 (ET-1), which was the original ET found in cultured endothelial cells. Endothelial cells predominately produce ET-1 while ET-2 and ET-3 are also expressed in a wide variety of cell types. ET genes (preproET genes) code for a large precursor-protein mRNA (preproET mRNA). Different stimuli modulate the transcription of the preproET-1 gene (Table 1). The translation of preproET-1 mRNA results in the formation of a 203-amino acid preproET-1 peptide, which is cleaved by a furin convertase [10] to the 38-amino acid peptide big ET-1. Big ET-1 is transformed to $ET\text{-}1_{1-21}$ through cleavage of the Trp21–Val22 bond by ET-converting enzyme-1 (ECE-1), which exists in four isoforms (a, b, c and d) [11] and by chymase and non ECE metalloproteases . In addition, chymase cleaves big ET-1 at the Tyr31–Gly32 bond, resulting in the formation of $ET\text{-}1_{1-31}$ [12]. The 31-residue ET-1 has vasoconstrictive effects but its exact physiologic and pathophysiologic roles are currently not clear.

Table 1
Stimuli that can promote or inhibit the expression of pre-pro ET-1 gene

Promoters	Inhibitors
Hypoxia	Nitric oxide
Ischemia	Prostacyclin
Shear stress	Atrial natriuretic peptides
Pulsatile stretch	Estrogens
pH	
Angiotensin II	
Vasopressin	
Cathecolamines	
Insulin	
LDL (oxidized), HDL	
Cytokines	
Growth factors	
Adhesion molecules	
Thrombin	

ET-1 is not stored in secretory granules within endothelial cells, and specific stimuli (Table 1) can induce the transcription of ET-1 mRNA and the synthesis and secretion of ET-1 within minutes. The half-life of the mRNA is approximately 15–20 min, and the plasma half-life of ET-1 is approximately 4–7 min; therefore, vascular cells can rapidly adjust ET-1 production even if its prolonged activity at the receptor level may prevent a substantial contribution to short-term adjustments of vasomotor tone.

The experiments that have lead to these observations have been performed in endothelial cells and/or isolated vessels of different organisms including humans, rats, pigs and cows and the contribution of these mechanisms may vary in diverse species.

2.2. Endothelin receptors

All three ETs bind to two types of receptors named ET_A and ET_B: in the cardiovascular system, ET_A-receptors are found in smooth muscle cells and cardiac myocytes, whereas ET_B-receptors are localized on both endothelial cells and in smooth muscle cells [13] (Figs. 1 and 2). The binding of ET-1 to smooth muscle cells ET_A- and ET_B-receptors activates phospholipase C, which leads to an

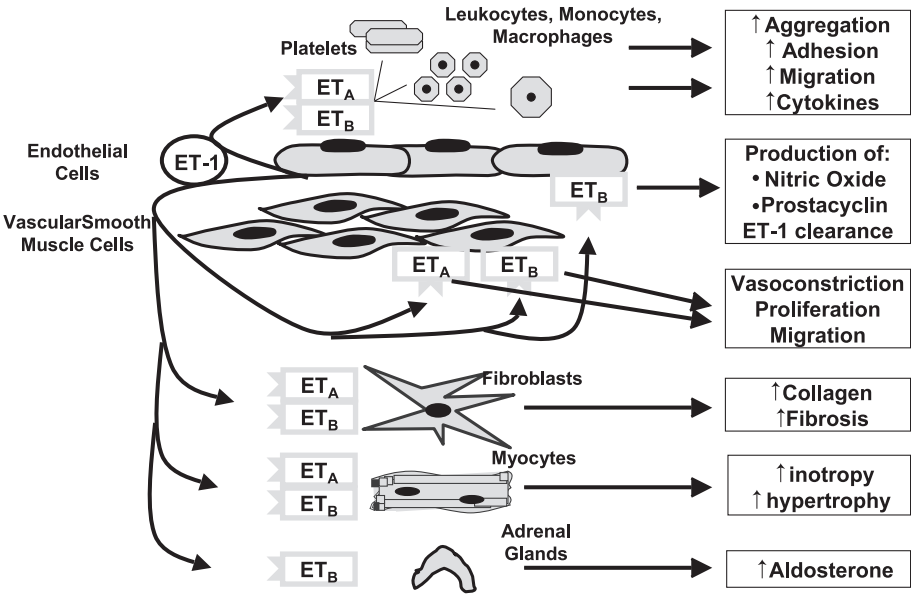

Fig. 2. Schematic representation of endothelin-1 effects in different cell types. ET-1 = endothelin-1, ET_A = endothelin-1 receptor A, ET_B = endothelin-1 receptor B.

