

PHARMACOTHERAPY

The Effect of the Endothelin-1 Receptor Antagonist, Bosentan, on Patients with Poorly Controlled Asthma: A 17-week, Double-Blind, Placebo-Controlled Crossover Pilot Study

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Objective. To determine the effect of bosentan on subjects with poorly controlled asthma. **Methods.** This was a double-blind, placebo-controlled crossover pilot study. Subjects were poorly controlled on anti-inflammatory and long acting β -agonist therapy, and had a baseline forced expiratory volume in 1 second (FEV₁) percent of predicted of 40–70%. Subjects were randomized to receive either bosentan or placebo at the therapeutic dose of 125 mg twice a day for 4 weeks, and then crossed over to the alternate therapy. The asthma control test, asthma symptom scores, rescue albuterol use, and FEV₁ were measured at baseline and during the last week of bosentan and placebo. Acute changes in FEV₁ were measured after the initial therapeutic bosentan and placebo dose. **Results.** Seven of eleven randomized subjects completed the protocol. There was no difference in change in FEV₁ after the bosentan phase when compared with placebo ($+0.08 \pm 0.31$ L and $+0.23 \pm 0.26$ L $p = .34$). Changes from baseline values in the asthma control test and asthma symptom scores were also similar in bosentan and placebo phases ($+1.71 \pm 3.99$ and $+4.57 \pm 4.39$ $p = .16$) and ($+0.14 \pm 9.3$ and -0.29 ± 5.28 $p = .93$). Rescue β -agonist use did not change significantly during the last week of the bosentan phase when compared with placebo phase (-5.86 ± 0.94 puffs and -5.14 ± 16.85 puffs $p = .94$). Additionally, there was no difference in the change in FEV₁ 4 hours after bosentan 125 mg and placebo (-0.08 L ± 0.07 vs. $+0.04$ L ± 0.20 $p = .20$). **Conclusions.** In this pilot study, 4 weeks of bosentan did not improve FEV₁, β -agonist use, asthma symptom score, or asthma control test score in patients with poorly controlled asthma when compared with placebo.

Keywords asthma, bosentan, endothelin-1, novel therapy, poorly controlled

INTRODUCTION

Asthma is a major healthcare concern, affecting 8.2% of the US population (24.6 million people), with adults accounting for 17.5 million of these patients (1). Asthma exacts a tremendous cost to US society, in terms of morbidity and mortality, as well as missed time from work and school. Data published in 2011 show that there were 10.5 million missed school days and 14.2 million missed work days related to asthma (1). Additionally, health care utilization for asthma accounted for 1.75 million asthma-related ED visits and 456,000 hospitalizations with 3447 deaths attributed to asthma (1).

Asthma is a disease of the airways caused by bronchoconstriction and chronic inflammation. Chronic inflammation may lead to airway thickening, mucous hypersecretion, and potentially irreversible airway remodeling. There are many proposed mediators of asthma including histamine, leukotrienes, TNF, various chemokines and interleukins, growth factors, and endothelin-1 (ET-1) (2).

ET-1 belongs to a family of peptides with vasoconstrictive and bronchoconstrictive properties (3). In the lung, the endothelin B (ET_B) receptor is found predominately in smooth muscle and to a less extent at other sites, suggesting smooth muscle is a primary target for ET-1 (4). ET-1 is 100 times more potent bronchoconstrictor than methacholine. Inhalation of ET-1 causes bronchoconstriction in

human asthmatics but not in normal subjects (5). During acute airway inflammation in rats, ET-1 bronchoalveolar lavage fluid (BALF) levels rise dramatically while plasma levels do not change (4). This rise in ET levels correlates with increased inflammatory cells in BALF (4). Furthermore, human asthmatics have elevated circulating and BAL concentrations of ET-1, and ET-1 levels increase further during an acute asthma exacerbation (6, 7). There is also evidence that ET-1 plays a role in airway inflammation, the hallmark of asthma, as it induces mucous secretion and stimulates the release of inflammatory mediators such as thromboxane (8) and various inflammatory prostanoids (9) from the airway in dogs and guinea pigs, respectively. In human cultured bronchial epithelial cells, ET-1 acting through the endothelin A (ET_A) receptor induces fibronectin release, suggesting a possible role of ET-1 in the airway remodeling seen in chronic asthma (10).

