Journal of the American College of Cardiology
© 2003 by the American College of Cardiology Foundation
Published by Elsevier Science Inc.

Vol. 41, No. 8, 2003
ISSN 0735-1097/03/$30.00
doi:10.1016/S0735-1087(03)00121-9

Effects of the Oral Endothelin-Receptor Antagonist Bosentan on Echocardiographic and Doppler Measures in Patients With Pulmonary Arterial Hypertension

Nazzareno Galiè, MD,* Alan L. Hinderliter, MD,† Adam Torbicki, MD,‡ Thierry Fourme, MD,§ Gerald Simonneau, MD,§ Tomas Pulido, MD,‖ Nilda Espinola-Zavaleta, MD,‖ Guido Rocchi, MD,* Alessandra Manes, MD,* Robert Frantz, MD,¶ Marcin Kurzyna, MD,‡ Sherif F. Nagueh, MD,# Robyn Barst, MD,** Richard Channick, MD,†† Karl Dujardin, MD,‡‡ Andrew Kronenberg, MD,† Isabelle Leconte, PHD,§§ Maurizio Rainisio, PHD,§§ Lewis Rubin, MD††

Bologna, Italy; Chapel Hill, North Carolina; Warsaw, Poland; Paris, France; Mexico City, Mexico; Rochester, Minnesota; Houston, Texas; New York, New York; San Diego, California; Leuven, Belgium; and Allschwil, Switzerland

OBJECTIVES The purpose of this study was to investigate the effects of bosentan (125 or 250 mg twice daily) on echocardiographic and Doppler variables in 85 patients with World Health Organization class III or IV pulmonary arterial hypertension (PAH).

BACKGROUND Bosentan, an orally active dual endothelin-receptor antagonist, improves symptoms, exercise capacity, and hemodynamics in patients with PAH.

METHODS Patients had primary pulmonary hypertension (84%) or PAH associated with connective tissue disease. Of these, 29 patients received placebo and 56 received bosentan (1:2 randomization). Six-minute walk tests and echocardiograms were performed at baseline and after 16 weeks of treatment.

RESULTS Baseline characteristics were similar in the placebo and bosentan groups, and echocardiographic and Doppler findings were consistent with marked abnormalities of right ventricular (RV) and left ventricular (LV) structure and function that were due to PAH. The treatment effect on 6-min walking distance was 37 m in favor of bosentan ($p = 0.036$). Treatment effects of bosentan compared with placebo on other parameters were as follows: Doppler-derived cardiac index = +0.4 l/min/m^2 ($p = 0.007$), LV early diastolic filling velocity = +10.5 cm/s ($p = 0.003$), LV end-diastolic area = +4.2 cm^2 ($p = 0.003$), LV systolic eccentricity index = −0.12 ($p = 0.047$), RV end-systolic area = −2.3 cm^2 ($p = 0.057$), RV:LV diastolic areas ratio = −0.64 ($p = 0.007$), Doppler RV index = −0.06 ($p = 0.03$), and percentage of patients with an improvement in pericardial effusion score = 17% ($p = 0.05$).

CONCLUSIONS Bosentan improves RV systolic function and LV early diastolic filling and leads to a decrease in RV dilation and an increase in LV size in patients with PAH. (J Am Coll Cardiol 2003; 41:1380–6) © 2003 by the American College of Cardiology Foundation

Pulmonary arterial hypertension (PAH) is defined, according to the World Health Organization (WHO) classification (1), as a group of diseases characterized by a progressive increase of pulmonary vascular resistance leading to right ventricle (RV) failure and death (2). Pulmonary arterial hypertension includes primary pulmonary hypertension (PPH) (2) and pulmonary hypertension associated with various conditions such as connective tissue disease (3). Recently, the orally active dual endothelin-receptor antagonist bosentan was reported to improve exercise capacity and cardiopulmonary hemodynamics in patients with PAH (4). In addition, bosentan improved exercise capacity and decreased the risk of clinical worsening in the Bosentan Randomized trial of Endothelin Antagonist Therapy for pulmonary hypertension (BREATHE-1), a randomized, placebo-controlled trial that included 213 patients with WHO class III or IV PAH (5).

Disease severity and the effects of treatment in patients with PAH can be assessed noninvasively by measuring exercise capacity (6,7). However, echocardiographic and Doppler evaluation of these subjects is also useful, not only for diagnostic purposes but also to assess the magnitude of the pathophysiologic adaptations of the heart and pulmonary circulation (8,9). In addition, echocardiography is effective in detecting changes in cardiac structure and function associated with medical and surgical treatments (10–16).