increase of inositol triphosphate, diacylglycerol and intracellular calcium and, consequently, to long-lasting vasoconstriction [14]. The increase of diacylglycerol and calcium stimulates also protein kinase C, which mediates the mitogenic action of ET-1 [15]. On the other hand, the activation of endothelial ET_B-receptors stimulates the release of NO and prostacyclin [16], prevents apoptosis [17], inhibits ECE-1 expression in endothelial cells and plays a minor role in endothelial-dependent vasodilatation [18]. ET_B-receptors also mediate the pulmonary clearance of circulating ET-1 [19] and the reuptake of ET-1 by endothelial cells (Fig. 2). There is a cross talk between ET_A- and ET_B-receptors apparently causing compensation when only one receptor is antagonized [20,21].

2.3. Endothelin effects

ET-1 plasma levels are low (1–2 pg/ml) in healthy adults, well below the pharmacological threshold. Therefore, under normal physiological conditions, ET-1 is not a circulating hormone, it rather acts as autocrine/paracrine factor at multiple sites. ET-1- and ET_A-receptors probably play a role in the maintenance of basal vasomotor tone and blood pressure in humans [22]. ET-1 is a direct smooth muscle cell mitogen acting by the activation of both ET_A- and ET_B-receptors, and it stimulates the production of cytokines and growth factors [7]. ET-1 induces the formation of extracellular matrix proteins and fibronectin, and it potentiates the effects of transforming growth factor-beta and platelet-derived growth factor [7]. The effects on collagen production seem to be mediated by both ET_A- and ET_B-receptors in dermal fibroblasts [21] and by ET_B-receptors in cardiac fibroblasts [23]. ET-1 possesses potent proinflammatory actions [24], induces platelet aggregation and stimulates the production of aldosterone by an ET_B-mediated mechanism [25]. Nanomolar concentrations of ET-1 have a positive inotropic [26] and chronotropic effect [27], and can induce cardiac hypertrophy by the stimulation of both ET_A- and ET_B-receptors.

2.4. Endothelin system and pulmonary circulation

The lungs represent a primary target for ET-1 effects and are a special site for ET-1 metabolic pathways. The highest content of immunoreactive ET-1 has been identified in the normal rat lung [28] with ET-1 mRNA levels being five times higher than in any other organ studied. ET-1-like immunoreactivity and mRNA expression have been demonstrated also in healthy human lung tissue [29], and ET_A- and ET_B-receptors are variably distributed in all components including vessels, bronchi and alveoli. ET_A-receptors predominate in the large human pulmonary arteries while ET_B-receptors prevail in airway smooth muscle, alveolar wall tissue and capillaries. The human lungs are the major site for both clearance and production of circulating ET-1: they remove about 50% of circulating ET-1 through the ET_B-receptors [19] and release through spillover a similar amount into circulation [30]. Therefore, in physiologic conditions, there is no arteriovenous ET-1 gradient across the pulmonary circulation [30,31]. When administered intravenously, ET-1 causes a biphasic response in the pulmonary circulation with initial mild vasodilation followed by sustained vasoconstriction [32]. The initial vasodilation is due to the release of NO and by activation of potassium channels [32]. Pulmonary vessel constriction is mediated by both ET_A- and ET_B-receptors because dual blockade is necessary to maximize the inhibition of ET-1-induced constriction in humans [33]. In addition, dual blockade allowed

a statistically significant improvement in survival in rats with monocrotalin-induced pulmonary hypertension as compared to controls, while the improvement observed with selective ET_A-receptor blockade was not statistically significant [34]. On the other hand, in a genetic rat model of ET_B-receptor deficiency, the ET_B-receptors seem to play a protective role in the pulmonary hypertensive response to chronic hypoxia [35].

3. Pulmonary arterial hypertension

PAH is defined, according to the WHO Classification (Table 2), as a group of diseases characterized by a progressive increase of pulmonary vascular resistance leading to right ventricular failure and death [36,37]. The median life expectancy from the time of diagnosis in patients with primary pulmonary hypertension (PPH) without targeted treatments is 2.8 years [38]. PAH includes PPH [36] and pulmonary hypertension associated with various conditions such as collagen vascular diseases, congenital systemic-to-pulmonary shunts, portal hypertension and HIV infection [37]. All these conditions share virtually identical obstructive pathologic changes of the pulmonary microcirculation [39] suggesting that also the pathobiological processes are shared among the disease spectrum of PAH.

