Review

Endothelin receptor antagonists for the treatment of pulmonary artery hypertension

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ABSTRACT

Aims: The demonstration that endothelin production is upregulated in pulmonary artery hypertension (PAH) served as the rationale for developing endothelin-receptor antagonists (ERAs) as a treatment for PAH. This article reviews the primary studies demonstrating efficacy of ERAs in PAH.

Main methods: Multicenter, placebo-controlled trials and open-label extension studies.

Key findings: Two orally active ERAs are currently approved for the treatment of PAH — the dual receptor antagonist bosentan, and the more selective ET_A receptor antagonist ambrisentan-based on multicenter randomized clinical trials demonstrating efficacy and safety. Long-term experience with both agents supports maintenance of therapeutic effects in most patients. Adverse effects, including altered liver function and edema may occur and require careful monitoring.

Significance: Despite failure to demonstrate efficacy of ERAs in other cardiopulmonary conditions, ERAs have a major role in the treatment algorithm for PAH.

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Contents

Introduction

The endothelins are a family of 21-amino acid peptides that play a key role in the regulation of vascular tone. The first member of this family identified was endothelin-1 (ET-1), a 2492 Da peptide with potent vasoconstrictor properties, isolated by Yanagisawa et al. (1988).

Two additional endothelin isopeptides, endothelin-2 (ET-2) and endothelin-3 (ET-3), were subsequently discovered (Inoue et al., 1989a). All three of these proteins share a high degree of amino acid homology. They also bear structural similarity to a family of peptides labeled sarafotoxins which were isolated from the venom of the snake *Atractaspis engaddensis*, suggesting a possible shared evolutionary origin (Takasaki et al., 1988).

Vascular endothelial cells are the major source of endothelins in humans. However, genes encoding the endothelin peptides are also found in a wide range of additional cell types including bronchial

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epithelium, macrophages, cardiac myocytes, glomerular mesangium, and glial cells, among others (Mattoli et al., 1990; Yu and Davenport, 1995; Ehrenreich et al., 1990; Miyauchi and Masaki, 1999).

The years following the discovery of endothelins saw an explosion of basic research into these compounds (Michael and Markewitz, 1996). This has led clinicians to postulate numerous potential applications to the manipulation of the endothelin system: including the treatment of renal diseases, systemic hypertension and cerebral vasospasm (Michael and Markewitz, 1996; Benigni and Remuzzi, 1999).

It is in the therapy of pulmonary vascular disease, however, that endothelin biology has, thus far, shown its greatest penetration into the clinical arena. This article will review the current understanding of the role of endothelins in the physiology and pathophysiology of the pulmonary circulation, as well as the clinical experiences gained from the use of endothelin receptor antagonists in the treatment of pulmonary arterial hypertension.

Physiology

Although marked structural similarity among the endothelins results in significant areas of overlapping biologic function, these compounds are not as similar as they first may appear. The human genes for ET-1, ET-2 and ET-3 are each located on different chromosomes (Michael and Markewitz, 1996). Furthermore, the distribution of the three endothelin proteins throughout different tissues appears to be quite heterogeneous. Endothelial cells, including those of the pulmonary circulation, predominantly generate ET-1. Kidney cells appear to express higher levels of ET-2 (Masaki, 1998). ET-3 has been found in high concentrations in the intestine and brain (Shinmi et al., 1989; Levin, 1995). Each of these three compounds seems to have a distinct physiologic role that guides their site(s) of expression. Our understanding of these unique yet overlapping roles remains incomplete.

Endothelin-1 production

The human ET-1 gene is located on the telomeric region of chromosome 6p (Michael and Markewitz, 1996). The ET-1 gene includes five exons that encode mRNA for a large precursor protein: preproendothelin-1 (PPET-1).

Unlike some other proteins, ET-1 is not kept in secretory granules (Nakamura et al., 1990) within cells. The rate-limiting step in the biosynthesis of ET-1 occurs at the level of transcription (Yanagisawa et al., 1988). Many stimuli that regulate ET-1 production have evolved through direct action upon transcription factors (Abstract figure). It appears that vascular endothelial cells are able to rapidly increase or inhibit ET-1 production to regulate vascular tone (Inoue et al., 1989b).

The majority of ET-1 secreted from cultured endothelial cells occurs from the abluminal side of the cells towards the adjacent vascular smooth muscle cells, which contain specific endothelin receptors (Yoshimoto et al., 1991). Thus, it is important to note that although circulating ET-1 can be detected in the plasma, and may have important clinical correlations with pulmonary vascular disease (Table 1), these

Table 1
Conditions associated with pulmonary hypertension in which ET-1 levels are increased.

