

Endothelin Antagonism in Pulmonary Arterial Hypertension

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ABSTRACT

The pathobiology of pulmonary arterial hypertension (PAH) reflects a multifactorial process and complex evolution that involves dysfunction of underlying cellular pathways and mediators. Among these, the endothelin system has been shown to be important in the pathogenesis of PAH. Endothelin-1 (ET-1), which is found in high levels in PAH, is a known potent vasoconstrictor with proliferative vascular remodeling properties. Left unchecked, endothelin excess, along with other derangements, may contribute to the development and perpetuation of PAH. There is now substantial evidence from clinical trials and long-term data that monotherapy with an endothelin receptor antagonist (ERA) is a beneficial, therapeutic approach in PAH. Combination therapy of an ERA with a prostanoid or phosphodiesterase-5 inhibitor, two drug classes that have different mechanisms of action, is conceptually appealing, but the evidence for its efficacy and safety are still being investigated. This review provides an overview of endothelin biology and the clinical use of ERAs for the treatment of PAH. The use of ERAs for other forms of pulmonary hypertension will not be reviewed here.

KEYWORDS: Pulmonary hypertension, endothelin, bosentan, sitaxsentan

Objectives: Upon completion of this article, the reader should be able to: (1) summarize the rationale for using endothelin receptor antagonists in the treatment of pulmonary arterial hypertension; and (2) understand the benefits and risks of using endothelin receptor antagonists.

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ENDOTHELIN SYSTEM BIOLOGY

A potent endothelial cell-derived vasoconstrictor was first isolated in 1985¹ and subsequently characterized as a 21-amino acid peptide, which was named endothelin.² There are three endothelin isoforms in humans: endothelin-1 (ET-1), ET-2, and ET-3, each of which contains two intramolecular disulfide bonds between

cysteine amino acid residues 1 through 15 and 3 through 11.³ Vascular endothelial cells secrete the majority of ET-1, 80% of which goes into the basolateral compartment away from the vascular lumen.⁴ The directionality of secretion toward the underlying smooth muscle layer supports the hypothesis that ET-1 acts as a paracrine regulator of smooth muscle function. In

Pulmonary Arterial Hypertension; Editor in Chief, Joseph P. Lynch, III, M.D.; Guest Editor, Victor F. Tapson, M.D. *Seminars in Respiratory and Critical Care Medicine*, volume 26, number 4, 2005. Address for correspondence and reprint requests: Richard N. Channick, M.D., Department of Medicine, Division of Pulmonary and Critical Care Medicine, University of California, San Diego, 9300 Campus Point Dr., MC 7381, La Jolla, CA 92037. E-mail: rchannick@ucsd.edu. ¹Department of Medicine, Division of Pulmonary and Critical Care Medicine, University of California, San Diego, La Jolla, California. Copyright © 2005 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. Tel: +1(212) 584-4662. 1069-3424,p;2005,26,04,402,408,ftx,en;srm00392x.

addition to vasoconstriction, ET-1 is thought to be a mediator in the vascular remodeling process by promoting smooth muscle proliferation,⁵ protein synthesis,⁶ and production of a variety of cytokines and growth factors.⁷

The endothelins bind two receptor subtypes: ET_A and ET_B.⁸ In vascular tissue, ET_A is located predominantly on smooth muscle cells, whereas ET_B is found on both endothelial and smooth muscle cells.⁷ Activation of ET_A by ET-1 leads to potent vasoconstriction due to an increase in cytosolic calcium levels via influx of extracellular calcium^{9,10} and release from intracellular stores.¹¹

The actions of ET_B are more complicated. Like ET_A, ET-1 activation of ET_B on vascular smooth muscle cells leads to vasoconstriction.^{12,13} Furthermore, some studies suggest that in the pulmonary hypertensive state, blockade of both ET_A and ET_B is necessary to achieve maximal vasodilation.^{14–16} Conversely, other studies suggest a protective role of ET_B in pulmonary hypertension.¹⁷ ET_B activation on endothelial cells can stimulate the local release of nitric oxide and prostacyclin, which are known potent vasodilators.^{18,19} Additionally, ET_B receptors play a prominent role in clearing circulating ET-1 across the pulmonary circulation, perhaps by as much as 50%^{20,21} (Fig. 1). Thus the question of whether nonselective, dual receptor antagonism or selective ET_A receptor antagonism is most beneficial in PAH is still unclear. Clinical studies comparing the two would be required to determine which approach is better.