The pathogenesis of PAH involves multiple and complex mechanisms including endothelial dysfunction in the pulmonary circulation, resulting in pulmonary vasoconstriction and vascular remodeling. The endothelial cells modulate smooth muscle cells activity by producing vasodilators/antimitotics, such as prostacyclin and NO, and vasoconstrictors/mitogens, such as thromboxane A_2 and ET-1 (Fig. 3). Endothelial dysfunction is a condition in which the physiologic balance between vasodilators/antimitotics stimuli and vasoconstrictors/mitogens substances is shifted towards the latter and this state has been clearly shown in PAH [40] (Fig. 3). In fact, an increase of 24-h excretion of a thromboxane A_2 metabolite and reduced excretion of a prostacyclin metabolite has been demonstrated in PAH patients [41]. Prostacyclin-synthase expression is reduced in pulmonary arteries of patients with PAH [42] and also endothelial NO-synthase expression is reduced in the lungs of patients with PPH [43].

Table 2
Diagnostic classification of pulmonary hypertension

1. Pulmonary arterial hypertension
   1.1. Primary pulmonary hypertension
      (a) Sporadic
      (b) Familial
   1.2. Related to:
      (a) Connective tissue diseases
      (b) Congenital systemic to pulmonary shunts
      (c) Portal hypertension
      (d) HIV infection
      (e) Drugs and (or) toxins
      (f) Persistent pulmonary hypertension of the newborn
2. Pulmonary venous hypertension
3. Pulmonary hypertension associated with disorders of the respiratory system and (or) hypoxemia
4. Pulmonary hypertension due to chronic thrombotic and (or) embolic disease
5. Pulmonary hypertension due to disorders directly affecting the pulmonary vasculature

4. The endothelin system in pulmonary arterial hypertension

Increased plasma levels of ET-1 were detected in patients with some forms of pulmonary hypertension a few years after the discovery of the peptide [44]. In addition, increased plasma levels of ET-1 were also reported in experimental models of PAH [45].

4.1. Primary pulmonary hypertension

In patients with PPH, ET-1 plasma levels are elevated [44] and the increase is correlated with right atrial pressure, pulmonary artery oxygen saturation and pulmonary vascular resistance [46–48]. In one study, ET-1 plasma levels were inversely correlated also with the survival of a group of PPH patients on conventional therapy [49]. Interestingly, continuous epoprostenol therapy which improves survival [50], and acute inhalation of iloprost had a beneficial effect on the abnormal balance between ET-1 and big ET-1 pulmonary clearance and release in PAH patients [51,52]. Although it is not clear if the increases of ET-1 plasma levels are a cause or a consequence of pulmonary hypertension [44], studies on tissue ET system expression support a prominent role in the pathogenesis of PAH. In fact, an increased staining for ET-1 compared with controls was detected on lung sections of patients with PPH as well as in patients with other forms of pulmonary hypertension [53]. ET-1 immunostaining was identified predominantly in endothelial cells of pulmonary arteries with medial thickening and intimal fibrosis and also in plexiform lesions and the intensity of ET-1-like immunoreactivity correlated with pulmonary vascular resistance. In addition increased expression of preproET-1 mRNA was identified in endothelial cells of the same vessels. The severity of structural abnormalities found on distal elastic pulmonary arteries by intravascular ultrasound assessment in pulmonary hypertension patients of various etiologies including also PAH was directly correlated with ET-1 plasma levels [54]. In PPH patients, immunoreactivity for ECE-1 is augmented in the endothelium of diseased pulmonary arteries [55] and ET_B-receptors are upregulated in the distal vessels [56]. Therefore, almost all components of the ET system are upregulated in the pulmonary arteries and are correlated with the severity of the disease.