Bosentan, a treatment for pulmonary hypertension, is an antagonist of both ET_A and ET_B and thus has potential to be useful in the treatment of asthma. Rats treated with bosentan prior to induction of airway inflammation had significantly lower BALF inflammatory cells as well as ET-1 levels (4). We also have previously reported a patient with severe sarcoidosis-related airways disease, whose symptoms of coughing and wheezing improved markedly after he was treated with bosentan for his sarcoid-related pulmonary arterial hypertension (11). Although sarcoidosis is not asthma, this response provides further support for a potential role of ET-1 antagonists in the treatment of airways disease. We therefore performed a double-blind

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placebo-controlled crossover pilot study in which we assessed the effect of bosentan on patients with asthma whose symptoms were poorly controlled, despite anti-inflammatory therapy.

METHODS

Subjects between the ages of 21–70 years with a history of asthma were eligible for enrollment. Eligible subjects had to be previously maintained on anti-inflammatory and long acting bronchodilator therapy (excluding tiotropium bromide), with a baseline forced expiratory volume in 1 second (FEV₁) percent of predicted of 40–70% and a minimum of 12% reversibility of FEV₁ (after administration of albuterol) documented within the prior 2 years. In addition, eligible subjects had poorly controlled asthma defined as symptoms including wheezing, chest tightness, or shortness of breath occurring at least three times a week or requiring use of “rescue” short-acting β -agonist at least three times a week. Subjects with recent respiratory infection, step up in asthma therapy, or acute medical visit for asthma during the 30 days prior to study were ineligible. Additionally, subjects with hepatic disease, renal disease, active cardiac disease, clinically significant anemia, or pulmonary disease other than asthma were ineligible. Subjects with greater than a ten pack year history of tobacco use were excluded as well. Subjects were permitted to use rescue β -agonists as needed. Asthma was diagnosed based on the guidelines from the National Asthma Education and Prevention Program: Expert Panel Report 3 (12). Morris-Polgar reference values were used for FEV₁ percent of predicted values. The protocol was approved by the University of Connecticut Health Center’s Institutional Review Board and was monitored by a data safety monitoring board. Funding was provided by Actelion, who had no role in the study design, analysis, or manuscript writing.

Subjects were screened for entry criteria by history, physical examination, and spirometry. During a 7- to 10-day run-in period, subjects recorded daily asthma symptom scores on a 1–5 Likert scale (1 being no symptoms and 5 being severe symptoms) according to the methods of Israel et al. (13) and recorded a daily medication diary of rescue inhaler use. Subjects were enrolled on Visit 1 if they had an asthma symptom score ≥ 12 (13) for the 7 days prior to Visit 1 and at least 14 puffs of rescue inhaler during the prior 7 days. Each use of nebulized short-acting bronchodilators was considered to be equivalent to 2 puffs of an inhaler. On Visit 1, after 7–10 days of placebo run-in, subjects were randomized to receive bosentan 62.5 mg twice daily for 4 weeks (Tracleer®, Actelion Pharmaceuticals, California, USA) followed by the therapeutic dose of bosentan 125 mg twice daily for an additional 4 weeks or identical shape and size placebo (See Figure 1). Randomization was determined by a computer-based randomization program. Subject allocation was concealed. Bosentan dosing was based on the dosage approved by the Food and Drug Administration for the

indication of pulmonary arterial hypertension, which specifies a subtherapeutic dose (half of the maintenance dose of 125 mg twice a day) for the first month of therapy. This dosing was chosen as the side effect profile at this dose is known. At week 8, subjects crossed over to the alternate therapy based on initial randomization. FEV₁, asthma control test (ACT), and mean asthma symptom scores were recorded at baseline and during the last week of 125 mg bosentan or placebo therapy. The acute bronchodilator affect was measured by spirometric changes in FEV₁ measured at 1, 2, and 4 hours, after the first day of bosentan 125 mg or placebo. Subjects were called the day prior to spirometry to remind them to refrain from rescue inhaler use on the day of measurement. Adherence was measured by pill count.

