

Endothelin Receptor Antagonists in Pulmonary Arterial Hypertension

Richard N. Channick, MD,* Olivier Sitbon, MD,† Robyn J. Barst, MD,‡ Alessandra Manes, MD,§ Lewis J. Rubin, MD*

San Diego, California; Paris, France; New York, New York; and Bologna, Italy

Endothelin receptor antagonism has emerged as an important therapeutic strategy in pulmonary arterial hypertension (PAH). Laboratory and clinical investigations have clearly shown that endothelin (ET)-1 is overexpressed in several forms of pulmonary vascular disease and likely plays a significant pathogenetic role in the development and progression of pulmonary vasculopathy. Oral endothelin receptor antagonists (ERAs) have been shown to improve pulmonary hemodynamics, exercise capacity, functional status, and clinical outcome in several randomized placebo-controlled trials. Bosentan, a dual-receptor antagonist, is approved by the U.S. Food and Drug Administration for class III and IV patients with PAH, based on two phase III trials. In addition to its efficacy as sole therapy, bosentan may have a role as part of a combination of drugs such as a prostanoid or sildenafil. The selective endothelin receptor-A antagonists sitaxsentan and ambrisentan are currently undergoing investigation. (J Am Coll Cardiol 2004;43:62S–67S) © 2004 by the American College of Cardiology Foundation

Since the last World Symposium on Pulmonary Hypertension in Evian, France, in 1998, endothelin receptor antagonism has emerged as a cornerstone of therapy for pulmonary arterial hypertension (PAH). Elucidation of the role of endothelin in the pathogenesis and progression of pulmonary vascular disease, the efficacy of endothelin receptor antagonists (ERAs) in randomized clinical trials, and long-term outcome data have placed this therapy at the forefront of the treatment armamentarium. We review here the current state of knowledge related to ERAs in the context of the treatment algorithm described elsewhere in this supplement.

ENDOTHELIN (ET)-1 AS AN IMPORTANT MEDIATOR IN PAH

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 ET-1, a 2492-dalton (Da) peptide with potent vasoconstrictor properties, isolated by Yanagisawa et al. in 1988 (1). Two additional endothelin isopeptides—ET-2 and ET-3—were subsequently discovered. The ET-1 appears to play the most prominent role in vascular control. Knowledge of the mechanisms and molecular aspects of ET-1 is important in understanding the therapeutic value of endothelin receptor antagonism.

PRODUCTION OF ET-1

The majority of ET-1 secreted from cultured endothelial cells occurs from the abluminal side of the cells toward the

adjacent vascular smooth muscle cells, which contain specific endothelin receptors (2). 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, these 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 (3). The two receptors have unique locations (4) and binding affinities (5) for the endothelin peptides. The ET_A receptors are expressed on pulmonary vascular smooth muscle cells, whereas ET_B receptors are located on both pulmonary vascular endothelial cells and smooth muscle cells.

When activated, the ET_A receptor located in pulmonary vascular smooth muscle cells mediates a potent vasoconstrictive response, thought to occur via G-protein-induced phospholipase C activation: 1,4,5-inositol triphosphate formation with the consequent release of Ca²⁺ from intracellular stores (3). There is also evidence that the ET_A receptor mediates increased intracellular calcium by activating non-selective calcium channels on the surface of the smooth muscle cell (6). 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 Ca²⁺ (7).

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 (8). It has been shown that the

From the *Pulmonary and Critical Care Division, University of California, San Diego, California; †Division of Pulmonary and Critical Care Medicine, University of Paris-Sud, Paris, France; ‡Division of Pediatric Cardiology, Columbia-Presbyterian Hospital, New York, New York; §Institute of Cardiology, University of Bologna, Bologna, Italy.

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Abbreviations and Acronyms

CTEPH	= chronic thromboembolic pulmonary hypertension
ERAs	= endothelin receptor antagonists
ET	= endothelin
ET _A	= endothelin receptor A
ET _B	= endothelin receptor B
IPAH	= idiopathic pulmonary arterial hypertension
6MWT	= 6-min walk test
PAH	= pulmonary arterial hypertension
WHO	= World Health Organization
NYHA	= New York Heart Association

mitogenic actions of ET-1 are mediated by both the ET_A (9) and ET_B (10) receptors.

In the *normal* pulmonary vasculature, ET_B receptors are predominantly expressed on endothelial cells (11,12). The ET_B receptors on endothelial cells mediate vasodilation via increased production of nitric oxide and prostacyclin (12–14). Nitric oxide and prostacyclin also negatively feed back on ET-1 activity by inhibition of preproendothelin-1 transcription. In addition, ET-1 is cleared by ET_B receptors.