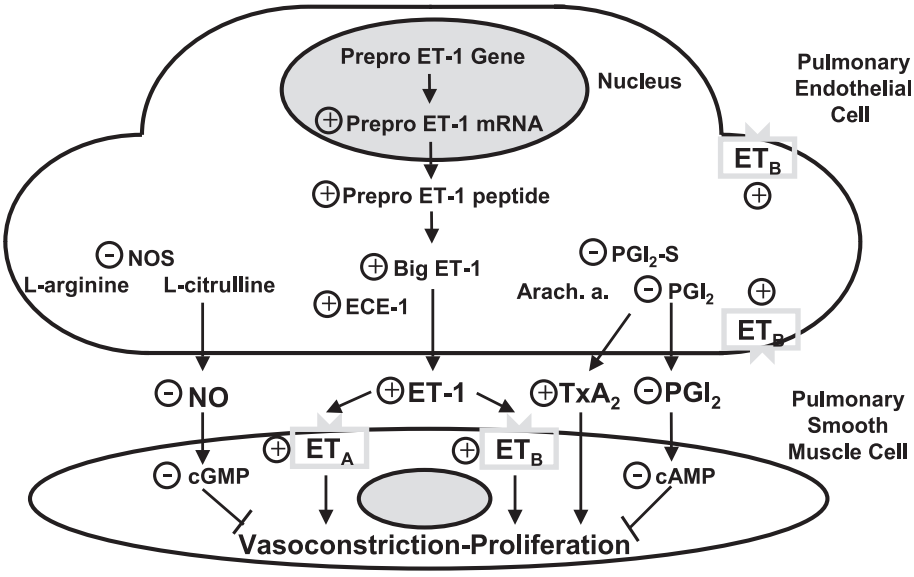

Fig. 3. Schematic representation of endothelial dysfunction in pulmonary arterial hypertension: a reduced activity of NOS and PGI_2-S and a decreased production of NO and PGI_2 are observed. Increased levels of TxA_2 and ET-1 are also detected. Almost all components of the endothelin system appear to be activated in the different types of pulmonary arterial hypertension. Arach. a. = arachidonic acid, ET-1 = endothelin-1, ET_A = endothelin-1 receptor A, ET_B = endothelin-1 receptor B. NOS = nitric oxide synthase, PGI_2 = prostacyclin, PGI_2-S = prostacyclin synthase, TxA_2 = thromboxane A_2.

4.2. Pulmonary arterial hypertension associated to connective tissue diseases

Connective tissue diseases can be complicated by pulmonary hypertension and/or lung fibrosis including systemic sclerosis, systemic lupus erythematosus, mixed connective tissue disease, dermatomyositis and rheumatoid arthritis. The spectrum of the lung involvement can span from isolated pulmonary hypertension to isolated lung fibrosis, to a variable combination of the two entities that reduce substantially the survival [57]. The clinical presentation and the pathologic changes of isolated pulmonary hypertension are undistinguishable from PPH and the incidence of pulmonary hypertension is greater in systemic sclerosis ranging from 10% to 15% [37]. In patients with systemic sclerosis, ET-1 plasma levels are greater as compared to controls [58], however without significant differences between patients with or without pulmonary hypertension and with or without lung fibrosis [59]. In contrast, ET-1 plasma levels were higher in patients with systemic lupus erythematosus and pulmonary hypertension as compared to those without this complication [60]. In patients with mixed connective tissue disease, ET-1 plasma levels were elevated and correlated to the levels of antiendothelial antibodies [61] even in absence of pulmonary hypertension. Interestingly, also patients with idiopathic pulmonary fibrosis without connective tissue disease have elevated ET-1 plasma levels [62] and have also increased expression of ET-1, big ET-1 and ET-1 mRNA in airway epithelium and type-II pneumocytes; ET-1-like immunoreactivity and mRNA were also present in pulmonary vascular endothelial cells, particularly in specimens from patients with concomitant pulmonary hypertension [29]. An increased level of ET-1 has been detected in macrophages from the bronco alveolar lavage of patients with systemic sclerosis and experimental studies have shown that ET-1 stimulates fibroblast proliferation and collagen synthesis [63].

From these data, it can be hypothesized that the ET system in connective tissue diseases may play a more complex pathophysiologic role not limited only to the development of pulmonary hypertension. In fact, ET-1 can affect all vascular, fibrotic and immunological changes typical of this group of diseases.

4.3. Pulmonary arterial hypertension associated to systemic-to-pulmonary shunts

PAH is a recognized complication of congenital cardiac diseases. Most originate from a systemic-to-pulmonary shunt such as atrial and ventricular septal defect or patent ductus arteriosus. With initial systemic-to-pulmonary shunting, the exposure of the pulmonary vasculature to increased blood flow as well as increased pressure may results in pulmonary vascular obstructive disease and the final pathological changes are identical to PPH. The consequent increase of pulmonary vascular resistance can eventually exceed systemic resistance and induce shunt reversal accompanied with oxygen-unresponsive hypoxemia, identified as Eisenmenger syndrome [64]. In animal models of systemic-to-pulmonary shunts in late gestation lambs, systemic ET-1 levels were elevated 4 weeks after delivery [65] and an increase of ECE and ET_A-receptors mRNA and protein, a reduction of ET_B-receptors mRNA and protein and no changes of mRNA and protein levels for preproET