The primary end point was change in mean daily asthma symptom score between baseline and during the last week of bosentan 125 mg. Secondary end points were change in ACT, change in FEV₁ after therapy, albuterol use during the last week of therapy, and acute bronchodilator effect of bosentan as assessed by measuring change in FEV₁ after bosentan 125 mg. For this analysis, subjects served as their own control. Test of statistical significance was performed using the 2 sided paired *t*-test.

RESULTS

Of the eleven subjects enrolled between March 2009 and April 2010, seven subjects completed the protocol. One subject was withdrawn due to pregnancy in the placebo phase, and another was withdrawn due to elevated transaminases, also during the placebo phase; neither had received bosentan. Two subjects were lost to follow up before completing the first phase of treatment and their data therefore were not included. Among the seven subjects who completed the study, there were no adverse events. The mean age of the seven subjects who completed the study was 50.9 ± 14.7 years and six (86%) were female. The mean baseline FEV₁ and FEV₁ percent of predicted before the bosentan phase and placebo phase were similar (Table 1). There were no statistically significant differences in the change in asthma control test, asthma symptom score, FEV₁, and β -agonist use during the bosentan phase compared to the placebo phase (Table 2, Figure 2). The acute FEV₁ response to bosentan and placebo at 1, 2, and 4 hours was also similar (Table 3).

DISCUSSION

Abundant preclinical data suggest a potential role of ET-1 in the pathogenesis of asthma, in addition to being a potent bronchoconstrictor (2). ET-1 bronchial epithelium expression is increased in asthma patients not receiving corticosteroids (14). ET-1 exhaled breath concentrate levels are higher in asthmatics compared to healthy controls, and are increased further in severe asthma (15).

Despite the promising preclinical data suggesting a role of ET-1 in asthma, our pilot crossover study demonstrated