The objective of the current study was to investigate the

From the *Institute of Cardiology, University of Bologna, Italy; †Department of Medicine, University of North Carolina, Chapel Hill, North Carolina; ‡Department of Chest Medicine, Institute of Tuberculosis and Lung Disease, Warsaw, Poland; §Division of Pulmonary and Critical Care Medicine, University of Paris-Sud, Paris, France; ‖Instituto Nacional de Cardiologia, Mexico City, Mexico; ¶Department of Cardiology, Mayo Clinic, Rochester, Minnesota; #Baylor College of Medicine and the Methodist Hospital, Houston, Texas; **Department of Pediatrics, Columbia University College of Physicians and Surgeons, New York, New York; ††Division of Pulmonary and Critical Care Medicine, University of California, San Diego, California; ‡‡Department of Cardiology, Gasthuisberg University Hospital, Leuven, Belgium; §§Actelion Ltd., Allschwil, Switzerland. This study was supported by Actelion Pharmaceuticals Ltd., Allschwil, Switzerland.

Manuscript received August 11, 2002; revised manuscript received December 20, 2002, accepted January 2, 2003.

Abbreviations and Acronyms

BREATHE-1 = Bosentan Randomized trial of Endothelin Antagonist Therapy for pulmonary hypertension
LV = left ventricle/ventricular
PAH = pulmonary arterial hypertension
PPH = primary pulmonary hypertension
RV = right ventricle/ventricular
WHO = World Health Organization

effects of bosentan on echocardiographic and Doppler measures in a subgroup of patients with PAH enrolled in the BREATHE-1 study.

METHODS

Patients. Of the 213 patients enrolled in the BREATHE-1 study, 85 were included in the echocardiographic substudy that was performed in 13 out of 27 centers. The centers included in the echocardiographic substudy volunteered to participate. Detailed inclusion and exclusion criteria have been described previously (5). Briefly, enrolled patients were in WHO functional classes (1) III or IV with either PPH or PAH associated with connective tissue disease. The study was conducted according to the Helsinki Declaration of 1975, as revised in 1983, and local good clinical practice guidelines. Written, informed consent was obtained from all patients.

Study protocol. Details of the BREATHE-1 study protocol have been described in a previous publication (5). Upon local ethics review committee approval at each specific site, all patients randomized in the BREATHE-1 study were also enrolled in the echocardiographic substudy. The 85 patients were randomized to receive either bosentan 62.5 mg b.i.d. for four weeks followed by the target dose (125 mg b.i.d. or 250 mg b.i.d.) or matching doses of placebo. A baseline echocardiogram was performed before randomization; according to the BREATHE-1 study protocol, exercise capacity was assessed by the 6-min walk test (6). Repeat evaluations were performed after 16 weeks of therapy.

Imaging protocol. Two-dimensional and Doppler ultrasound examinations were performed with a defined imaging protocol. Recordings were analyzed with the use of an offline quantification system. Measurements were performed at the core echocardiography laboratory by experienced observers who were unaware of each patient's clinical history or treatment assignment and whether the examination was at baseline or following blind treatment. Measurements were made on three representative beats and the results were averaged.

Echocardiographic variables. The following echocardiographic variables were analyzed.

- The RV and left ventricle (LV) end-diastolic and end-systolic areas were measured in the apical four-chamber view by tracing the endocardial edges and the plane of the atrioventricular valves at end-diastole and end-systole (17).
- The ratio of the ventricular areas (RV to LV areas ratio) was calculated as the ratio of the RV end-diastolic and LV end-diastolic areas.
- The RV percent change in area (10,18) was calculated from the RV end-diastolic and end-systolic areas as: RV percent change in area = 100 × (RV end-diastolic area − RV end-systolic area)/RV end-diastolic area.
- The LV eccentricity index was measured at end-diastole and end-systole from the parasternal short-axis views of the LV at the level of the chordae tendineae. This was calculated by the method of Ryan et al. (19) as: LV index = D2/D1, where D2 is the minor-axis dimension parallel to the septum and D1 is the minor-axis diameter perpendicular to and bisecting the septum.
- The minimum diameter of the inferior vena cava during respiration was measured from subcostal images.
- The pericardial effusion size was determined from parasternal long-axis and short-axis views. Effusions were graded and assigned a score as follows (10): absent (score = 0), trace (score = 1; separation of pericardial layers in both systole and diastole), small (score = 2; diastolic separation <1 cm), moderate (score = 3; diastolic separation of 1 to 2 cm), or large (score = 4; diastolic separation >2 cm).

Doppler variables. The following Doppler variables were analyzed.

- Right ventricle acceleration time was measured from the pulsed-wave Doppler flow velocity profile of the RV outflow tract and defined as the interval from the onset to the maximal velocity of forward flow.
- Right ventricle ejection time was measured from the RV outflow pulsed-wave Doppler signal as the interval from the onset of forward flow to pulmonic valve closure.
- Doppler RV index, a global measure of RV function, was calculated as described by Tei (20). This index is defined as: (isovolumetric contraction time + isovolumetric relaxation time)/RV ejection time. The tricuspid closing to opening time, that is, the sum of isovolumetric contraction, isovolumetric relaxation, and ejection times, was measured as the duration of the continuous-wave tricuspid regurgitant signal. Because the sum of the isovolumetric times equals the tricuspid closing to opening time minus RV ejection time, the Doppler RV index was calculated as (tricuspid closing to opening time minus RV ejection time)/RV ejection time.
- The maximal tricuspid regurgitant jet velocity was measured by determining the peak regurgitant velocity in the continuous-wave Doppler flow profile obtained from the cardiac apex.
- Cardiac output was determined from pulsed-wave measurements of the LV outflow tract velocity profile (21). The diameter of the LV outflow tract was measured at the