in lung tissue were detected. In this model, additional administration of ET-1 increased pulmonary vascular resistance as compared to a reduction observed in controls [65]. Thus, in this model, the response of ET-1 was modified from pulmonary vasodilatation to vasoconstriction suggesting a role for the ET system in the pathogenesis of pulmonary hypertension. In another model of overcirculation-induced PAH in piglets, increases of circulating plasma ET-1, pulmonary mRNA for ET-1, ET_B-receptor and pulmonary ET-1 were detected [66]. In children with congenital cardiac shunts, some studies demonstrate a significant correlation between plasma ET-1 levels pulmonary arterial pressure and pulmonary blood flow [67]. In other series, although plasma ET-1 levels were elevated they were not higher in those with pulmonary hypertension and did not correlate with hemodynamics [68]. Correction of the defect have been shown to reduce both pulmonary hypertension and ET-1 levels [67]. In ''low flow–high resistance'' subjects ET_A-receptor density in pulmonary arteries and parenchyma was higher as compared to ''high flow–low resistance'' patients and ET-1 immunoreactivity in lung artery walls tended to be correlated to ET_A-receptor density [69]. In contrast, ET_B-receptor expression remained low and unrelated to the above factors. In conclusion, an upregulation of the ET system is present in patients with congenital cardiac shunts while a variable correlation with the hemodynamic changes is detectable. On the other hand, the development of pulmonary hypertension in patients with systemic-to-pulmonary shunts is related to many variables including the size and the type of the defect and the ''individual susceptibility'' that can explain also the discrepancies between the hemodynamic changes and ET system activation.

4.4. Persistent pulmonary hypertension of the newborn

Persistent pulmonary hypertension of the newborn (PPHN) is a severe condition (included among the PAH forms) of near-term gestation newborns that occurs in 1–2 per 1000 live births and may be idiopathic or complicate a variety of neonatal disorders including congenital diaphragmatic hernia and parenchymal lung diseases such as hyaline membrane disease, pneumonia and meconium aspiration [70]. PPHN is characterized by severe pulmonary hypertension resulting in pulmonary-to-systemic shunting of blood, and marked hypoxemia due to the lack of the normal fall in pulmonary vascular resistance at birth [70].

In an animal model of PPHN, an increase in lung ET-1 protein content [71] and of ET_A-receptor density and a reduction of ET_B-receptor activities has been shown. In addition an increase of preproET-1 mRNA a decrease of ET_B mRNA expression, without change in ECE-1 or ET_A mRNA expression has also been shown [72] . Interestingly, in the late-gestation ovine fetus, prolonged selective ET_B inhibition with BQ-788 caused severe PH and vascular remodeling, with high ET-1 levels, supporting the importance of ET_B-receptors in maintaining the low pulmonary vascular resistance in the normal fetal circulation [73].

In patients with PPHN, plasma levels of ET-1 are elevated compared with normal newborns [74] and a linear correlation has been shown between ET-1 plasma levels and both alveolar-arterial oxygen gradient and mean airway pressure. The increase of plasma ET-1 concentrations were detected at <12 and 24 h of age but were not any more present at 5 days of age as compared to normal newborns [75], while the opposite was true for NO metabolites. Thus, limited endogenous NO synthesis and elevated endogenous ET-1 production during the first few days of life may contribute to pulmonary hypertension in infants with PPHN. In this case, the initial vasoconstriction might promote pulmonary-to-systemic shunting resulting in hypoxemia, itself releasing additional mediators that cause vasoconstriction, thereby perpetuating the cycle [70].

4.5. Other forms of pulmonary hypertension

The ET system is activated not only in PAH but also in other forms of pulmonary hypertension including pulmonary venous hypertension, pulmonary hypertension associated with disorders of the respiratory system and (or) hypoxemia and pulmonary hypertension due to chronic thrombotic and (or) embolic disease. The detailed discussion of these conditions is beyond the scope of the current review.

5. Endothelin receptor antagonism

The ET system can be antagonized by the inhibition of the ECE and by the blockade of ET receptors. The inhibition of the ECE reduces the production of ET-1 [76] but the effectiveness of these drugs is limited by independent pathways contributing to ET-1 formation, such as chymase and metalloproteases (Fig. 1). Therefore, the more efficient way to antagonize the ET system is the use of ET-1 receptor antagonists that can block either ET_A- or ET_A- and ET_B-receptors. Currently, several peptides and nonpeptide compounds that block ET receptors are available [7], and some have been tested in both animal models and clinical trials in patients with PAH.