Clinically, several lines of evidence have demonstrated a strong relationship between endothelin system dysfunction and pulmonary arterial hypertension (PAH). Patients with idiopathic PAH (IPAH) have high plasma levels of ET-1,²² which positively correlate with right atrial pressure and negatively correlate with

pulmonary artery oxygen saturation.²³ There is also increased staining for ET-1 in the endothelial cells of pulmonary arteries with medial thickening and intimal fibrosis, the intensity of which is strongly correlated with pulmonary vascular resistance in patients with IPAH.²⁴

Given the prominent role of the endothelin system in PAH, there is sound rationale for the use of endothelin receptor antagonists (ERAs) in the treatment of PAH.

DUAL ENDOTHELIN RECEPTOR ANTAGONISM

After the introduction of intravenous epoprostenol in 1995 for the specific treatment of PAH, it was not until six years later in 2001 that the U.S. Food and Drug Administration approved the second specific treatment for PAH. Bosentan, a dual ERA of both ET_A and ET_B, was approved for use in patients with PAH who are in New York Heart Association (NYHA) functional class III or IV. Approval was largely based on the results of two double-blind, randomized, placebo-controlled studies showing efficacy on a variety of clinically relevant parameters. Bosentan represents a significant addition to the treatment armamentarium against PAH because it is orally active and exerts its effects through a separate mechanism than does epoprostenol.

The first multicenter, randomized, double-blind, placebo-controlled trial of bosentan was published by Channick et al in 2001.²⁵ In this pilot trial, 32 patients in functional class III with IPAH or PAH associated with scleroderma were randomized in a 2:1 fashion to bosentan or placebo. Patients received a starting dose of 62.5 mg twice daily for 4 weeks followed by the target dose of 125 mg twice daily. At week 12, the difference in

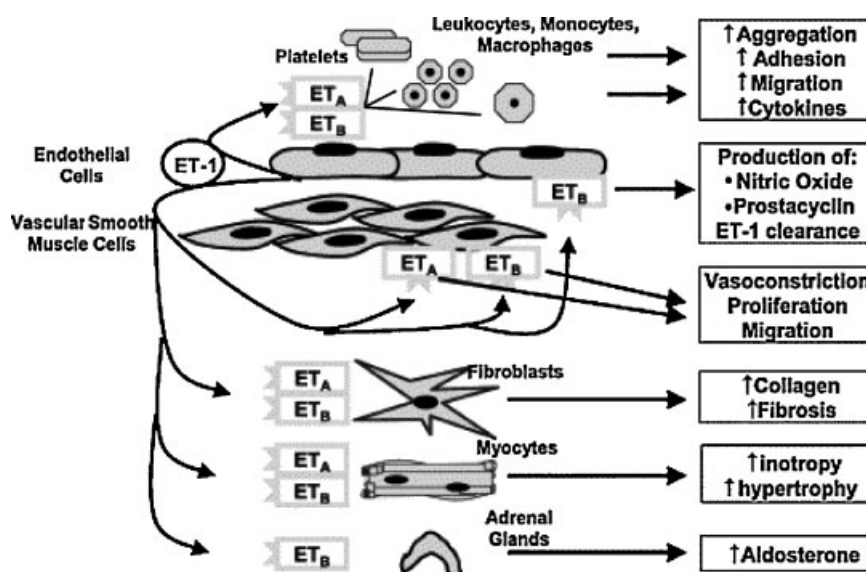


Figure 1 Schematic representation of endothelin-1 effects in different cell types. ET-1, endothelin-1; ET_A, endothelin-1 receptor A; ET_B, endothelin-1 receptor B. (Reprinted with permission from Galie et al.⁷)

6-minute walk test (6MWT) distance between the two groups was statistically significant (difference 76 m; bosentan +70 m versus placebo -6 m, relative to respective baselines). Further analysis showed that these differences were present as early as week 8 and maintained through week 20. Secondary end points of functional class, hemodynamics (cardiac index, pulmonary vascular resistance, pulmonary artery pressure, and mean right atrial pressure), and time to clinical worsening between the two groups were also significant at week 12, with the bosentan group showing improvement and the placebo group showing worsening.

