Original Research

Bosentan for Patients with Echocardiographic Evidence of Pulmonary Hypertension Due to Long-Standing Rheumatic Mitral Stenosis

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Bosentan, endothelin receptor antagonists, mitral stenosis, pulmonary hypertension, rheumatic fever.

Introduction: Vasoconstrictive endothelin signaling is not limited to idiopathic pulmonary arterial hypertension, but has also been implicated in pulmonary hypertension due to valvular heart disease. The efficacy and safety of endothelin receptor antagonists in these patients is unknown. We investigated the effects of bosentan in patients with transthoracic echocardiographic (TTE) evidence of pulmonary hypertension due to mitral stenosis associated with rheumatic fever.

Methods: This was a prospective, single-center, open-label, uncontrolled study of bosentan in outpatients with uncorrected mitral stenosis due to rheumatic fever. The primary endpoint was exercise capacity at six months, as determined by six-minute walking distance (6MWD) and maximal oxygen uptake (VO_{2max}). Secondary endpoints were the BORG dyspnea index (BDI), echocardiographic left ventricular ejection fraction (LVEF) and pulmonary arterial pressure (PAP), serum pro-brain natriuretic peptide (proBNP), and adverse events at six months.

Results: Ten patients (eight females; mean age 75 years) were enrolled. Bosentan was well tolerated by nine, whereas one withdrew from treatment. By intention-to-treat analysis, bosentan resulted in a marked increase in 6MWD (+32 m, p=0.015), but a significant reduction in VO_{2max} (-0.45 mL/min/kg, p=0.011). Peak PAP did not change significantly, but mean PAP dropped by 16% (p=0.03) and LVEF increased by 6.5% (p=0.003). Profound reductions were observed in BDI and serum proBNP (-67% and -37%, p=0.002 and p=0.011, respectively). Adverse events included minor reductions in body mass and hematocrit.

Conclusion: The results of this pilot study suggest that endothelin receptor antagonism improves the functional status of patients with TTE evidence of pulmonary hypertension due to valvular heart disease. Randomized controlled trials are needed to confirm the results.

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According to the 4th World Symposium on Pulmonary Hypertension (PH) held in Dana Point, California, in 2008, PH is clinically classified into arterial, chronic thromboembolic, miscellaneous, and associated with lung or left heart disease.[1] Although pulmonary arterial hypertension (PAH) is regarded as the prototype, and most available treatments have been developed for these patients, PH associated with left heart disease probably represents the most frequent type of PH, affecting millions of patients worldwide.[2] Only a few studies using medications approved for PAH have been performed in this patient population, and the efficacy and safety of anti-PAH medications remain unknown.[1,3]

Left heart disease-associated PH may result either from left ventricular systolic or diastolic dysfunction, or from left heart valvular disease.[1] Although in some of

these patients pulmonary vascular resistance is normal and PH results from the passive transmission of backpressure from the left atrium via the pulmonary venous circulation to the pulmonary arterial bed, a significant percentage (20-40%) display elevation of pulmonary arterial pressure out of proportion to that expected from left atrial pressure.[2,4] Some patients with left heart valvular disease, especially mitral stenosis, can develop severe PH of the same magnitude as that seen in PAH, indicating increased pulmonary artery vasomotor tone and/or pulmonary vascular remodeling.

Endothelin 1, a powerful endogenous vasoconstrictor and mitogen, is critically involved in the vasoconstriction and vessel remodeling observed with PAH, and endothelin receptor antagonism is beneficial for such patients.[8,9] However, endothelin 1 is also systemically produced in rheumatic mitral stenosis-related PH, suggesting that endothelin signaling may be involved in the pathogenesis of PH in this subpopulation.[10,11] We hypothesized that this pathophysiological mechanism contributes to their elevated pulmonary pressure, as well as their poor functional status. To this end, we investigated the clinical and hemodynamic effects of bosentan treatment on patients with PH associated with long-standing rheumatic mitral stenosis.