Data suggest 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 (15). The vasoconstrictive actions of the ET_B receptor may become more pronounced in the pathologic setting of pulmonary hypertension (16), possibly due to upregulation of ET_B receptors in states of pulmonary hypertension (17). The functions of both receptors under pathologic conditions may therefore determine whether antagonizing one or both receptors is preferable.

In patients with PAH, several derangements in ET-1 expression and activity have been demonstrated. Patients with idiopathic pulmonary arterial hypertension (IPAH, formerly called primary pulmonary hypertension) have been shown to have higher serum levels of ET-1 and higher arterial-to-venous ratios of ET-1 than do healthy controls (18). Endothelin levels have also been shown to correlate with pulmonary hemodynamics (19). In addition, lung specimens from patients with IPAH (formerly primary pulmonary hypertension), when compared to those from patients without pulmonary hypertension, exhibit increased ET-1 staining of the muscular pulmonary arteries (20). Also, PAH associated with congenital cardiac disease has been shown in human investigations to correlate with high levels of ET_A receptor density and circulating ET-1, which in some instances decrease following surgical correction of the cardiac lesions (21–23).

Chronic thromboembolic pulmonary hypertension (CTEPH) has also been associated with increased activity of the ET-1 system in both animal (24,25) and human (26) pathologic studies. Pulmonary hypertensive changes were attenuated in the presence of combined ET_A/ET_B receptor blockade in a canine model of CTEPH (24). It is known

that many patients with CTEPH have a concomitant small vessel vasculopathy that can limit the hemodynamic improvement following pulmonary thromboendarterectomy, suggesting a pathogenetic role for endothelin in this process.

CLINICAL USE OF ERAs

Given the prominent role that ET-1 appears to play in several forms of pulmonary hypertension, ERAs have a strong rationale. There is now one approved ERA, oral bosentan, for the treatment of PAH. Other ERAs are currently under investigation.

Bosentan. Bosentan is an antagonist of both the ET_A and ET_B receptors, with only slightly higher *in vitro* affinity for the ET_A receptor. Two randomized clinical trials led to U.S. Food and Drug Administration (FDA) approval of bosentan for PAH patients who are functional class III or IV.

The first multicenter randomized placebo-controlled study of chronic oral bosentan was performed by Channick and colleagues in 32 patients with IPAH (*n* = 27) or with PAH related to scleroderma (*n* = 5) (27). Recruited patients were all World Health Organization (WHO) functional class III, and there was 2:1 randomization to the bosentan group in relation to placebo. Patients in the bosentan group received the drug at a dose of 62.5 mg twice daily for four weeks followed by 125 mg twice daily. Concurrent therapy with digoxin, anticoagulants, diuretics, and calcium channel blockers was permitted; however, patients receiving epoprostenol were excluded. The primary end point was exercise capacity as measured by the 6-min walk test (6MWT) and secondary end points included hemodynamic improvement by right heart catheterization, change in functional class, and time to clinical worsening—all measured at 12 weeks. The intention-to-treat analysis demonstrated statistically significant improvements in the bosentan group compared to placebo in 6MWT, with a mean treatment effect of 76 m and pulmonary hemodynamics (cardiac output, pulmonary vascular resistance, mean pulmonary arterial pressure) (Fig. 1).

A subsequent larger double-blind, placebo-controlled study of bosentan in PAH by Rubin *et al.* (28) enrolled 213 patients with IPAH (*n* = 150) or PAH related to scleroderma (*n* = 47) or systemic lupus erythematosus (*n* = 16). All patients belonged to WHO functional class III or IV. Baseline parameters included mean 6MWT of ~330 m, and mean pulmonary artery pressures of ~55 mm Hg. Patients randomized to the bosentan group received 62.5 mg twice daily for four weeks, then either 125 mg twice daily (*n* = 74) or 250 mg twice daily (*n* = 70) for an additional 12 weeks, in comparison to placebo (*n* = 69). The primary end point was functional status as measured by the 6MWT at 16 weeks. This trial also showed a statistically significant improvement in 6MWT in both bosentan groups in comparison to placebo (Fig. 2). Analysis of secondary measures of efficacy revealed a trend in the bosentan groups toward lower Borg dyspnea indices and