5.1. Effects on animal models

In the rat, monocrotalin administration causes acute pulmonary vascular endothelial injury followed by the gradual development of pulmonary hypertension, evident after 2 weeks. Even if this model has no human equivalent, ET circulating levels increase before the development of pulmonary hypertension and remain elevated thereafter [77]. In these cases, the administration of both selective ET_A-receptor antagonists and dual ET_A- and ET_B-receptor antagonists are able to partially prevent and possibly reverse

pulmonary hypertension and right ventricular hypertrophy and to reduce mortality [34,77–79]. Pulmonary hypertension is also prevented and reversed by ET-1 receptor antagonists in overcirculation-induced models [65,66]. The mechanisms of the effects of ET-1 receptor antagonists in animal models include the direct reduction of pulmonary vascular tone, the reduction of medial hypertrophy and the increase of luminal diameter, and the improvement of the endothelium dependent regulation of vascular tone [78]. Theoretically, the antagonism of ET_B-receptor, in addition to ET_A-receptor blockade can be favorable or detrimental according to the dual effect of the ET_B stimulation. Currently, the net effect of the additional ET_B antagonism is not clear even if a more complete blockade of the vasoconstrictive, proliferative and profibrotic effects of ET-1 seems to be advantageous.

The hypothesis that some differences may exist in the effect of a dual ET_A- and ET_B-receptor antagonist versus a selective ET_A-receptor antagonist has been tested in a comparative study [34]. In this monocrotalin rat model, the dual antagonist doubled survival compared with the untreated animals and increased it by 10% compared with the animals receiving the selective antagonist [34]. There was also a reduction of right ventricular hypertrophy only in the animals receiving the dual antagonist. These two findings, however, were not accompanied by a greater reduction of right ventricular systolic pressure measured in vivo in the surviving animals. In addition, the pressure-flow curves were similarly improved in both treated groups. The authors therefore did not conclude that one agent was superior to the other even if a trend in favor of the dual antagonist was present.

5.2. Clinical experience

The limited oral treatment options for PAH patients include long-term anticoagulant therapy and therapy with calcium-channel blockers in a reduced number of patients that responds to acute vasoreactivity tests [80]. In fact, even if about 20% of subjects can be considered responders to acute vasoreactivity tests [80], less than half of them responds favourably to long-term calcium-channel blockers treatment. Beneficial effects on survival have been reported with continuous intravenous infusion of epoprostenol (prostacyclin), but this treatment is quite invasive because it requires tunnelled catheters and portable pumps [50]. Prostacyclin analogues that can be inhaled (Iloprost) or administered subcutaneously (Treprostinil) or orally (Beraprost) have also proven beneficial effects and are currently available for clinical use only in selected countries [81].

The clear evidence of the activation of the ET system in PAH patients and the favorable effects of ET-1 receptor antagonists in animal models provide a sound rationale for testing this form of treatment in humans. Accordingly, in the past few years, an extensive experience has been collected on the effects of the ET-1 receptor antagonists in patients with PAH (Table 3).

Table 3
Prospective clinical studies with ET-1 receptor antagonists in patients with PAH[a]

Trial	Bosentan pilot [82]	Bosentan long term [83]	BREATHE-1 [84,86]	Sitaxentan pilot [92]	STRIDE-1 [93,94]
Patients *n*	32	29	213	20	178
Trial type	Controlled	Open label	Controlled	Open label	Controlled
Drug	Bosentan	Bosentan	Bosentan	Sitaxentan	Sitaxentan
Duration (months)	3	8 to 22	4	3	3
Primary end points	6-min walk	6-min walk	6-min walk	6-min walk	Peak-VO2
NYHA functional class (%)					
II	–		–	40	33
III	100	96	91	55	66
IV	–	4	9	5	1
Etiology (%)[b]					
PPH	85	83	70	40	53
CTD	15	17	30	10	24
CHD	–	–	–	50	24
HIV	–	–	–	–	–
Treatment effect					
Peak VO2 (% predicted)	N/A	N/A	N/A	N/A	+3%[c]
6-min walk change (m)	+76	+60[d]	+44	+40[d]	+34
Hemodynamics	Improved	Improved	N/A	Improved	Improved
Clinical events	Reduced	Reduced	Reduced	N/A	Reduced[e]

BREATHE-1: *B*osentan *R*andomized trial of *E*ndothelin *A*ntagonist *The*rapy for pulmonary hypertension; CHD = congenital heart disease (congenital systemic to pulmonary shunts); CTD = connective tissue disease; N/A = not available. PPH = primary pulmonary hypertension; STRIDE-1: *S*itaxsentan *to* *R*elieve *I*mpaire*d* *E*xercise study.

[a] Data on a pilot trial with ambisentan are not yet available.