Methods

Study design

This was a prospective, single-center, open-label, uncontrolled phase I/II trial of regular-dosed oral bosentan in patients with long-standing rheumatic mitral stenosis who showed evidence of PH on transthoracic echocardiography (TTE). The primary endpoint was exercise capacity at six months, determined by six-minute walking distance (6MWD) and maximal oxygen uptake (VO_{2max}). Secondary endpoints were BORG dyspnea index (BDI), echocardiographic left ventricular ejection fraction (LVEF) and pulmonary arterial pressure (PAP), serum pro-brain natriuretic peptide (proBNP), and adverse events at six months. At enrollment, patients' histories were recorded and they underwent physical assessment, body weight and height determinations, pulse oximetry, dyspnea assessment using BDI, 6MWD measurement, cardiopulmonary exercise testing, echocardiographic assessment of LVEF and PAP, as well as blood testing for routine cell counts, biochemistry, and proBNP. Patients received oral bosentan for six months, starting at 62.5 mg twice daily and switched to 125 mg twice daily after four weeks. Adherence to therapy was confirmed by monthly follow-up visits initiated six months before bosentan commencement and continued until study conclusion. Post-treatment evaluation at six months consisted of the same tests, plus additional history questions, physical examination, and cross-referencing of blood tests in order to identify possible adverse events related to bosentan therapy.

Patients

The study was conducted according to the principles set forth in the 1983 revision of the 1975 Declaration of Helsinki and was approved by the scientific board of the General Hospital of Chalkida (registry #29/22-09-2008). Patients were eligible for the study if they were outpatients, 60-85 years of age, with a stable disease and with a New York Heart Association (NYHA) congestive heart failure functional class IIIb/IV. Their history had to include non-operated mitral valve stenosis as a result of rheumatic fever at age <16 years, and their mean pulmonary artery pressure (PAP) had to be >40 cm H_2O as measured by TTE. Exclusion criteria were a history of treatment with an endothelin receptor antagonist, hospitalization during the previous year, and mitral valve repair. Eligible patients were identified between December 2008 and December 2010 among outpatients of the General Hospital of Chalkida. In January 2011 the trial was registered with ClinicalTrials.gov (#NCT01270750), and all participants gave written informed consent. Patients were then prospectively enrolled between February and April 2011. The study was concluded in October 2011.

Tests

The 6MWD tests were performed in the Department of Pulmonary Medicine according to the guidelines of the American Thoracic Society.[12] LVEF and PAP (peak and mean) were assessed using TTE at rest, performed by a specialist cardiologist using a General Electric® Vivid-7 device (General Electric, Fairfield, CT, USA). VO_{2max} was determined by the Astrand 6-minute Cycle Test (Static Bike). Blood samples were analyzed for cell counts using an Abbott Cell Dynn Sapphire® analyzer (Abbott Laboratories, Abbott Park, IL, USA), serum biochemistry (including electrolytes, liver and renal function tests, serum

lipids and various enzymes) using a Cobas® e601 bioanalyzer (F. Hoffmann-La Roche Ltd., Basel, Switzerland), and serum pro-BNP using a Hitachi Roche Elecsys® 2010 (F. Hoffmann-La Roche Ltd., Basel, Switzerland).

Analysis

Power calculations* determined that ten subjects were necessary to detect a 25% difference in the primary study endpoint (exercise capacity), assuming 25% SD, 5% α-error, and 35% β-error (i.e. with 65% power). Discontinuation of study medication because of clinical deterioration was analyzed with the patient's assessment at the time of premature withdrawal.

Normally distributed data are presented as mean [95% confidence interval (CI)]. Non-normally distributed data are presented as median (interquartile range [IQR]). To compare variables pre- and post-treatment, Fisher's exact test, the paired Student t-test, and the Mann–Whitney U-test were used for categorical, continuous normally distributed, and continuous non-normally distributed variables, respectively. Probability values are two-tailed, with a value of $p<0.05$ being considered significant. Analyses were performed using Prism Version 5.0 (GraphPad, La Jolla, CA).