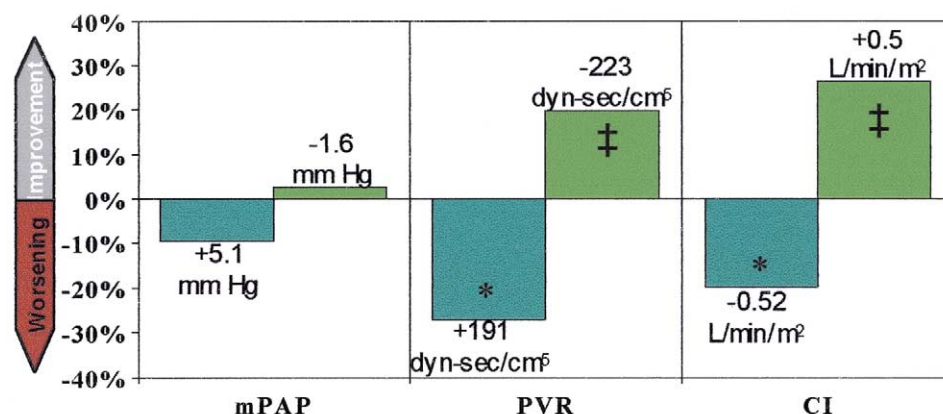


Figure 1. Hemodynamic effects of bosentan versus placebo at 12 weeks. Significant improvements in cardiac index (CI), pulmonary vascular resistance (PVR), and mean pulmonary arterial pressure (mPAP) were all noted. The placebo group worsened over the 12-week period. Adapted from Channick et al. (27)—original work. Control group is shown in blue; bosentan group is shown in green. ‡Significant improvement versus baseline; *Significant decline versus baseline.

improved functional class. There was also a statistically significant increase in the bosentan groups in time to clinical worsening (Fig. 3), as measured by the composite end point of time to death, lung transplantation, hospitalization, or study dropout because of worsening pulmonary hypertension, need for epoprostenol therapy, or atrial septostomy. In addition, in a subgroup of 85 patients enrolled in an echocardiographic substudy, bosentan improved different echocardiographic and Doppler parameters related to the right ventricular systolic function and the left ventricular early diastolic filling (29).

Data on the long-term efficacy of bosentan are now available. A recent report by Sitbon et al. (30) demonstrated sustained improvement in functional class and pulmonary hemodynamics for at least one year.

Mortality data for 169 patients treated with bosentan as first-line therapy was recently presented by McLaughlin et al. (31). In that report, three-year survival was 86% compared to a predicted survival of 48% for these individuals based on a validated National Institutes of Health (NIH) survival equation. In this cohort, at two years, 70% of patients were still maintained on bosentan alone.

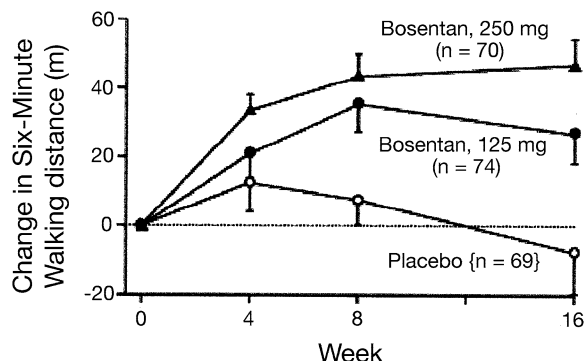


Figure 2. Six-minute walk distance improvement seen in study of 213 patients demonstrating significant treatment effect of bosentan. (From Rubin et al. [28]).

Safety. Bosentan is primarily eliminated by hepatic metabolism through the P450 enzyme systems CYP2C9 and CYP3A4. Steady-state levels are usually achieved after three to five days with twice daily dosing. Upon reaching steady-state, the elimination half-life becomes constant. One metabolite of bosentan (Ro 48-5033) is pharmacologically active but is believed to contribute <20% of the clinical response to bosentan. Renal clearance of bosentan appears to be negligible.

Clinical evidence suggests that bosentan administration can precipitate hepatocellular injury, particularly at higher doses. Combined data from existing clinical trials reveal greater than threefold elevations of the aminotransferases in 11% of bosentan patients (n = 658) compared with 2% of patients given placebo (n = 280). This effect was observed both early and late in treatment. The more severe elevations in aminotransferases were observed in the patients receiving 250 mg twice daily or higher. The liver abnormalities were often asymptomatic and all resolved with dose reduction or cessation. In some patients, reintroduction of bosentan did not lead to recurrent hepatic enzyme elevations. Studies in rats have revealed that bosentan-induced liver injury is likely mediated by drug-induced inhibition of the hepatocellular bile-salt export pump (32).