Table 1. Baseline Demographic, Clinical, Hemodynamic, and Exercise Characteristics by Treatment Group

Characteristics	Placebo (N = 29)	Bosentan Groups Combined (N = 56)	125 mg of Bosentan (N = 29)	250 mg of Bosentan (N = 27)
Age, yrs	44.9 ± 19.2	45.1 ± 15.6	48.8 ± 16.2	41.1 ± 14.3
Gender, no. (%)				
Men	5 (17.2)	8 (14.3)	5 (17.2)	3 (11.1)
Women	24 (82.8)	48 (85.7)	24 (82.8)	24 (88.9)
Etiology of PAH, no. (%)				
Primary	23 (79.3)	48 (85.7)	27 (93.1)	21 (77.9)
Associated with scleroderma	4 (13.8)	7 (12.5)	2 (6.9)	5 (18.5)
Other	2 (6.9)	1 (1.8)	–	1 (3.7)
WHO functional class, no. (%)*				
III	28 (96.6)	49 (87.5)	25 (86.2)	24 (88.9)
IV	1 (3.4)	7 (12.5)	4 (13.8)	3 (11.1)
6-min walk distance, m	334 ± 76	335 ± 83	319 ± 87	352 ± 77
Hemodynamic parameters				
Mean right atrial pressure, mm Hg	10 ± 5	10 ± 6	11 ± 6	9 ± 6
Mean pulmonary artery pressure, mm Hg	58 ± 19	56 ± 15	56 ± 12	57 ± 17
Cardiac index, l/min/m^2	2.3 ± 0.7	2.4 ± 0.8	2.2 ± 0.6	2.5 ± 0.9

Values with a plus/minus symbol are the mean ± SD
PAH = pulmonary arterial hypertension; WHO = World Health Organization.

aortic annulus from inner edge to inner edge, utilizing zoom images from the parasternal long-axis view. The cross-sectional area of the outflow tract (CSA_{ot}) was calculated as $3.14r^2$, assuming a circular shape. Outflow tract time velocity integral (TVI_{ot}) was determined by digitizing the Doppler signals, tracing the black/white interface. Stroke volume was then calculated as $CSA_{ot} \times TVI_{ot}$ and was multiplied by heart rate to obtain cardiac output. Cardiac output was divided by body surface area to obtain cardiac index.

- Left ventricle filling was assessed from pulsed-wave Doppler mitral inflow signals. Computerized quantification included measurements of mitral E- and A-wave velocities, and the time-velocity integral.

Statistical analysis. The null hypothesis of the study was that there is no difference between the combined bosentan arms and placebo in the distributions of the change from baseline in echocardiographic and Doppler parameters. The LV eccentricity indexes were considered the primary parameters because of favorable changes shown in a previous pharmacologic trial in patients with PPH (10). As a consequence the sample size of the study was based on these changes. The dose-response for efficacy was analyzed descriptively. The changes from baseline to treatment values of each echocardiographic and Doppler parameter were calculated for individual patients in whom technically adequate studies were available at baseline and at 16 weeks. Two patients in the placebo group and one patient in the bosentan group discontinued study medication because of clinical worsening or death and were analyzed using the worst rank value. Statistical analyses were based on the intent to treat population (full analysis set). Baseline values and mean changes at week 16 of 6-min walking distance and echocardiographic and Doppler parameters of the placebo and combined bosentan arms were compared by the Mann-Whitney *U* test (two-sided). A p value of <0.05 was considered significant. The parameters were analyzed exploratorily and corrections for multiple comparisons were not performed.

Multiple stepwise regression analysis was performed to identify among the echocardiographic and Doppler parameters those that better predict the changes in 6-min walking distance.

RESULTS

Of the 85 patients included in the study, 56 received bosentan (29 received 125 mg b.i.d. and 27 received 250 mg b.i.d.) and 29 received placebo.

The placebo and bosentan groups were well matched with respect to baseline demographic and clinical characteristics, exercise capacity, and hemodynamic measurements (Table 1). Baseline echocardiographic and Doppler values for the placebo and bosentan groups are shown in Tables 2 and 3, respectively. There were no statistically significant differences between the groups in any of these parameters. In comparison with previously published echocardiographic data in normal subjects (10,22), the patients with PAH in the present study had severe dilation and hypokinesis of the right ventricle, reduced size of the left ventricle and marked septal displacement in both diastole and systole. Similarly, our patients with PAH (23–25) had shorter RV acceleration and ejection times, lower stroke volume, higher maximal tricuspid regurgitant velocity, and greater Doppler RV index than observed in normal subjects (20).