Results

Outpatient screening identified ten eligible patients between December 2008 and December 2010; no patients were excluded. All the eligible patients resided in unprivileged areas of Evoia and Voiotia, Greece and had suffered rheumatic fever prior to the age of 16. In all cases, at least 40 years had elapsed between the rheumatic fever episode and the diagnosis of mitral stenosis. Cardiac surgery evaluation, as well as the possibility for interventional therapy, had been offered repeatedly, but all patients refused to undergo interventional procedures. Table 1 summarizes the baseline patient characteristics. The mean age of our patient population was 74.8 years. Since we included patients with "fixed", uncorrected mitral valve stenosis secondary to rheumatic heart disease, it was normal that our population consisted of elderly people who had suffered from the infection before the routine use of antibiotics and who lived in areas with poor access to medical services. Pre-treatment evaluation revealed impaired LVEF, elevated PAP, and increased serum proBNP (Table 2). All patients had enlarged left atria and right ventricles and tricuspid regurgitation, six had atrial fibrillation, and one had a patent *foramen ovale*.

Table 1. Enrolment data obtained from ten patients with secondary pulmonary hypertension due to severe mitral valve dysfunction.

Age (years)	74.8 [70.8-78.8]
Sex (male/female)	2/8
Height (m)	1.62 [1.58-1.67]
Body mass (kg)	64.1 [56.7-71-5]
Dyspnea/dizziness/edema/chest pain	10/2/7/3
Prior supplemental oxygen/diuretic/angiotensin-converting enzyme inhibitor/vitamin K antagonist/β-adrenergic blocker/digitalis/aspirin treatment	2/10/6/6/5/7/2
Heart rate (/min)	80.8 [74.6-87.0]
Respiratory rate (/min)	14.8 [14.1-15.5]
SpO_2 (%)	93.7 [91.2-96.2]
Systolic ABP (mmHg)	139.5 [127.8-151.2]
Diastolic ABP (mmHg)	82.5 [74.6-90.5]

Data are given as either mean [95% CI] or as number of observations (n). SpO_2 – hemoglobin pulse oxygen saturation; ABP – arterial blood pressure.

Bosentan treatment was well-tolerated by nine patients. One patient withdrew from treatment within one month of bosentan commencement because of subjective clinical deterioration. She was evaluated and included in the intention-to-treat analysis. All other patients concluded post-treatment evaluation at six months while still receiving bosentan. After six months of treatment, bosentan resulted in a significant increase of 32 [8-57] m in 6MWD, but a significant decrease of 0.44 [0.13-0.76] mL/min/kg in VO_{2max} (Table 2 and Figure 1). Functional status was improved by bosentan treatment, as indicated by the disappearance of dyspnea at rest in four patients, by the steep decrease of 2.3 (1.5-3.1) in BDI at rest and of 1691 [498-2883] U/L in serum proBNP, and by the down-staging of NYHA functional class in six patients (Table 2 and Figure 2). In addition, echocardiographic left ventricular function and pulmonary pressure were also improved after six months of bosentan: LVEF increased by 2.6 [1.1-4.1]%, mean PAP decreased by 7.5 [0.9-14.2] cmH_2O, and peak PAP decreased in all but one patient (Table 2 and Figure 2).

Regarding the safety of bosentan, no life-threatening or serious adverse events were observed in these patients with TTE evidence of PH related to

* http://www.dssresearch.com/toolkit/sscalc/size_a2.asp; last accessed on Jan 25, 2011

Table 2. Summary of bosentan's effects in ten patients with secondary pulmonary hypertension due to severe mitral valve dysfunction.