This study was followed by the larger *Bosentan Randomized trial of Endothelin Antagonist Therapy* (BREATHE-1), which involved 213 patients with IPAH or PAH associated with scleroderma or systemic lupus erythematosus.²⁶ This pivotal trial confirmed the beneficial effects of bosentan in patients with PAH. The design for BREATHE-1 was similar to the previous study except for the inclusion of both class III and IV patients, an additional 250 mg twice daily bosentan dosing group, and 6MWT primary end point assessment at week 16.

Again, the difference in 6MWT, along with all the secondary end points of Borg dyspnea index, functional class, and time to clinical worsening, were significantly better for the bosentan group (Fig. 2). Although there was a trend toward increased 6MWT for the bosentan 250 mg group compared with the 125 mg group, it was not significantly different and was associated with more frequent aminotransferase abnormalities. This pivotal trial showed that bosentan was efficacious and safe, with the preferable dose being 125 mg twice daily.

Interestingly, in a subgroup analysis, bosentan increased the 6MWT in those with IPAH, whereas it prevented deterioration in those with scleroderma

(compared with each group's respective placebo arms). Clearly, treatment with bosentan was beneficial to those with scleroderma, but these results confirm that scleroderma associated PAH represents a greater challenge for new therapeutic agents. This finding is also consistent with a prior study demonstrating a poorer prognosis for this patient population.²⁷

Long-term survival data are now available from an open-label extension study of these two randomized, controlled trials (RCTs).²⁸ Kaplan-Meier survival estimates were 96%, 89%, and 86% at 1, 2, and 3 years, respectively. These were significantly higher than the "natural history" survival estimates predicted by the validated equation derived from the National Institutes of Health Primary Pulmonary Hypertension Registry: 1-year 69%, 2-year 57%, and 3-year 48%²⁹ (Fig. 3). At 2 years, 70% of patients remained on bosentan monotherapy. The others received prostanoids and/or other oral therapies either in addition to or instead of bosentan. Bosentan continued to show survival benefit in the long-term without the need for treatment augmentation or replacement in the large majority of cases. Not surprisingly, those in functional class IV and those who had 6MWT below the median at baseline (358 m), had a poorer outcome.

Data regarding use of bosentan in other forms of PAH are limited. Sitbon and colleagues recently published the results of BREATHE-4, a phase IIIb prospective, noncomparative cohort study of 16 patients with class III-IV PAH associated with human immunodeficiency virus (HIV) infection.³⁰ At 16 weeks, there were significant improvements in 6MWT, echocardiographic parameters, quality of life scores, and functional class, with 14 of the 16 patients improving at least one class from baseline. Bosentan was safe despite several patients being coinfecting with either hepatitis B or hepatitis C virus, and the large majority receiving

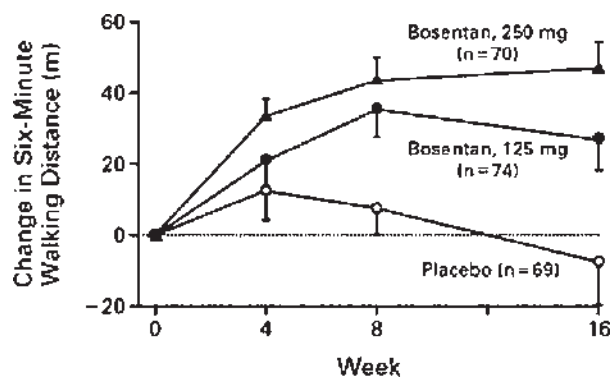


Figure 2 Mean ($\pm$ SE) change in 6-minute walking distance from baseline to week 16 in the placebo and bosentan groups. $p < .01$ for the comparison between the 125 mg dose of bosentan and placebo, and $p < .001$ for the comparison between the 250 mg dose and placebo by the Mann-Whitney U test. There was no significant difference between the two bosentan groups ($p = .18$ by the Mann-Whitney U test). (Reprinted with permission from Rubin et al.²⁶)