In the patients enrolled in the echocardiographic substudy, bosentan improved the distance walked in 6 min and the mean difference between treatment groups was 37 m in

Table 2. Baseline Echocardiographic Variables of Patients by Treatment Group

Variables	Placebo		Bosentan Groups Combined		125 mg of Bosentan		250 mg of Bosentan	
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
RV end-diastolic area, cm^2	24	27 ± 8	44	30 ± 11	21	32 ± 11	23	28 ± 11
RV end-systolic area, cm^2	24	22 ± 8	42	25 ± 10	21	26 ± 10	21	24 ± 9
RV percent change in area, %	24	18 ± 11	42	18 ± 11	21	21 ± 13	21	15 ± 8
LV end-diastolic area, cm^2	19	16 ± 7	37	14 ± 6	18	13 ± 5	19	16 ± 6
LV end-systolic area, cm^2	18	11 ± 6	37	10 ± 4	18	9 ± 4	19	11 ± 5
LV systolic eccentricity index	27	1.86 ± 1.35	52	1.65 ± 0.38	27	1.67 ± 0.37	25	1.64 ± 0.40
LV diastolic eccentricity index	28	1.82 ± 1.03	52	1.75 ± 0.45	27	1.83 ± 0.47	25	1.66 ± 0.43
RV:LV diastolic areas ratio	19	2.22 ± 1.54	37	2.44 ± 1.48	18	2.84 ± 1.39	19	2.06 ± 1.50
IVC minimum diameter, cm	19	1.29 ± 0.54	42	1.45 ± 0.54	23	1.51 ± 0.62	19	1.38 ± 0.43
Pericardial effusion score, %*	29	45*	56	45*	29	48*	27	41*

*% of patients with any degree of pericardial effusion.
IVC = inferior vena cava; LV = left ventricle; RV = right ventricle.

favor of bosentan (95% confidence interval: 0.5 to 72.7 m, p = 0.036).

A comparison of changes in echocardiographic and Doppler variables after 16 weeks of treatment in the bosentan and placebo groups is shown in Tables 4 and 5. Patients in the placebo group tended to have a greater increase in RV area and a greater worsening of RV percent area change than those treated with bosentan. Therapy with bosentan was associated with an increase of LV areas and with improvement of LV systolic eccentricity index and RV to LV diastolic areas ratio (Fig. 1). The placebo group had a greater increase of inferior vena cava minimum diameter and a smaller percentage of patients with an improvement in the pericardial effusion score. Treatment with bosentan increased RV ejection time, stroke volume, and cardiac index (Fig. 2), and was associated with an improvement in Doppler RV index (Fig. 3) and of parameters related to early diastolic LV filling (peak E velocity, E to A ratio, and time-velocity integral of the transmitral flow profile). There was a trend towards a reduction of the maximal tricuspid regurgitant velocity in patients treated with bosentan, although the difference from the placebo group did not reach statistical significance.

Both bosentan dosages induced beneficial treatment effects and no consistent dose response for efficacy could be ascertained. (Tables 4 and 5; Figs. 1, 2, and 3). Multiple stepwise regression analysis showed that the changes in 6-min walking distance correlated with changes in peak E mitral flow velocity (r = 0.65, p = 0.001) and pericardial effusion score (r = −0.43, p = 0.004).

DISCUSSION

The present study is the first double-blind, randomized, placebo-controlled trial to evaluate the effects of a medical treatment on echocardiographic and Doppler variables in patients with PAH. The administration of the orally active dual endothelin-receptor antagonist bosentan for 16 weeks resulted in an increase in exercise capacity and an improvement in echocardiographic and Doppler parameters associated with RV and LV structure and function. Compared with patients randomized to placebo, patients treated with bosentan had a decrease in RV dilation; increases in LV size, stroke volume, and cardiac index; and improvements in RV ejection and LV early diastolic filling characteristics. Bosentan also had beneficial effects on inferior vena cava diameter and pericardial effusion size.

The increase in cardiac index in patients treated with bosentan may be explained by a decrease in RV afterload due to the effects of the drug on pulmonary vascular

Table 3. Baseline Doppler Variables of Patients by Treatment Group

Variables	Placebo		Bosentan Groups Combined		125 mg of Bosentan		250 mg of Bosentan	
	n	Mean ± SD	n	Mean ± SD	N	Mean ± SD	n	Mean ± SD
RV acceleration time, ms	27	76 ± 21	50	75 ± 24	25	78 ± 30	25	72 ± 17
RV ejection time, ms	27	283 ± 40	50	291 ± 42	25	298 ± 39	25	283 ± 44
Doppler RV index	26	0.50 ± 0.24	45	0.54 ± 0.19	22	0.50 ± 0.17	23	0.58 ± 0.21
Maximal TV regurgitant velocity, cm/s	25	438 ± 70	46	427 ± 57	23	431 ± 54	23	423 ± 60
LV stroke volume, ml	26	50 ± 19	55	52 ± 18	28	49 ± 17	27	55 ± 18
Heart rate, beats/min	27	86 ± 14	55	81 ± 13	28	80 ± 12	27	82 ± 14
Cardiac index, $l/min/m^2$	26	2.51 ± 0.90	55	2.47 ± 0.83	28	2.31 ± 0.69	27	2.64 ± 0.94
Doppler MV peak E velocity, cm/s	24	49 ± 16	53	53 ± 20	27	54 ± 22	26	52 ± 19
Doppler MV E/A ratio	24	0.73 ± 0.25	53	0.81 ± 0.33	27	0.82 ± 0.39	26	0.80 ± 0.25
Doppler MV time-velocity integral, cm	24	12.3 ± 2.7	53	13.0 ± 4.0	27	13.2 ± 4.3	26	12.75 ± 3.7

LV = left ventricle; MV = mitral valve; RV = right ventricle; TV = tricuspid valve.