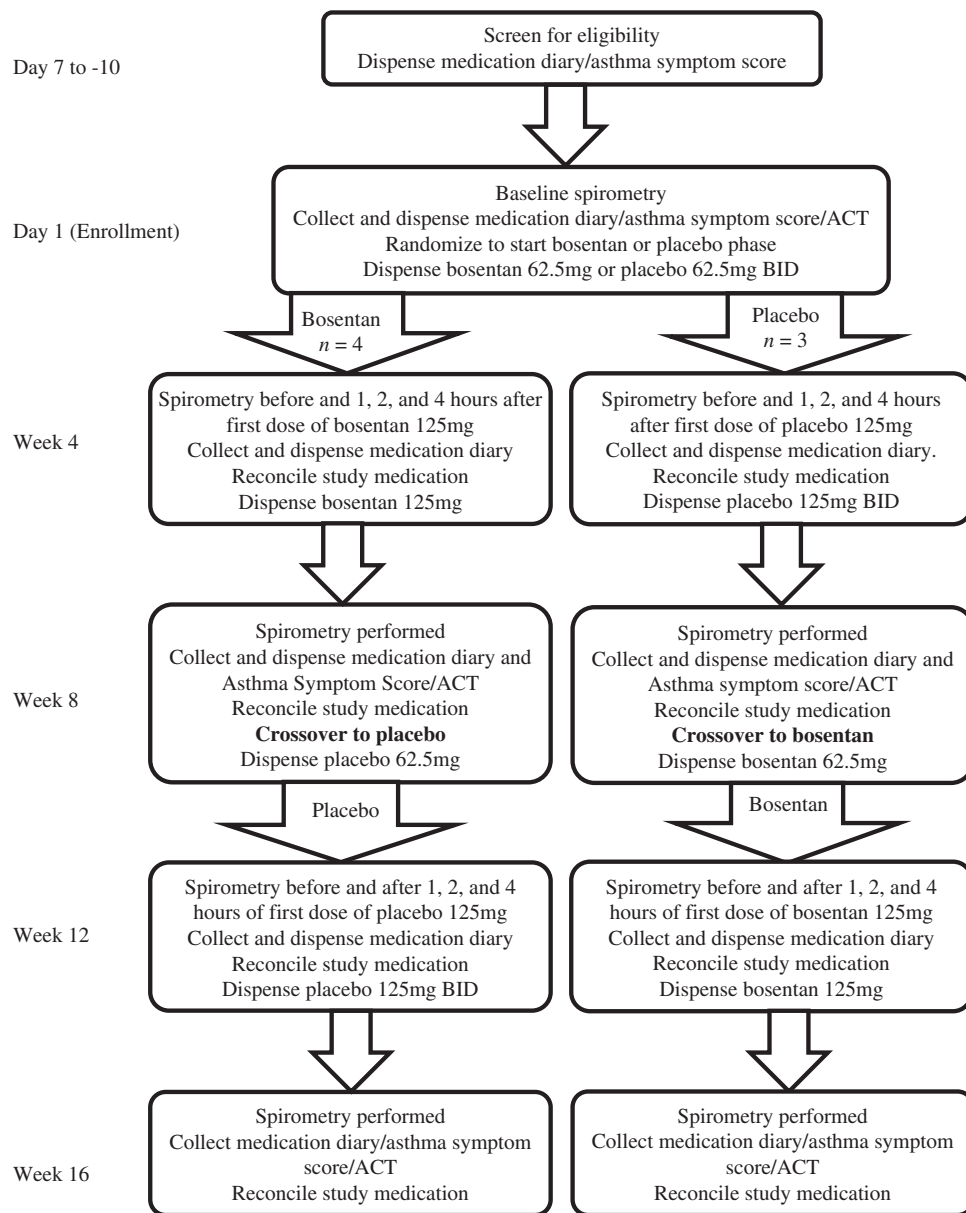


FIGURE 1.—Study design.

TABLE 1.—Baseline clinical characteristics.

	Placebo	Bosentan	p-value
Asthma control test score	13.6 ± 5.0	13.1 ± 3.9	.76
Asthma symptom score	19.7 ± 7.6	20.1 ± 3.5	.86
FEV ₁ (L)	1.46 ± 0.40	1.49 ± 0.47	.86
FEV ₁ percent of predicted	56 ± 16%	59 ± 13%	.45
Puffs of rescue β-agonist use (1 week)	34.57 ± 19.17	36.29 ± 18.92	.64

TABLE 2.—Effect of treatment with bosentan versus placebo.

	Placebo	Bosentan	p-value
Change in asthma control test	+4.57 ± 4.39	+1.71 ± 3.99	.16
Change in asthma symptom score	−0.29 ± 5.28	+0.14 ± 9.30	.93
Change in pre-bronchodilator FEV ₁ (L)	+0.23 ± 0.26	+0.08 ± 0.31	.34
Change in rescue β-agonist use (1 week)	−5.14 ± 16.85	−5.86 ± 0.94	.94

no improvement in asthma physiology or symptoms with the use of the ET-1 receptor antagonist, bosentan when compared with placebo. Furthermore, we did not demonstrate an acute bronchodilator response with the use of bosentan.

Although this pilot study suggests the possibility that ET-1 blockade may not be clinically useful in asthma patients, there are several other potential reasons for the observed lack of response to ET-1 receptor blockade. Bosentan is a non-selective ET-1 antagonist that binds

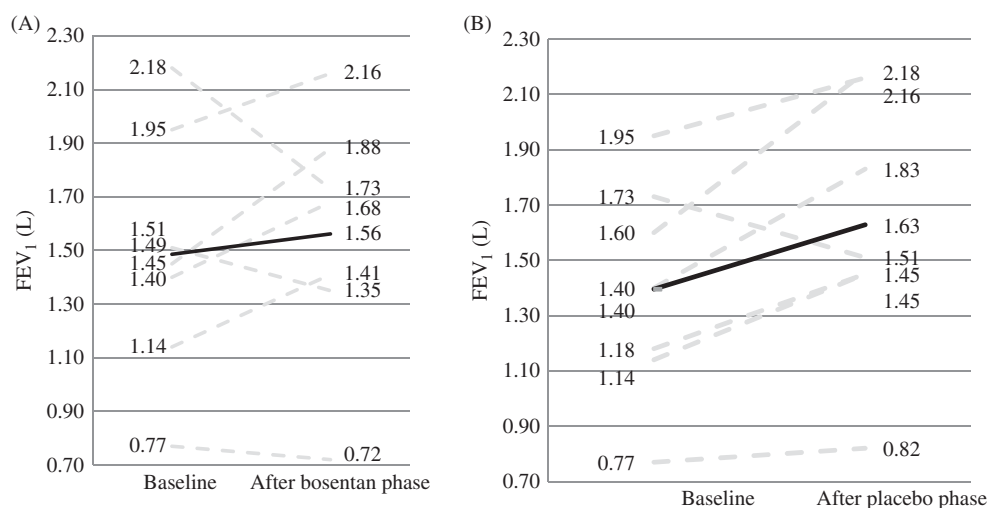


FIGURE 2.—(A) Individual and mean FEV₁ (shown in bold) measured in liters at baseline and after 4 weeks of bosentan 125 mg and (B) individual and mean (shown in bold) FEV₁ at baseline and after 4 weeks of placebo. There was no statistically significant change in response to bosentan when compared with placebo ($p = .86$).