Patients on bosentan must undergo monitoring of the alanine aminotransferase and aspartate aminotransferase before drug initiation and monthly thereafter. Patients with significant baseline hepatic dysfunction should not be given bosentan. In patients with hepatic congestion from right heart failure, aggressive diuresis may correct abnormal aminotransferases occurring solely on this basis, and consequently requalify these patients for bosentan.

Bosentan is contraindicated in pregnancy. Animal models reveal that the endothelin peptides appear to play an important role in fetal development. In one study (33) ET-1 was implicated in the closure of the ductus arteriosus at birth. Mice with ET-1 deficiency (34) and those given

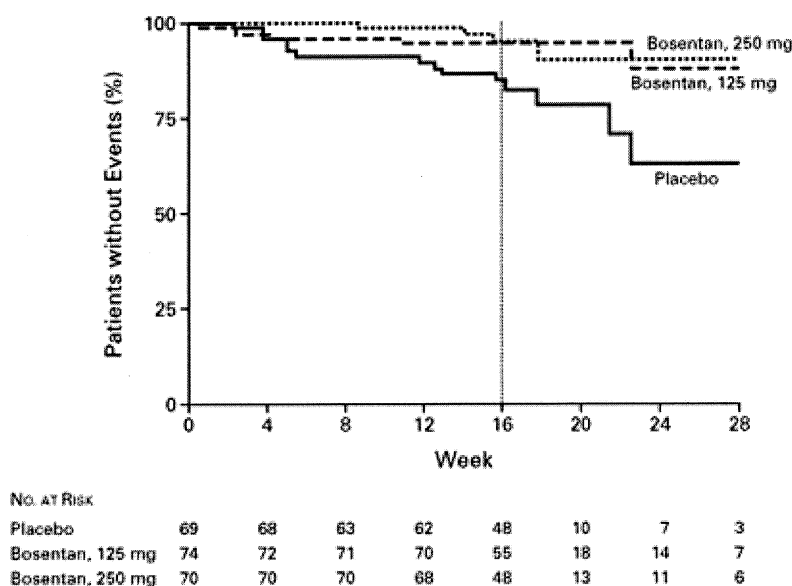


Figure 3. Delay in clinical worsening in bosentan-treated patients. Clinical worsening was defined as death, premature withdrawal from study, hospitalization for worsening pulmonary arterial hypertension, or initiation of epoprostenol. (From Rubin *et al.* [28]).

bosentan (product monograph, Actelion Pharmaceuticals, Allschwil, Switzerland) as fetuses develop severe craniofacial abnormalities. Pregnancy must be excluded before therapy with bosentan and prevented thereafter with reliable contraception. Hormonal forms of contraception may not be reliable in the setting of bosentan therapy, and thus should not be the sole form of contraception in females of childbearing potential.

Other common side effects observed with bosentan include a dose-related decrease in hemoglobin of unknown etiology, headaches, and flushing.

Several drugs have been shown to interact with bosentan through the P450 system. Glyburide and cyclosporine A are contraindicated with concurrent bosentan therapy. Although a small study has shown that humans given bosentan 500 mg twice daily have reduced warfarin effect (35), no influence on warfarin activity has been seen in the clinical trials using the 125-mg and 250-mg twice-daily doses of bosentan.

Role in the context of existing treatments for PAH.

Bosentan received approval in 2001/2002 by a number of regulatory agencies, including those in Canada and the U. S. The approved indications are PAH, with functional class III or IV. For WHO functional class III and possibly early class IV patients who are not acutely vasoreactive or who have failed calcium channel blocker therapy, bosentan should be considered the initial treatment of choice, based on compelling short- and long-term data. For patients with significant hemodynamic decline, especially with signs of overt right ventricular failure, or those who progress to WHO functional class IV, epoprostenol remains the initial therapy of choice. These recommendations are outlined elsewhere in this supplement as part of a consensus treatment algorithm.

The addition of bosentan to epoprostenol is a potentially

attractive approach, as the two agents work through different and possibly complementary mechanisms. A randomized controlled trial of combined epoprostenol–bosentan found a trend toward greater percent reduction in total pulmonary resistance with the combination versus repoprostenol alone (36).