Table 4. Changes From Baseline in Echocardiographic Variables by Treatment Group and Treatment Effect

	Placebo		Bosentan Groups Combined		Treatment Effect		125 mg of Bosentan		Treatment Effect		250 mg of Bosentan		Treatment Effect	
Variables	**N**	**C ± SEM***	**n**	**C ± SEM***	**Mean**	**p†**	**n**	**C ± SEM***	**Mean**	**p†**	**n**	**C ± SEM***	**Mean**	**p†**
RV end-diastolic area, cm^2	24	2.30 ± 0.99	44	0.49 ± 0.85	−1.81	0.122	21	−0.22 ± 1.18	−2.51	0.07	23	1.13 ± 1.23	−1.16	0.399
RV end-systolic area, cm^2	24	3.29 ± 0.97	42	0.97 ± 0.74	−2.32	0.057	21	0.82 ± 1.04	−2.47	0.064	21	1.12 ± 1.07	−2.17	0.168
RV percent change in area, %	24	−6.70 ± 1.77	42	−1.86 ± 1.69	4.84	0.094	21	−4.36 ± 2.05	2.34	0.424	21	0.64 ± 2.61	7.34	0.043
LV end-diastolic area, cm^2	19	−2.95 ± 0.98	37	1.24 ± 0.69	4.19	0.003	18	0.50 ± 1.02	3.45	0.026	19	1.94 ± 0.93	4.89	0.004
LV end-systolic area, cm^2	18	−2.13 ± 0.77	37	0.56 ± 0.64	2.70	0.016	18	−0.41 ± 0.82	1.73	0.187	19	1.48 ± 0.94	3.61	0.006
LV systolic eccentricity index	27	0.09 ± 0.16	52	−0.03 ± 0.05	−0.12	0.047	27	0.03 ± 0.07	−0.05	0.228	25	−0.10 ± 0.05	−0.19	0.027
LV diastolic eccentricity index	28	0.00 ± 0.08	52	−0.06 ± 0.04	−0.07	0.174	27	−0.09 ± 0.06	−0.09	0.142	25	−0.03 ± 0.04	−0.04	0.406
RV:LV diastolic areas ratio	19	0.40 ± 0.16	37	−0.24 ± 0.13	−0.64	0.007	18	−0.29 ± 0.21	−0.69	0.015	19	−0.20 ± 0.16	−0.60	0.027
IVC minimum diameter, cm	19	0.34 ± 0.10	42	0.12 ± 0.08	−0.22	0.033	23	0.13 ± 0.10	−0.21	0.130	19	0.11 ± 0.11	−0.23	0.027
Pericardial effusion score, %‡	29	28‡	56	11‡	−17‡	0.053	29	10‡	−17‡	0.159	27	11‡	−17‡	0.069

*C ± SEM denotes mean change ± standard error of the mean. †p values are for the comparison with the placebo group. ‡percent of patients with improvement of at least one degree of pericardial effusion score.
IVC = inferior vena cava; LV = left ventricle; RV = right ventricle

Table 5. Changes From Baseline in Doppler Variables by Treatment Group and Treatment Effect

	Placebo		Bosentan Groups Combined		Treatment Effect		125 mg of Bosentan		Treatment Effect		250 mg of Bosentan		Treatment Effect	
Variables	**n**	**C ± SEM***	**n**	**C ± SEM***	**Mean**	**p†**	**n**	**C ± SEM***	**Mean**	**p†**	**n**	**C ± SEM***	**Mean**	**p†**
RV acceleration time, ms	27	−2.41 ± 3.40	50	3.46 ± 2.63	5.87	0.169	25	−0.56 ± 3.21	1.85	0.59	25	7.48 ± 4.08	9.89	0.073
RV ejection time, ms	27	−3.81 ± 5.72	50	18.34 ± 4.03	22.15	0.007	25	14.8 ± 5.48	17.89	0.052	25	22.6 ± 5.90	26.41	0.007
Doppler RV index	26	−0.01 ± 0.03	45	−0.08 ± 0.02	−0.06	0.034	22	−0.06 ± 0.02	−0.05	0.075	23	−0.09 ± 0.03	−0.07	0.070
Maximal TV regurgitant velocity, cm/s	25	6.40 ± 6.45	46	−4.50 ± 5.50	−10.90	0.280	23	−8.48 ± 8.35	−14.88	0.155	23	−0.52 ± 7.27	−6.92	0.681
LV stroke volume, ml	26	0.06 ± 2.04	55	7.43 ± 1.45	7.37	0.007	28	5.94 ± 2.14	5.88	0.071	55	8.97 ± 1.96	8.91	0.005
Heart rate, beats/min	27	−2.11 ± 2.82	55	−4.42 ± 1.45	−2.31	0.118	28	−2.32 ± 1.46	−0.21	0.274	27	−6.59 ± 2.49	−4.48	0.109
Cardiac index, l/min/m^2	26	−0.18 ± 0.11	55	0.19 ± 0.07	0.37	0.007	28	0.18 ± 0.09	0.36	0.020	27	0.20 ± 0.12	0.38	0.021
Doppler MV peak E velocity, cm/s	24	−2.54 ± 2.82	53	7.91 ± 2.40	10.45	0.003	27	3.74 ± 3.49	6.28	0.062	26	12.23 ± 3.13	14.77	0.001
Doppler MV E/A ratio	24	−0.03 ± 0.05	53	0.15 ± 0.04	0.18	0.004	27	0.11 ± 0.06	0.15	0.024	26	0.18 ± 0.05	0.21	0.006
Doppler MV time-velocity integral, cm	24	−0.27 ± 0.48	53	2.07 ± 0.40	2.34	0.001	27	1.15 ± 0.52	1.42	0.054	26	3.02 ± 0.56	3.29	0.000