[b] Sum of % may not be 100% for rounding to the nearest unit, 0.5 is rounded to the upper unit.

[c] Only for 300 mg dose.

[d] Improvement from baseline.

[e] Only for 100 mg dose.

5.2.1. Dual receptor antagonism

Bosentan is an oral active dual ET_A- and ET_B-receptor antagonist and is the first molecule of this class of drugs to be synthesized [18].

In the first pilot study, 32 NYHA class III patients were randomized 2:1 to receive either 125 mg twice daily of bosentan or placebo [82]. After 12 weeks, a treatment effect of 76 m in favor of bosentan was observed in 6-min walking distance and the difference was maintained after 20 weeks. An improvement of right atrial pressure, cardiac index, mean pulmonary artery pressure and pulmonary vascular resistance was also assessed. Clinical endpoints such as Borg dyspnea index, NYHA functional class and clinical worsening also improved. Increases in plasma levels of aminotransferases were seen in two patients but the levels returned to normal without change of dose. Twenty-nine of the original 32 patients received bosentan in an extension study: at month 6, assessed patients maintained the improvement in walk distance and long-term treatment with bosentan for >1 year was associated with an improvement in hemodynamic parameters and NYHA functional class [83].

In the larger *B*osentan *R*andomized trial of *E*ndothelin *A*ntagonist *The*rapy for pulmonary hypertension (BREATHE-1) study 213 NYHA classes III and IV patients were randomized 1:1:1 to receive placebo or 62.5 mg of bosentan twice daily for 4 weeks followed by either bosentan 125 or 250 mg bid for a minimum of 12 weeks [84]. At week 16, patients treated with bosentan improved their 6-min walking distance and the mean treatment effect was 44 m. Although both bosentan dosages induced a significant treatment effect, the placebo-corrected improvement ended to be more pronounced for the 250-mg bid than for the 125-mg bid dosage (+54 and +35 m, respectively). However, no formal dose response for efficacy could be ascertained. Although a similar treatment effect was achieved in patients with PPH and in those with PAH associated with scleroderma, bosentan improved the walking distance from baseline in PPH patients (+46 m in the bosentan group versus −5 m in the placebo group), whereas it prevented walk distance deterioration of the scleroderma patients (+3 m in the bosentan group versus −40 m in the placebo group). Bosentan also improved the Borg dyspnea index, functional class and increased the time to clinical worsening. Increases in hepatic aminotransferases occurred in 10% of the subjects, were found to be dose-dependent and reversible after dose reduction or discontinuation. In fact, abnormal hepatic function was more frequent and severe in the 250-mg dose group and a decrease in transaminases concentrations was observed in all cases in which the bosentan dose was reduced. Based on these results, the recommended target therapeutic dose of bosentan was confirmed as 125 mg twice daily. The most likely mechanism for the liver enzyme changes after bosentan is a dose-dependent competition by bosentan and its metabolites with the biliary excretion of bile salts, resulting in a retention of bile salts that can be cytotoxic to hepatocytes [85].

An echocardiographic substudy was performed in 85 patients enrolled in 13 centers. Several echocardiographic and Doppler parameters related to PAH were improved in bosentan treated patients as compared to the placebo group including Doppler derived cardiac index (+0.4 l/min/m^2), Tei index, right and left ventricles dimensions and pericardial effusion score [86].

In the open label extension of the bosentan studies, 169 patients have received the drug for a mean of 2.1 ± 0.5 years. Preliminary reports [87] show that survival was 92% at 26 months as compared to a predicted survival of 56% based on historical controls [38]. Differences between observed and predicted values at 6, 12, 18, 24 and 26 months were significant ($p < 0.001$). Only 10% of patients required epoprostenol administration as rescue treatment [87].

Combination treatment is attractive for the potential of correcting concurrently different pathophysiologic mechanisms. The efficacy and safety of the combination of bosentan and epoprostenol were investigated in 33 patients with severe PAH enrolled in a placebo-controlled prospective study (BREATHE-2). All patients started epoprostenol and were randomized for 16 weeks in a 2:1 ratio to bosentan (62.5 mg twice daily for 4 weeks then 125 mg twice daily) or placebo. Improved hemodynamics, exercise capacity and functional class were observed in both groups. A preliminary report shows that in the combination treatment group, there was a trend for a greater (though non-significant) improvement in all hemodynamic parameters [88].

In clinical practice, the combination of bosentan with prostanoids may be of interest, in case of unsatisfactory clinical efficacy of the latter therapy. In an open series of patients with PAH where bosentan was added to a long-standing prostanoid treatment, improvements of 6-min walk distance and peak oxygen consumption were observed [89].