Idiopathic pulmonary artery hypertension, persistent pulmonary hypertension of the newborn
CREST syndrome
Eisenmenger syndrome
Mitral stenosis
Congestive heart failure
Chronic obstructive pulmonary disease, interstitial lung disease
High altitude exposure
Obstructive sleep apnea
Pulmonary hypertension after heart surgery (cardiopulmonary bypass)

plasma levels may not necessarily reflect the paracrine action of ET-1 on adjacent smooth muscle cells.

Endothelin receptors

There are two distinct receptors for the endothelin family of peptides, endothelin receptor A (ET_A) and endothelin receptor B (ET_B). The endothelin receptors belong to the family of receptors connected to guanine nucleotide-binding (G) proteins (Takuwa et al., 1990). The two receptors have unique locations (Benigni, 1995) and binding affinities (Masaki, 1998) for the endothelin peptides. ET_A receptors are expressed on pulmonary vascular smooth muscle cells, and have high affinity for ET-1 and ET-2, with less affinity for ET-3. ET_B receptors are located on both pulmonary vascular endothelial cells and smooth muscle cells. ET_B receptors bind all three endothelin isoforms with nearly equal affinity.

When activated, the ET_A receptor located in the pulmonary vascular smooth muscle cells mediates vasoconstriction. The mechanism is thought to occur via G protein induced phospholipase C activation; 1,4,5-inositol triphosphate (IP_3) formation; and the consequent release of Ca^{2+} from intracellular stores (Takuwa et al., 1990). There is some evidence that ET_A receptor may also increase intracellular calcium by activating non-selective calcium channels on the surface of the smooth muscle cell (Iwamuro et al., 1999). The vasoconstriction induced by ET_A has been shown to persist even after ET-1 is removed from the receptor, likely due to persistently elevated concentrations of intracellular Ca^{2+} (Clarke et al., 1989).

In addition to its powerful vasoconstricting effects, ET-1 is known to be a potent mitogen, with the ability to induce cell proliferation in a number of cell types, including vascular smooth muscle cells (Chua et al., 1992). It has been shown that the mitogenic actions of ET-1 are mediated by both the ET_A (Davie et al., 2002) and ET_B (Sugawara et al., 1996) receptors. In the pathogenesis of PAH, ET-1 is thought to both produce vasoconstriction and stimulate vasoproliferation. In the pulmonary vasculature, ET_B receptors are predominantly expressed on endothelial cells (Sakurai et al., 1990; Hirata et al., 1993). ET_B receptors on endothelial cells mediate vasodilation via increased production of nitric oxide and prostacyclin (Hirata et al., 1993; De Nucci et al., 1988; Filep et al., 1991). Nitric oxide and prostacyclin also negatively feedback on ET-1 activity by inhibition of PPET-1 transcription: In the setting of PAH, where both endothelial nitric oxide and prostacyclin production are impaired, the pathogenic properties of overexpressed ET-1 are, therefore, unopposed by these counter-regulatory molecules. Inflammation may also play a pathogenic role in some forms of PAH, and endothelin may be upregulated in this setting, as well.

ET_B receptors contribute to the clearance of circulating ET-1, likely due to internalization of the ET-1/ET_B receptor complex into the cell after binding (Dupuis et al., 1996b).

It has been observed that the normal human lung removes roughly 50% of circulating ET-1, and that it releases a similar quantity, resulting in the lack of an arterial-to-venous ET-1 gradient across the pulmonary vasculature in the normal state (Dupuis et al., 1996a).

There are data suggesting that the ET_B receptor does not exclusively mediate pulmonary vasodilation. Under some circumstances it may actually contribute to pulmonary vasoconstriction, through a population of ET_B receptors located on vascular smooth muscle cells (Masaki, 1995). The vasoconstrictive actions of ET_B receptors may become more pronounced in the pathologic setting of pulmonary hypertension (Dupuis et al., 2000) than in the normal pulmonary vasculature. It has been postulated that this action may result from down-regulation of ET_A receptors in states of pulmonary hypertension, possibly as an adaptive response to high levels of circulating ET-1 (Kuc and Davenport, 2000).

The vasoconstrictive actions of the ET_B receptor may confer a therapeutic advantage to the strategy of dual ET_A/ET_B receptor blockade over

selective ET$_A$ receptor blockade in the treatment of pulmonary arterial hypertension.

Pathophysiology

The endothelins are thought to participate in the pathophysiology of a spectrum of pulmonary vascular diseases. The extent to which the endothelin system is involved in each disease affecting the pulmonary circulation is not completely understood, however similarities in the pathogenesis of this family of disorders suggest that endothelin biology has broad applicability. The evidence for the role of the endothelin system in the pathophysiology of several individual pulmonary vascular diseases will be subsequently reviewed.