*C ± SEM denotes mean change ± standard error of the mean. †p values are for the comparison with the placebo group.
MV = mitral valve; LV = left ventricle; RV = right ventricle; TV = tricuspid valve.

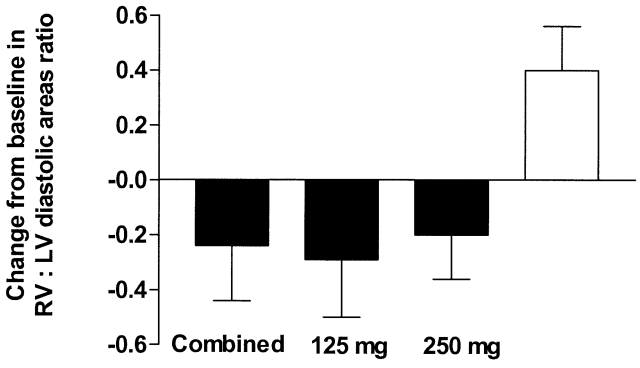

Figure 1. Change and standard error of the mean from baseline to week 16 in right ventricle to left ventricle (RV:LV) diastolic areas ratio in bosentan- and placebo-treated patients. The treatment effect was statistically significant for combined dose group ($p = 0.007$) and for 125 mg ($p = 0.015$) and 250 mg dose subgroups ($p = 0.026$). **Black bar** = bosentan; **white bar** = placebo.

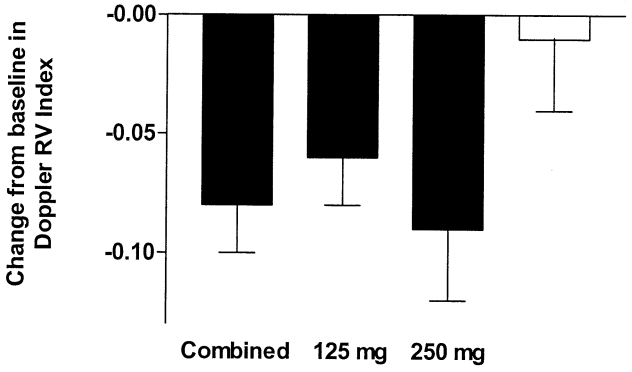

Figure 3. Change and standard error of the mean from baseline to week 16 in Doppler right ventricle (RV) index in bosentan- and placebo-treated patients. The treatment effect was statistically significant for the combined treatment group ($p = 0.034$) and of borderline statistical significance for both 125 mg ($p = 0.074$) and 250 mg ($p = 0.070$) dose subgroups. **Black bar** = bosentan; **white bar** = placebo.

resistance, as observed by invasive hemodynamic measurements performed in previous studies (4).

Treatment with bosentan prevented further enlargement of the RV and reduction of RV percent change in area as compared with placebo. A similar trend was observed in a randomized, unblinded study assessing the effects of epoprostenol (10). These data suggest that the reduction of RV afterload in actively treated patients is sufficient to prevent additional RV dilation and deterioration of RV function, but may not be large enough to cause an improvement in these parameters.