	Pre-treatment	Post-treatment	p
Somatometric data:			
Body weight (kg)	64.1 [56.7-71.5]	62.7 [55.5-70.0]	0.007*
Body mass index (kg/m^2)	24.2 [22.3-26.1]	23.7 [21.8-25.6]	0.008*
Functional status:			
Dyspnea present	10	6	0.515‡
BORG dyspnea index	3.00 (1.75-4.25)	1.00 (0.00-1.25)	0.002†
NYHA class (IV/IIIb/IIIa/IIb)	4/6/0/0	3/1/5/1	0.021‡
Hemodynamic parameters:			
Ejection fraction (%)	40 [33-47]	43 [35-51]	0.003*
Peak PAP (cmH_2O)	70 [65-76]	60 [48-72]	0.062*
Mean PAP (cmH_2O)	46 [42-50]	39 [32-45]	0.030*
Exercise capacity:			
6MWD (m)	195 [91-299]	227 [116-338]	0.015*
VO_{2max} (mL/min/kg)	4.19 [4.02-4.35]	3.74 [3.40-4.09]	0.011*
Blood tests:			
ProBNP (U/L)	4533 [3055-6011]	2842 [1531-4154]	0.011*
Hemoglobin (g/dL)	13.3 [12.8-13.9]	12.8 [12.2-13.3]	0.0003*
Hematocrit (%)	39.8 [37.6-42.0]	38.1 [35.9-40.3]	0.001*

Data are given as mean [95% CI], median (interquartile range), or number of observations (n). *By paired Student's t-test. †By Mann–Whitney U-test. ‡By Fisher's exact test. NYHA – New York Heart Association; PAP – pulmonary artery pressure; 6MWD – six-minute walking distance; VO_{2max} – maximal oxygen uptake; proBNP – pro-brain natriuretic peptide.

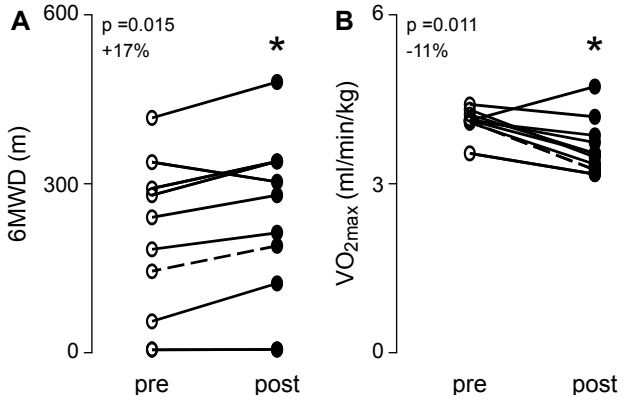

Figure 1. Effects of bosentan treatment on exercise capacity (primary study endpoint). (A) Six-minute walking distance (6MWD). (B) Maximal oxygen uptake (VO_{2max}). Circles, raw data; Lines, mean; boxes, 95% CI; p, probability of no difference pre- and post-treatment; *p<0.05 for comparison pre- and post-treatment. The dashed line indicates the patient who withdrew from treatment after one month, but was included in the intention-to-treat analyses.

rheumatic mitral stenosis. No changes in serum biochemistry were noted, including hepatic and renal function (data not shown). No symptoms or signs indicative of congestive heart failure were evident in the patient who withdrew from treatment and was evaluated prematurely. However, a small but statistically significant loss of body mass, -1.4 [0.5-2.3] kg, was detected post-treatment (Table 2 and Figure 3). In addition, a minor but statistically significant drop in peripheral blood hemoglobin content of 0.58 [0.35-0.81] g/dL was observed (Table 2 and Figure 3).

Discussion

In this study we report the effects of endothelin receptor antagonism in patients with TTE evidence of PH associated with rheumatic mitral stenosis. The rationale for bosentan administration in patients with long-standing rheumatic mitral stenosis was based on the hypothesis that these patients exhibit increased pulmonary artery vasomotor tone and pulmonary vascular remodeling mediated via endothelin signaling. Despite the fact that these patients' pathophysiology had persisted for more than 40 years without intervention, six months of bosentan treatment resulted in favorable changes in 6MWD, mean PAP, LVEF, proBNP, and symptom control, as well as overall functional status. At the same time, the effects of bosentan on maximal oxygen uptake and peak PAP were unfavorable or equivocal. The present report presents the first clinical study of the use of endothelin receptor antagonists in patients with TTE evidence of PH due to left heart valvular disease, and one of the few attempts to apply any anti-PAH medication in this patient group.