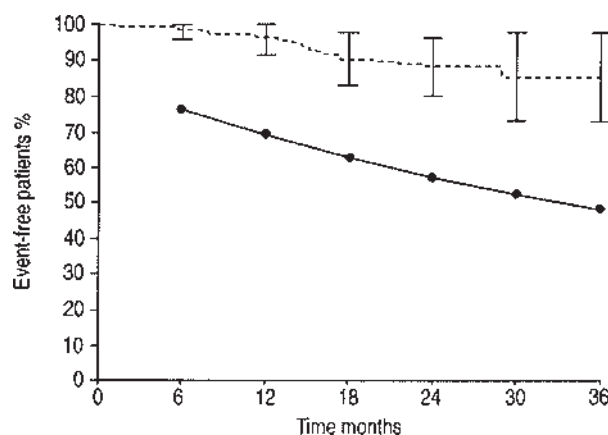


Figure 3 Kaplan-Meier estimates with 99.9% confidence intervals and predicted survival using the D'Alonzo equation.²⁹ - - -, observed survival; —, predicted survival. (Reprinted with permission from McLaughlin et al.²⁸)

potentially hepatotoxic antiretroviral therapy. Thus, at least in the short term, bosentan appears efficacious in the treatment of PAH associated with HIV infection, although a confirmatory trial and long-term data with more patients would be welcome to establish it as a definitive treatment option in this patient group.

Studies investigating the use of bosentan in PAH associated with Eisenmenger's syndrome (BREATHE-5) and functional class II patients with PAH (Endothelin Antagonist Trial in Mildly Symptomatic [EARLY]) are ongoing and should shed further light into the role of bosentan in these conditions.

The major adverse effect associated with bosentan is hepatocellular injury, which is dose dependent. Aminotransferase elevations occur in ~11% of patients treated with bosentan.³¹ Other commonly reported side effects included edema, flushing, and headache. Nonspecific decreases in hemoglobin concentration are also seen in ~5% of patients. Both glyburide and cyclosporin are contraindicated with bosentan, given the increased risk of liver toxicity. Liver function tests are required monthly whereas complete blood counts are recommended every 3 months.

Bosentan is a pregnancy category X medication. Women of child-bearing potential must be monitored with pregnancy tests before and regularly during treatment with bosentan. In addition, those using a hormonally based method of contraception must use a second form of birth control because bosentan may potentially decrease the efficacy of the former.

BOSENTAN VERSUS EPOPROSTENOL

Although direct comparisons between bosentan and epoprostenol are not available, a retrospective analysis of class III IPAH patients has shown that survival estimates up to 36 months were not significantly different in those who were initially treated with bosentan compared with those initially treated with epoprostenol.³² This suggests that an initial strategy employing bosentan in class III patients does not adversely affect their long-term survival when compared with those treated with epoprostenol.

Although bosentan is approved for use in class IV patients, it is still not clear whether treatment with bosentan or epoprostenol as an initial strategy provides the best outcomes for this patient group. Most experts consider continuous intravenous epoprostenol therapy as first-line therapy in class IV patients, which is consistent with the most current evidence-based treatment algorithm published.³³

SELECTIVE ET_A-RECEPTOR ANTAGONISM

Given the opposing actions of ET_A and ET_B, it may be advantageous to selectively antagonize the ET_A receptor

while allowing the ET_B receptor to remain unopposed. This has the theoretical advantage of blocking the deleterious vasoconstrictive and vascular smooth muscle proliferative effects mediated through ET_A while maintaining the beneficial vasodilatory and ET-1 clearance actions of ET_B.

The safety and efficacy of sitaxsentan, an orally active, selective ET_A receptor antagonist, was first evaluated in a small, open-label pilot trial involving 20 patients with class II–IV PAH.³⁴ At week 12, significant improvements were seen in 6MWT and various hemodynamic parameters. There were two cases of acute hepatitis, one of which was fatal.