The increase of RV acceleration and ejection times in the bosentan group and their shortening in patients randomized to placebo probably represent the effects of opposite changes in afterload on RV ejection. It is difficult to assess whether the improvement in the Doppler RV index in the bosentan group was due to prolongation of RV ejection time caused by decreased RV afterload or to improved intrinsic RV performance, or both. The Doppler RV index is influenced by both systolic and diastolic performance of RV (23), and is independently correlated with survival in patients with PPH. An improvement of the Doppler RV index has been

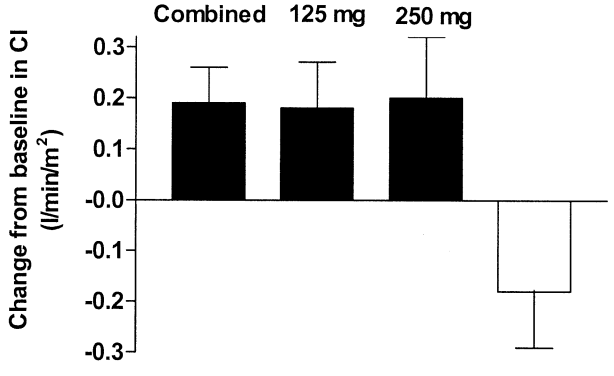

Figure 2. Change and standard error of the mean from baseline to week 16 in Doppler-derived cardiac index (CI) in bosentan- and placebo-treated patients. The treatment effect was statistically significant for combined dose group ($p = 0.007$) and for 125 mg ($p = 0.019$) and 250 mg dose subgroups ($p = 0.020$). **Black bar** = bosentan; **white bar** = placebo.

observed also after substantial reductions of RV afterload such as those obtained by successful pulmonary thromboendoarterectomy (15).

The treatment effect observed on maximal tricuspid regurgitant velocity, a parameter related to pulmonary artery systolic pressure, was not statistically significant. It is possible that the reduction in pulmonary arterial systolic pressure was minimized by the increase in stroke volume and cardiac index.

The smaller increase of inferior vena cava minimum diameter and the reduction of pericardial effusion score in a greater proportion of patients treated with bosentan probably reflect a favorable effect on the right atrial pressure (26). Previous studies suggests that pericardial effusion score is a predictor of adverse outcomes and mortality in patients with PPH (22,26).

The increase in LV dimensions in the bosentan group may be explained by an increase of LV diastolic filling due to increased RV output, and/or to a decrease in the septal displacement toward the LV. The first mechanism is supported by the increase of peak E velocity, E to A ratio, and time-velocity integral of the mitral Doppler flow profile in the bosentan group. Interestingly, an increase of diastolic mitral flow indices was also observed after successful thromboendoarterectomy in patients with chronic thromboembolic pulmonary hypertension (27,28). However, we also observed a trend toward less septal displacement, as reflected by a decrease in the eccentricity indexes, in bosentan-treated patients. The improvement in the RV to LV diastolic areas ratio observed in the bosentan group has also been observed following interventions that significantly reduce RV afterload, such as pulmonary thromboendoarterectomy (13) or lung transplantation (12,18).

The correlation between 6-min walking distance changes and peak E mitral flow velocity and pericardial effusion score changes observed in this study may be explained by the influence of RV pump function determinants such as cardiac output and right atrial pressure on these simple echocardio-

graphic parameters. In fact, diastolic mitral flow indices are correlated with cardiac output and RV afterload changes in patients with precapillary pulmonary hypertension (28). In addition, pericardial effusion score was correlated with exercise capacity as well as with right atrial pressure in another series of patients with PPH (26).

Conclusions. Our study demonstrates that a 16-week treatment with the orally active dual endothelin-receptor antagonist bosentan improves echocardiographic and Doppler parameters in patients with PAH. Bosentan therapy improves RV systolic function and LV early diastolic filling and leads to a decrease in RV dilation and an increase in LV size. In addition, bosentan favorably influences parameters that predict survival in patients with PAH, such as Doppler RV index and pericardial effusion size. This study supports a significant role for bosentan in decreasing the rate of disease progression in patients with PAH.

Reprint requests and correspondence: Dr. Nazzareno Galiè, Istituto di Cardiologia, Università di Bologna, via Massarenti, 9, 40138-Bologna, Italy. E-mail: n.galie@bo.nettuno.it.