Idiopathic pulmonary arterial hypertension

Patients with idiopathic pulmonary arterial hypertension (IPAH) demonstrate higher serum levels of ET-1 and higher arterial-to-venous ratios of ET-1 than healthy controls (Stewart et al., 1991). This phenomenon may represent increased production of ET-1 by the lung, reduced clearance by the lung, or a combination of these processes. Lung specimens from patients with IPAH, when compared to those from patients without pulmonary hypertension, exhibit increased ET-1 staining of the muscular pulmonary arteries and increased expression of PPET-1 in the endothelial cells of the same vessels (Giaid et al., 1993). There is furthermore a correlation between the intensity of staining for ET-1 and the patients' hemodynamic measurements of pulmonary vascular resistance. Recent studies have shown increased Endothelin Converting Enzyme-1 (ECE-1) in the pulmonary vascular endothelial cells of IPAH patients (Giaid, 1998), and increased net pulmonary clearance of ET-1 in patients with IPAH treated with continuous intravenous epoprostenol (Langleben et al., 1999).

Other pulmonary vascular diseases

Pulmonary hypertension from chronic hypoxia has been shown in animal models to be associated with increased ET-1 and ET$_A$ expression (Chen and Oparil, 2000; Chen et al., 1997). In these models, it is also notable that dual ET$_A$/ET$_B$ receptor blockade resulted in amelioration of pulmonary hypertensive changes (Eddahibi et al., 1995). Interestingly, rat models have also demonstrated regional differences in endothelin expression throughout the lung, leading some authors to suggest that heterogeneity of the endothelin system may help to regulate local responses to hypoxia in the pulmonary circulation (Takahashi et al., 2001a, 2001b). Detailed human investigations into the role of the endothelin system in chronic hypoxemia have not been reported to date.

Pulmonary hypertension from congenital cardiac disease has been shown in human investigations to correlate with high levels ET$_A$ receptor density and circulating ET-1, which in some instances decreased following surgical correction of the cardiac lesions (Bando et al., 1997; Ishikawa et al., 1995; Lutz et al., 1999). The development of hypoxemia in patients with congenital shunts may be an additional factor which magnifies the detrimental effects of ET-1 (Allen et al., 1993).

Chronic Thromboembolic Pulmonary Hypertension (CTEPH) has been associated with increased activity of the ET-1 system in both animal (Kim et al., 2000a, 2000b) and human (Bauer et al., 2002) pathologic studies. Pulmonary hypertensive changes were attenuated in the presence of dual ET$_A$/ET$_B$ receptor blockade in a canine model of CTEPH (Kim et al., 2000a). It is known that many patients with CTEPH have a concomitant small vessel vasculopathy which can limit the hemodynamic improvement following pulmonary endarterectomy. These data suggest that endothelin may play a role in this process.

Persistent Pulmonary Hypertension of the Newborn (PPHN) has been associated with increased ET-1 expression and ETA receptor activity in a number of animal studies, involving several different models of PPHN (Ivy et al., 1994, 1998; Okazaki et al., 1998; Shima et al., 2000). Clinical studies of human babies with PPHN (Christou et al., 1997; Kumar et al., 1996; MacDonald et al., 1999; Rosenberg et al., 1993) have also revealed elevated levels of circulating ET-1, which appear to correlate with other markers of disease severity.

Clinical use of endothelin receptor antagonists

There are currently 2 endothelin receptor antagonists commercially available for the treatment of PAH, ambrisentan, and bosentan. These agents were approved based on results of 12–16 week randomized, placebo-controlled trials demonstrating their efficacy in improving exercise capacity, as measured by the 6-minute walk test. Long-term, open label studies have confirmed the benefits of this class of medication. A third ERA, sitaxsentan, a highly selective ET$_A$-receptor antagonist, was approved for marketing in Europe based on two randomized trials demonstrating similar effects on 6-minute walk test, hemodynamics, and quality of life parameters as the other two agents; however, sitaxsentan was withdrawn from marketing and further development when several cases of fatal hepatic failure were reported with its use.

Ambrisentan

Ambrisentan is a specific ET$_A$ receptor antagonist approved for PAH, functional classes II and III at doses of 5 mg or 10 mg once daily. Following a Phase 2, dosing study showing favorable pulmonary hemodynamic effects (Galie et al., 2005), two randomized controlled trials (ARIES 1 and ARIES 2) of ambrisentan (ARIES 1: 5 mg, 10 mg, placebo; ARIES 2: 2.5 mg, 5 mg, placebo) were performed, enrolling a total of 394 patients (Galie et al). Both trials achieved the primary endpoint of placebo-corrected improvement in 6-minute walk distance. In ARIES-2, there was a significant improvement in time to clinical worsening in the treatment group as compared with placebo. There was a trend towards improvement in time to clinical worsening in the ARIES-1 study, but it was not statistically significant (p = 0.307). World Health Organization (WHO) functional class improvement was significant in ARIES-1 and there was trend towards improvement in ARIES-2 but did not reach statistical significance (p = 0.117). 298 patients were enrolled and followed in the long-term extension study over 48 weeks. Eighteen patients required additional therapies (prostanoids or phosphodiesterase type-5 [PDE-5] inhibitors). Of the 280 patients continued on ambrisentan monotherapy, the improvement in 6-minute walk test (6-MWT) at 12 weeks was 40 m and maintained at 39 m. Although there were no patients with elevations in serum aminotransferases >3 times upper limit of normal while on ambrisentan, in the trials, long-term follow-up has revealed cases of transaminase elevations which resolve upon discontinuation of ambrisentan.