This was followed by a much larger randomized, double-blind, placebo-controlled trial of placebo versus sitaxsentan 100 mg a day or 300 mg a day [Sitaxsentan to Relieve Impaired Exercise (STRIDE-1)].³⁵ 178 patients with IPAH or PAH associated with connective tissue disease or congenital heart disease in class II–IV were enrolled. The primary end point was change in peak oxygen consumption, with secondary end points of 6MWT, functional class, hemodynamics, time to clinical worsening, quality of life, and various cardiopulmonary exercise testing variables.

At week 12, peak oxygen consumption was significantly increased only for the sitaxsentan 300 mg dose group (+3.1%), compared with placebo. Both sitaxsentan dosing groups demonstrated similar improvements in 6MWT, functional class, pulmonary vascular resistance, and cardiac index, compared with placebo. There were no differences seen between groups in quality of life measures, time to clinical worsening, and several other variables derived from cardiopulmonary exercise testing.

The incidence of aminotransferase abnormalities was dose-dependent: 0% for the 100 mg group, and 10% for the 300 mg group. In the open-label extension that randomized patients to the 100 mg or 300 mg dose (median duration 26 weeks), the incidence of abnormalities increased to 5% for the 100 mg group and 21% for the 300 mg group.

Another caution with the use of sitaxsentan is the interaction with warfarin, reported in some cases to cause marked elevation of the international normalized ratio (INR) and need for dose reduction of warfarin.

A small 1-year follow-up study from a Canadian cohort of 11 patients who took part in STRIDE-1 and continued to receive open-label sitaxsentan 100 mg/d under a compassionate use protocol continued to show significant improvements in 6MWT, functional class, and hemodynamics, compared with baseline.³⁶

Several other studies are under way with sitaxsentan. STRIDE-2 is investigating the safety and efficacy of sitaxsentan compared with placebo and open-label bosentan. This trial provides a direct comparison of sitaxsentan to bosentan. STRIDE-6 is another RCT of sitaxsentan in patients who have failed therapy with

bosentan due to clinical deterioration or liver function test abnormalities. Ambrisentan, another selective ET_A-receptor blocker, is currently in phase III trials. These studies will hopefully shed light on the utility, or lack thereof, of selective endothelin receptor blockade.

ENDOTHELIN RECEPTOR ANTAGONISTS VERSUS SILDENAFIL

Conceivably, in the near future, physicians may be faced with the challenge of choosing from the available oral agents for initial treatment of PAH. However, little head-to-head comparison data are available. In a recently published, small, double-blind study, Wilkins et al randomized 26 patients with World Health Organization (WHO) class III IPAH or PAH associated with connective tissue disease to treatment either with sildenafil, a phosphodiesterase-5 inhibitor (PDE-5; 50 mg twice daily for 4 weeks, then 50 mg three times daily) or bosentan (62.5 mg twice daily for 4 weeks, then 125 mg twice daily) for 16 weeks.³⁷ One patient in the sildenafil group died unexpectedly. When analyzed by intention-to-treat with imputed data for the patient who died, there were no significant differences between the treatment groups with respect to the main outcome measures of right ventricle mass, 6MWT, cardiac function by echocardiogram, brain natriuretic peptide (BNP), and Borg dyspnea index.

COMBINATION/ADD-ON THERAPY

As is commonplace in the treatment of systemic hypertension, using a combination of drugs that targets different pathways in the management of PAH may increase the efficacy of treatment while potentially minimizing dose-related side effects. In the case of prostanoids, ERAs, and PDE-5 inhibitors, all three employ different intracellular pathways to exert their effects; however, there may be important interactions between pathways. For example, prostanoids³⁸ and nitric oxide³⁹ have been shown to inhibit the release of ET-1, suggesting the possibility of an interaction between the endothelin system and prostanoids or PDE-5 inhibitors.