Since the discovery of endothelin, the endothelin/endothelin receptor axis has been established as

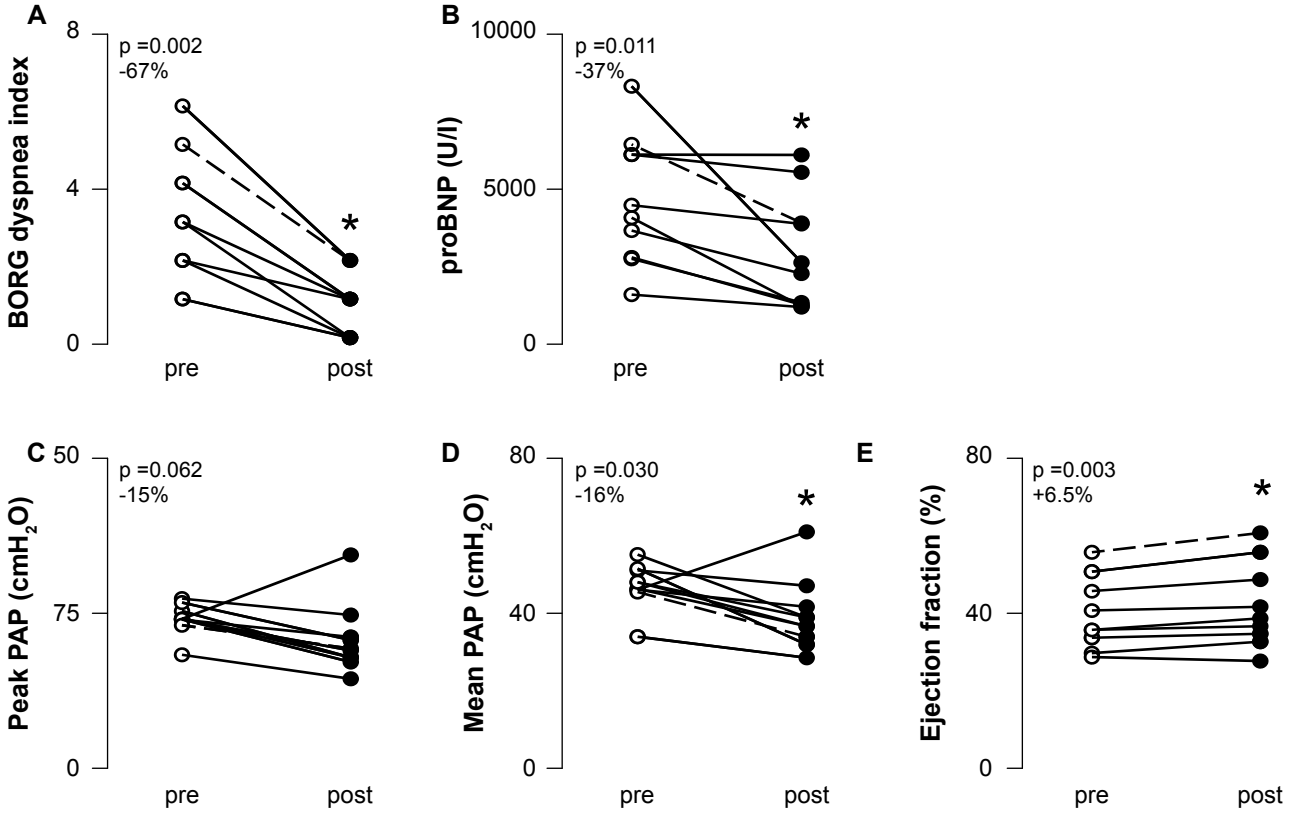

Figure 2. Effects of bosentan treatment on secondary study endpoints. (A) BORG dyspnea index. (B) Serum pro-brain natriuretic peptide (proBNP) levels. (C) Peak pulmonary artery pressure (PAP). (D) Mean PAP. (E) Ejection fraction. Symbols, etc., as in Figure 1.