Bosentan

Bosentan is a potent, non-peptide, oral endothelin A and B receptor antagonists, with higher affinity for the ET$_A$ subtype receptor. Bosentan received Food and Drug Administration (FDA) approval November 2001 for patients with WHO functional class III or IV PAH. The first double-blind, placebo-controlled trial randomized 32 patients with IPAH (84%) or PAH associated with scleroderma with NYHA class III to bosentan or placebo for 12 weeks (Channick et al., 2001). The primary endpoint was the placebo-corrected change in 6-MWT, with secondary endpoints including change in pulmonary hemodynamics, WHO functional class, *Borg* dyspnea index and clinical worsening. The placebo-corrected improvement in 6-MWT was 76 m in favor of the bosentan group, In addition, pulmonary hemodynamics, especially cardiac index and pulmonary vascular resistance were favorably affected by bosentan. There were asymptomatic increases in liver

aminotransferases in 2 patients on bosentan, but these returned to baseline without discontinuing or changing the dose.

The larger BREATHE-1 (bosentan randomized trial of endothelin antagonist therapy) trial, which randomized 213 patients with IPAH (70%) and pulmonary hypertension associated with connective tissue disease with WHO functional classes III and IV to placebo or bosentan at 125 mg or 250 mg twice daily, confirmed the efficacy and safety of bosentan over 16 weeks (Rubin et al., 2002). At 16 weeks, a placebo-corrected 6MWD improvement of 44 m was noted (p<0.001). There were also improvements in the *Borg* dyspnea score and time to clinical worsening in both bosentan groups. Increases in liver aminotransferases greater than 8 times upper limit of normal was again noted in the bosentan group and was dose-dependent with 2 patients in the 125 mg group and 5 patients in the 250 mg group.

In addition to the above "pivotal" trials of bosentan, a randomized controlled trial of bosentan in PAH patients less functionally impaired (WHO class II), the EARLY trial, demonstrated a benefit in reducing pulmonary vascular resistance and preventing clinical worsening at 6 months. No statistically significant effect on 6-minute walk distance was seen, although baseline walk distance was greater than 400 m, confirming that this cohort had better baseline exercise capacity (Galiè et al., 2008).

Bosentan has also been studied in a controlled fashion in patients with congenital heart disease (Galiè et al., 2006). The BREATHE 5 study demonstrated that, compared to placebo, bosentan improved six minute walk distance without worsening hypoxemia.

Long-term survival data in patients on bosentan, although uncontrolled, has been published. Of the 169 IPAH patients enrolled in the 2 pivotal trials of bosentan, estimated survival at 1 and 2 years was 96% and 89% respectively, as compared to the predicted survival of 69% and 57% (McLaughlin et al., 2005). (based on a validated NIH equation calculating predicted survival from baseline hemodynamics). It should be acknowledged that there are no prospective controlled survival data with the newer agents, given obvious ethical concerns about such trials in the era of existing therapy.

As noted above, ERAs as a class have a propensity for inducing liver function abnormalities, with a spectrum of severity ranging from sitaxsentan as the most frequent and causing the most severe hepatic dysfunction to ambrisentan causing less severe dysfunction and with greater infrequency. Current regulatory guidelines mandate monthly monitoring of liver function in patients receiving bosentan, while this requirement has been removed for ambrisentan based on a review of safety data from a large cohort of patients treated for several years. Nevertheless, periodic monitoring of liver function in patients receiving ambrisentan is still advised.

Future directions

The next steps in the research of endothelin receptor antagonists for the therapy of PAH appear to have promise. Questions which remain to be answered include the role of endothelin receptor antagonists in: (a) long-term efficacy on morbidity and mortality of ERAs in combination with other agents (b) upfront combination therapy strategies, for example with a prostanoid or PDE-5 inhibitor, and (c) expanded disease indications, such as for CTEPH or fibrotic lung disease.

Conflict of interest statement

The author is a consultant of Actelion, Gilead, Pfizer, United Therapeutics, GeNO, and AIRES.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.lfs.2012.07.033.

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