REFERENCES

1. Nomenclature Committee. Nomenclature and Classification of Pulmonary Hypertension. Rich S. Primary pulmonary hypertension: executive summary from the World Symposium-Primary Pulmonary Hypertension 1998, 25–27. World Health Organization. Available at: http://www.who.int/ncd/cvd/pph.html.
2. Rubin LJ. Primary pulmonary hypertension. N Engl J Med 1997;336: 111–7.
3. MacGregor AJ, Canavan R, Knight C, et al. Pulmonary hypertension in systemic sclerosis: risk factors for progression and consequences for survival. Rheumatology (Oxford) 2001;40:453–9.
4. Channick RN, Simonneau G, Sitbon O, et al. Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomised placebo-controlled study. Lancet 2001; 358:1119–23.
5. Rubin LJ, Badesch DB, Barst RJ, et al. Bosentan therapy for pulmonary arterial hypertension. N Engl J Med 2002;346:896–903.
6. Guyatt GH, Sullivan MJ, Thompson PJ, et al. The 6-minute walk: a new measure of exercise capacity in patients with chronic failure. Can Med Assoc J 1985;132:919–23.
7. Miyamoto S, Nagaya N, Satoh T, et al. Clinical correlates and prognostic significance of six-minute walk test in patients with primary pulmonary hypertension. Comparison with cardiopulmonary exercise testing. Am J Respir Crit Care Med 2000;161:487–92.
8. Naeije R, Torbicki A. More on the noninvasive diagnosis of pulmonary hypertension: Doppler echocardography revisited. Eur Respir J 1995;8:1449.
9. Bossone E, Duong Wagner TH, Paciocco G, et al. Echocardiographic features of primary pulmonary hypertension. J Am Soc Echocardiogr 1999;12:655–62.
10. Hinderliter AL, Willis PW, Barst RJ, et al. Effects of long-term infusion of prostacyclin (epoprostenol) on echocardiographic measures of right ventricular structure and function in primary pulmonary hypertension. Primary Pulmonary Hypertension Study Group. Circulation 1997;95:1479–86.
11. Ritchie M, Waggoner AD, Dávila RV, Barzilai B, Trulock EP, Eisenberg PR. Echocardiographic characterization of the improvement in right ventricular function in patients with severe pulmonary hypertension after single-lung transplantation. J Am Coll Cardiol 1993;22:1170–4.
12. Xie GY, Lin CS, Preston HM, et al. Assessment of left ventricular diastolic function after single lung transplantation in patients with severe pulmonary hypertension. Chest 1998;114:477–81.
13. Menzel T, Wagner S, Kramm T, et al. Pathophysiology of impaired right and left ventricular function in chronic embolic pulmonary hypertension: changes after pulmonary thromboendarterectomy. Chest 2000;118:897–903.
14. Menzel T, Kramm T, Bruckner A, Mohr-Kahaly S, Mayer E, Meyer J. Quantitative assessment of right ventricular volumes in severe chronic thromboembolic pulmonary hypertension using transthoracic three-dimensional echocardiography: changes due to pulmonary thromboendarterectomy. Eur J Echocardiogr 2002;3:67–72.
15. Menzel T, Kramm T, Mohr-Kahaly S, Mayer E, Oelert H, Meyer J. Assessment of cardiac performance using Tei indices in patients undergoing pulmonary thromboendarterectomy. Ann Thorac Surg 2002;73:762–6.
16. Menzel T, Kramm T, Wagner S, Mohr-Kahaly S, Mayer E, Meyer J. Improvement of tricuspid regurgitation after pulmonary thromboendarterectomy. Ann Thorac Surg 2002;73:756–61.
17. Kaul S, Tei C, Hopkins J, Shaw P. Assessment of right ventricular function using two-dimensional echocardiography. Am Heart J 1984; 107:526–31.
18. Ritchie M, Waggoner AD, Dávila RV, Barzilai B, Trulock EP, Eisenberg PR. Echocardiographic characterization of the improvement in right ventricular function in patients with severe pulmonary hypertension after single-lung transplantation. J Am Coll Cardiol 1993;22:1170–4.
19. Ryan T, Petrovic O, Dillon JC, Feigenbaum HF, Conley MJ, Armstrong W. An echocardiographic index for separation of right ventricular volume and pressure overload. J Am Coll Cardiol 1985;5: 918–24.
20. Yeo TC, Dujardin KS, Tei C, Mahoney DW, McGoon MD, Seward JB. Value of a Doppler-derived index combining systolic and diastolic time intervals in predicting outcome in primary pulmonary hypertension. Am J Cardiol 1998;81:1157–61.
21. Marshall SA, Weyman AE. Doppler estimation of volumetric flow. In: Weyman AE, editor. Principle and Practice of Echocardiography. Philadelphia, PA: Lea and Febiger, 1994:978–95.
22. Raymond RJ, Hinderliter AL, Willis PW, et al. Echocardiographic predictors of adverse outcomes in primary pulmonary hypertension. J Am Coll Cardiol 2002;39:1214–9.
23. Tei C, Dujardin KS, Hodge DO, et al. Doppler echocardiographic index for assessment of global right ventricular function. J Am Soc Echocardiogr 1996;9:838–47.
24. Bossone E, Rubenfire M, Bach DS, Ricciardi M, Armstrong WF. Range of tricuspid regurgitation velocity at rest and during exercise in normal adult men: implications for the diagnosis of pulmonary hypertension. J Am Coll Cardiol 1999;33:1662–6.
25. Bossone E, Avelar E, Bach DS, Gillespie B, Rubenfire M, Armstrong WF. Diagnostic value of resting tricuspid regurgitation velocity and right ventricular ejection flow parameters for the detection of exercise induced pulmonary arterial hypertension. Int J Card Imaging 2000; 16:429–36.
26. Hinderliter AL, Willis PW, Long W, et al. Frequency and prognostic significance of pericardial effusion in primary pulmonary hypertension. PPH Study Group. Primary pulmonary hypertension. Am J Cardiol 1999;84:481–4.
27. Menzel T, Wagner S, Kramm T, et al. Pathophysiology of impaired right and left ventricular function in chronic embolic pulmonary hypertension: changes after pulmonary thromboendarterectomy. Chest 2000;118:897–903.
28. Mahmud E, Raisinghani A, Hassankhani A, et al. Correlation of left ventricular diastolic filling characteristics with right ventricular overload and pulmonary artery pressure in chronic thromboembolic pulmonary hypertension. J Am Coll Cardiol 2002;40:318–24.