a central pathway to the pulmonary arterial vasoconstriction and remodeling observed with PAH.[8,13] The endothelin receptor antagonist bosentan has been proven to be safe and effective against several forms of PAH and has been approved for the condition by European and North American authorities.[9,14-18] Although some pathophysiological mechanisms and alterations seen with PAH may apply to other forms of PH, the application of PAH medications, including bosentan, to other types of non-arterial PH still needs to be clinically investigated.[1] In this regard, bosentan has been successfully used in chronic thromboembolic PH, but has failed to prove of any use in PH associated with chronic obstructive pulmonary disease.[19,20] Only one study has addressed the safety and efficacy of bosentan in patients with PH due to left heart disease.[3] The authors concluded that endothelin receptor antagonism is unsafe and ineffective in patients with PH, owing to systolic left ventricular dysfunction.[3] Another study reported on the effects of epoprostenol on PH after mitral valve replacement.[21] However, no newer PAH medication, such as an oral endothelin receptor antagonist, has previously been applied to patients with PH due to left heart valvular disease. Hence, our data add to the very limited literature on endothelin blockade in heart disease-associated PH and we are the first to describe the effects of bosentan on PH related to left heart valvular disease.

Mitral stenosis is a common disease, most frequently resulting from rheumatic fever of streptococcal origin;[2] the related PH represents one of the most common forms of PH.[1] Although mitral stenosis mainly impacts the developing world, an atypical form is increasingly seen in developed countries.[22] In addition, although interventional mitral valve repair or replacement is the treatment of choice, the development of medical therapies is also necessary, since many patients do not have access to surgical or endovascular treatment, because of their poor socioeconomic status, or are unfit for such modalities.[22] A better understanding of the pathophysiology of mitral stenosis-associated PH is needed in order to define

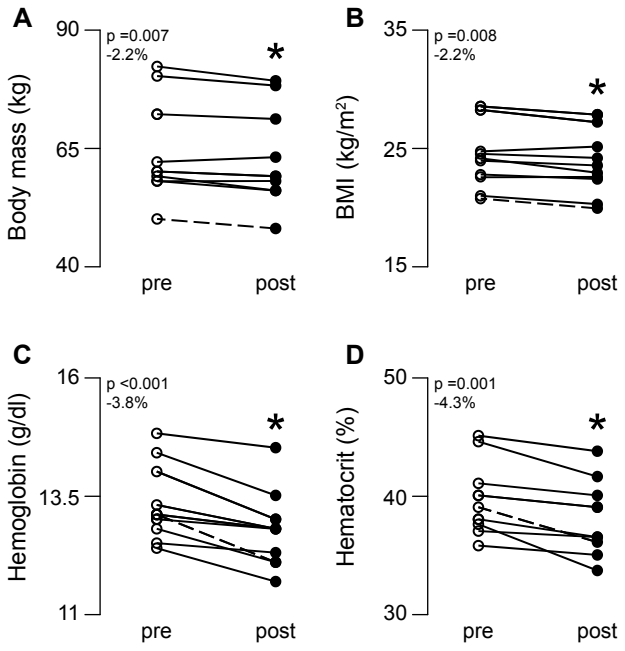

Figure 3. Untoward effects of bosentan. (A) Body mass. (B) Body mass index (BMI). (C) Peripheral blood hemoglobin content. (D) Peripheral blood hematocrit. Symbols, etc., as in Figure 1.

the most suitable medical treatment. Passive backpressure transmission from a stenotic mitral orifice to the pulmonary circulation is essential; but increased vasomotor tone and pulmonary vascular remodeling appear to be involved in the process, presenting a marked therapeutic target for existing PAH pharmacotherapies.[1] Moreover, endothelin signaling is present in mitral stenosis-associated PH, irrespectively of whether the mediator is produced in the pulmonary or the systemic circulation, setting a rational basis for the trial of bosentan or other endothelin receptor antagonists.[10,11]