BREATHE-2 investigated the combination of epoprostenol plus bosentan or placebo in 33 patients who were newly starting treatment.⁴⁰ Patients were in NYHA class III–IV with IPAH or PAH associated with connective tissue disease. After 16 weeks, hemodynamics, 6MWT, and functional class improved in both groups relative to their own baselines, likely due to epoprostenol. Although there was a trend toward improved hemodynamics in the epoprostenol/bosentan group compared with the epoprostenol/placebo group, there were no significant differences in 6MWT or functional class. The statistical outcome may have been affected by the higher number of study withdrawals

from the epoprostenol/bosentan group, all of which were judged to be related to the underlying PAH and not the study itself.

Other combination studies have included the addition of bosentan to a failing regimen of iloprost or beraprost,⁴¹ and sildenafil onto bosentan.⁴² Although sildenafil has yet to receive approval by any regulatory agency for use in PAH, the combination of bosentan and sildenafil is already being used in clinical practice. In healthy volunteers, there is a pharmacokinetic interaction between the two drugs: bosentan decreases the sildenafil area under the concentration-time curve (AUC-t) by 63%; conversely, sildenafil increases bosentan's AUC-t by 50%.⁴³ The implications for patients with PAH are unknown at this time. Studies investigating the use of bosentan to promote dose reduction or cessation of prostanoid therapy have reported varying success but are limited to small case series or retrospective analyses with varying protocols.^{44–47}

APPLICATION OF BOSENTAN TO CLINICAL PRACTICE

Published evidence-based algorithms for the initial treatment of PAH are now available.^{33,48} In general, bosentan is indicated in patients who are functional class III or possibly early class IV and not acutely vasoreactive. Bosentan is started at 62.5 mg twice daily for 1 month and then increased to the target dose of 125 mg twice daily as long as liver function tests are within the recommended limits.

If, after 3 months of therapy, poor prognostic signs such as 6MWT less than 375 m or persistence of functional class III–IV⁴⁹ are present, it may be reasonable to consider adding a prostanoid or sildenafil, depending on the clinical circumstance. By doing this, one hopes to gain additional benefit from a class of medication that has a different mechanism of action from that of ERAs. Data are emerging to support this combination approach. In general, we do not withdraw bosentan even when reassessed parameters do not seem to have improved because bosentan has been shown to stabilize the rate of deterioration (i.e., decrease the time to clinical worsening).^{25,26}

With the recent U.S. Food and Drug Administration approval of inhaled iloprost and the announcement of positive results from the Sildenafil Use in Pulmonary Arterial Hypertension study at a late-breaking clinical trials session at the American College of Chest Physicians' CHEST 2004 meeting, the decision about initial treatment strategy in class III–IV patients will become more complicated. Further studies assessing the long-term durability of benefit of these two medications and head-to-head comparisons are needed to make definitive recommendations about initial treatment choices. In the absence of these data, provider

expertise and experience, available resources, patient preference, and tolerability of side effects or adverse events will likely dictate initial treatment strategies.

In the case of unstable class IV patients, most experts would agree that first-line treatment with intravenous epoprostenol is indicated. Patients in functional class II are followed closely for progression of symptoms and/or hemodynamic worsening. A study of bosentan in class II patients is under way and should shed further light on the issue of early treatment.

CONCLUSIONS

From the first identification of ET-1 as a potent vasoconstrictor 20 years ago, state of the art therapy today for PAH in many instances now includes treatment with ERAs. Bosentan has proven its safety and efficacy in pivotal RCTs, and long-term survival data have now confirmed the durability of benefit. Intravenous epoprostenol is the only other medication for which the same can be said. Other ERAs are proceeding through the investigational pipeline.

Despite this remarkable progress, several questions remain to be answered: (1) how best to use ERAs in relation to the other therapies; (2) the value of selective versus nonselective ERAs; and (3) the role of ERAs for other forms of PAH (e.g., portal hypertension, drugs/toxins) and pulmonary hypertension due to lung disease or chronic thromboembolic disease.

ACKNOWLEDGMENTS

This work was supported in part by a National Institutes of Health Ruth L. Kirschstein National Research Service Award (F32 HL078099-01) and American Lung Association of California Research Fellowship Training Grant (RT-1009-N).

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