Oral bosentan has been recently proposed also as a transition therapy in patients displaying severe and/or unbearable side effects of prostanoid therapy including sepsis with intravenous epoprostenol [90].

An open-label, uncontrolled single and multiple-dose study has been performed in children 4–17 years of age with PAH (BREATHE-3) to assess pharmacokinetics, tolerability and safety of oral bosentan. In this preliminary study a significant improvement of hemodynamics was observed after 12 weeks of treatment in the 18 enrolled children either with bosentan alone or in combination with epoprostenol [91].

Bosentan has been approved for the treatment of NYHA classes III and IV PAH patients in the USA, Canada and Europe.

5.2.2. Selective ET_A-receptor antagonism

The safety and efficacy of sitaxsentan, a selective ET_A-receptor antagonist, were preliminarily evaluated in a 12-week, open-label trial of 20 patients with NYHA classes II, III and IV PAH [92]. Sitaxsentan was administered orally at

100–500 mg bid and after 12 weeks a significant improvement in exercise capacity as assessed by the 6-min walk distance (+49 m as compared to baseline). Mean pulmonary artery pressure and pulmonary vascular resistance also improved. Serious adverse events included two cases of acute hepatitis (fatal in one patient).

A second larger controlled clinical trial on sitaxsentan (STRIDE-1: *S*itaxsentan *t*o *R*elieve *I*mpaire*d* *E*xercise) was performed on 178 patients with NYHA classes II, III and IV PAH [93,94]. Etiology included PPH and PAH associated with connective tissue diseases and congenital heart diseases. Patients were randomized 1:1:1 to placebo, sitaxsentan 100 mg or sitaxsentan 300 mg given orally once daily for 12 weeks. The primary endpoint was the change from baseline to week 12 in % of predicted peak-VO_2. Secondary endpoints included 6-min walk distance and NYHA class. Preliminary data recently presented [93,94] show that sitaxsentan 300 mg (but not 100 mg) improved peak-VO_2 by 3.1% of predicted as compared with placebo. Sitaxsentan 100 and 300 mg also significantly improved 6-min walk distance (100 mg: +35 m, 300 mg: +33 m), and NYHA class. The different statistical results on peak-VO_2 and 6-min walk distance could be addressed when the study data will be fully published. Both sitaxsentan doses resulted in significant improvements in cardiac index and pulmonary vascular resistance. Incidence of abnormal liver function tests, which reversed in all cases, was 0% for 100 mg and 9.5% for 300 mg.

The safety and efficacy of ambisentan, a selective ET_A-receptor antagonist, were evaluated in a Phase II, randomized, double-blind, dose-ranging 12-week study in 64 PAH patients. The drug was administered once a day to four dose groups (1, 2.5, 5 and 10 mg). The primary efficacy endpoint of the study was the change from baseline in the 6-min walk distance. Preliminary reports show that a comparable improvement in exercise capacity and hemodynamics was observed in all dose groups.

5.2.3. Comparison

The current experience testifies the efficacy of ET-1 receptor antagonist drugs in improving symptoms, exercise capacity, hemodynamics and outcome of patients with PAH. The largest knowledge has been collected with the dual receptor antagonist bosentan that is currently available in many countries for clinical use. ET_A-receptor selective drugs appear to be also effective even if the experience is still preliminary and additional pivotal studies are underway. Comparisons between trials with diverse compounds (Table 3) are complicated by differences in baseline characteristics of patient populations and in study designs. For example, NYHA functional class II patients and subjects with congenital heart diseases were enrolled only in the sitaxentan studies. To detect any differences in efficacy and safety between dual and selective ET-1 receptor antagonists, direct comparative trials would be required. On the other hand, the design characteristics of these studies (sample size, endpoints and statistics) appear to be complex and difficult to implement.

6. Conclusions

An inappropriate activation of the ET-1 system has been clearly shown in patients with almost all types of PAH. The attempts to antagonize the ET-1 system in this condition have been successful in both animal models and clinical trials. In fact, the orally active dual ET-1 receptor antagonist bosentan has improved symptoms, exercise capacity, hemodynamics, echocardiographic parameters and the outcome of patients with severe PAH. The selective ET_A-receptor antagonist sitaxentan has improved exercise capacity and hemodynamics of PAH patients in two preliminary studies. The main side effect of ET-1 antagonists is the increase of liver enzymes likely due to an accumulation of bile salts cytotoxic to hepatocytes. Additional trials with these and other ET-1-receptor antagonist are currently ongoing. ET-1 receptor antagonism is an effective treatment in a PAH patients.

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