TABLE 3.—Acute bronchodilator effect of therapy.

	Placebo	Bosentan	<i>p</i> -value
Change in FEV ₁ at 1 hrs (L)	+0.04 ± 0.13	−0.02 ± 0.09	.28
Change in FEV ₁ at 2 hrs (L)	+0.08 ± 0.16	−0.04 ± 0.06	.12
Change in FEV ₁ at 4 hrs (L)	+0.04 ± 0.2	−0.08 ± 0.07	.2

both ET_A and ET_B receptors. In a recent study, mice treated with an ET_A antagonist had increased BAL lymphocytes and decreased eosinophils, whereas mice treated with an ET_B antagonist showed no change in BAL lymphocytes or eosinophils (16). Additionally, mice treated with the ET_A antagonist showed a reduction in airway hyperresponsiveness and mucus production, while treatment with an ET_B antagonist had no significant effect (16). The non-selective nature of bosentan may be one of the reasons why we saw no response in our subjects. Also, it may have been that the dose chosen was insufficient to measure a response. Finnsnes et al. demonstrated decreased airway inflammation and eosinophils in Wistar rats treated with oral or intravenous bosentan (17). However the dose given was 30 mg/kg intravenous and 100 mg/kg orally, much higher dose than the dose chosen for our study, the FDA approved dose for pulmonary hypertension. The safety profile of such high dosing is not known in human subjects, however. While it is possible that a longer course of therapy could have resulted in measurable benefit, this is unlikely, as bosentan reaches steady state in approximately 5 days and patients received 4 weeks of therapy at the therapeutic dose (18). Carry-over effect from an insufficient washout period after bosentan treatment may have been an issue although in that case one would expect this to lead to improvement in FEV₁ or symptoms. This however was not seen in either group, making this effect less likely. Additionally, bosentan has

a half-life of 5 hours, so 4 weeks of placebo 62.5 mg twice daily followed by placebo 125 mg daily for 3 weeks should have been more than adequate time to allow for washout prior to recording FEV₁ and asthma symptom scores (18).

Finally, our study is limited by its small sample size, and the fact that only seven of eleven of enrolled subjects completed the study, increasing the possibility of confounding and/or a type II error. However, crossover designs are considered statistically efficient and generally require fewer subjects compared to non-crossover studies. As this was a crossover study, each subject served as his own control, reducing the influence of confounding covariates. No a priori power calculations were performed for this study, as it was a pilot study. Given that there was no prior existing human data, any effect size would have been speculative. However, we performed a post hoc power calculation. Because of the expense of this drug and its associated toxicity, we assumed that it would be unlikely to be used unless it had a profound benefit. Therefore, assuming a 50% reduction in asthma symptom score, using the observed standard deviations for values of sigma, and an alpha of 0.05, there was a 59% power to reject the null hypothesis. We note that bosentan was associated with a slight worsening of asthma symptom score (albeit non-significant), further lessening the chance of a type II error resulting in failure to detect an actual benefit of bosentan.

CONCLUSION

We have performed the first clinical trial investigating the use of an ET-1 receptor antagonist in human asthmatics. In this double-blind crossover pilot study, 4 weeks of bosentan therapy did not improve asthma symptom score, ACT, FEV₁, or albuterol use in poorly controlled asthmatics when compared with placebo. Although the small sample size limits our ability to conclude that non-selective ET-1

blockade has no role in asthma treatment, our results suggest that if there is benefit, the magnitude may not be large. Further, larger studies may be warranted, perhaps with a selective ET-1_A receptor antagonist.

DECLARATION OF INTEREST

The authors report no conflicts of interest and are solely responsible for the content of this paper.

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