SELECTIVE ET_A ANTAGONISTS

Selective antagonists of the ET_A receptor are currently undergoing investigation, with the rationale that the “favorable” actions of the ET_B receptor will remain intact and efficacy further improved. Sitaxsentan sodium, a potent endothelin receptor antagonist that has oral bioavailability and a long duration of action ($t_{1/2}$, 5 to 7 h), is approximately 6,500-fold more selective as an antagonist for ET_A compared with ET_B receptors. In the first randomized, double-blind, placebo-controlled trial with sitaxsentan in PAH, 178 New York Heart Association (NYHA) functional class II, III, and IV patients with either PPH, PAH related to connective tissue disease, or PAH related to congenital systemic to pulmonary shunts were equally randomized to receive placebo, sitaxsentan 100 mg, or sitaxsentan 300 mg, orally once daily (37). Although the primary efficacy end point of maximum oxygen consumption was not improved by sitaxsentan compared to placebo, the drug did improve exercise capacity (6-min walk distance) (Fig. 4) and functional class after 12 weeks of treatment. These functional benefits occurred with both the 100-mg and the 300-mg doses. Treatment effects in the sitaxsentan groups were 35 m ($p < 0.01$) for the 100-mg dose and 33 m ($p < 0.01$) for the 300-mg dose. The NYHA functional class improved in 16 of 55 (29%) patients in the 100-mg group and in 19 of 63 (30%) patients in the 300-mg group. In

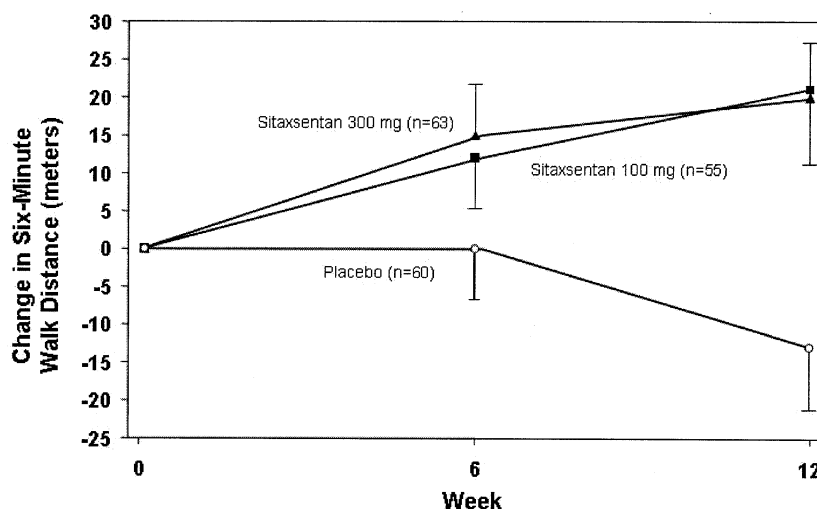


Figure 4. Six-minute walk distance improvement seen in study of 178 patients demonstrating significant treatment effect of sitaxsentan. (From Barst et al. [37]).

contrast, only 9 of 60 (15%) patients in the placebo group had improvement in NYHA functional class. Improvements in pulmonary vascular resistance and cardiac index were also noted in the sitaxsentan group.

As with bosentan, liver function abnormalities occurred (10% in the 300-mg group). It should be noted that, in an earlier pilot study, sitaxsentan was associated with fatal hepatitis when used at higher doses (38). In the larger randomized trial, the most frequently reported clinical adverse events with sitaxsentan treatment (and more frequent than in placebo) were headache, peripheral edema, nausea, nasal congestion, and dizziness, reactions previously noted with ET-receptor antagonists. The most frequently reported laboratory adverse event was increased international normalized ratio or prothrombin time, related to the effect of sitaxsentan on inhibition of CYP2C9 P450 enzyme, the principal hepatic enzyme involved in the metabolism of warfarin. A second phase III trial of sitaxsentan is currently underway.

Another ET_A antagonist, ambrisentan, is currently in phase III clinical trials in patients with PAH, and information on relative safety and efficacy will be forthcoming in the near future.

FUTURE DIRECTIONS IN ERA THERAPY

Endothelin receptor antagonists for the therapy of PAH appear to have great promise. Questions that remain to be answered include 1) the role of ERAs in early PAH (i.e., WHO class I and II); 2) ERAs as part of combination therapy—for example, with epoprostenol, treprostinil, or sildenafil; 3) the value of selective versus nonselective endothelin receptor antagonism; and 4) the role of ERAs in treating other conditions such as CTEPH or fibrotic lung disease.

Reprint requests and correspondence: Dr. Richard N. Channick, UCSD Medical Center, 9300 Campus Point Drive, La Jolla, California 92037. E-mail: rchannick@ucsd.edu.

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