Our results suggest that these mechanisms are indeed important in patients with TTE evidence of mitral stenosis-related PH: bosentan treatment was able to reduce PAP, to improve LVEF, and to inhibit systemic release of proBNP in ten patients with long-standing ("fixed") mitral stenosis. The data indicate that increased pulmonary vasomotor tone is a reversible, treatable component in mitral valve stenosis pathophysiology. In addition to indices of cardiac function and of pulmonary pressure, bosentan treatment improved the functional status of the patients, as reflected by increased 6MWD (one of the two primary endpoints of our study), attenuated symptoms, and improved NYHA functional class. Although these results indicate efficacy, the other primary endpoint of the present study, maximal oxygen uptake, was not improved, but was significantly reduced by bosentan treatment. These contradictory results most probably reflect an unfortunate or overoptimistic choice of primary endpoints in the present work. Most published randomized controlled trials of PH have used 6MWD as the primary endpoint, since this is the most pertinent and physiologically relevant measure of exercise capacity.[9] Moreover, VO_{2max} more accurately reflects ventilator efficacy rather than exercise capacity, and more closely mirrors physiological ventilatory and circulatory reserve at maximal rather than everyday workload.[23] The discrepant results become even more puzzling since 6MWD and VO_{2max} usually change in a parallel fashion, as in a study of the effects of everyday exercise training of patients with idiopathic PAH and chronic thromboembolic PH.[24] One possible explanation of the observed reduction in VO_{2max} after six months on bosentan treatment is the combined decrease of body mass and hemoglobin content by 2.2% and 3.8%, respectively, which might synergistically lead to a ~10% reduction in VO_{2max}, close to what was observed in the study. Whatever the cause of the discrepant results, we believe that the beneficial effect of bosentan on 6MWD more accurately reflects the post-treatment improvement in functional status. Moreover, our results and the existing literature indicate that exercise capacity determined by the 6MWD presents a superior outcome measurement for PH studies.[9,23]

It is true that most of the patients in NYHA functional class IV remained in class IV. The improvement in NYHA functional class was not included in our primary or secondary endpoints. It seems that ultimately the inclusion of patients in NYHA class IIIB and IV rather weakens our results and that endothelin receptor antagonists should probably be studied in patients with heart failure who belong to earlier NYHA class groups.

The present study suffered from several limitations that do not allow firm conclusions to be drawn. It was conducted in a single tertiary healthcare center with no access to cardiac catheterization facilities. This was a major limitation, since the diagnosis of PH requires confirmation by right-heart catheterization. Thus, we had to rely on Doppler echocardiography for the measurement of pulmonary pressure. The non-blinded study design may have led to bias in patient wellbeing, as well as the physician's inter-

pretation of the findings. The lack of placebo-treated control patients further limits the implications of the data. The non-blinded, uncontrolled study design was dictated by the rarity of patients with uncorrected, long-standing rheumatic mitral stenosis in our region. Hence, to compensate for the lack of controls, we compared the pre- and post-treatment assessment of each patient in a paired manner. We realize, however, that the number of patients is too small to draw definite conclusions. Intention-to-treat analysis was used and secondary outcome measurements were included to help interpret the primary endpoint results.

Despite the limitations, we believe this pilot study contains critical evidence for a beneficial impact of bosentan in patients with TTE evidence of mitral stenosis-associated PH. These results should encourage randomized controlled trials of endothelin receptor antagonists and/or other anti-PAH drugs against non-arterial PH. In addition to the clinical significance of non-arterial PH pharmacotherapy development that has been outlined above, the pathophysiological implications of our results are not to be overlooked. Our data suggest that active vasoconstrictor signals are consistently involved in the pathophysiology of mitral stenosis-related PH and may also operate in other forms of "passive" PH. In addition to endothelin, other vasomotor and endothelial mitogenic pathways may play an important role in non-idiopathic, non-arterial PH and deserve to be elucidated by future research.

In conclusion, our data suggest that oral endothelin receptor antagonism can exert favorable functional, hemodynamic, and physiological effects in patients with long-standing mitral stenosis due to rheumatic fever, opening a discussion on the use of these drugs in this patient subpopulation. Pending randomized controlled trials, bosentan and other anti-PAH-directed therapies may prove to be beneficial for patients with non-arterial PH due to left heart valvular disease.

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