Long-term effects of bosentan therapy in adult patients with pulmonary arterial hypertension related to congenital heart disease (Eisenmenger physiology): safety, tolerability, clinical and haemodynamic impact

Michele D'Alto, Carmine Dario Vizza, Emanuele Romeo, Roberto Badagliacca, Giuseppe Santoro, Roberto Poscia, Berardo Sarubbi, Massimo Mancone, Paola Argiento, Fabio Ferrante, Maria Giovanna Russo, Francesco Fedele and Raffaele Calabrò

Heart published online 29 Nov 2006;
doi:10.1136/hrt.2006.097360

Heart Online First, published on November 29, 2006 as 10.1136/hrt.2006.097360

Long-term effects of bosentan therapy in adult patients with pulmonary arterial hypertension related to congenital heart disease (Eisenmenger physiology): safety, tolerability, clinical and haemodynamic impact.

Corresponding author:
Michele D'Alto, MD, PhD
Via D. Fontana, 81
80128, Naples – Italy
Tel. +390817062683; Fax number: +390817064275
e-mail address: mic.dalto@tin.it

Authors:
Michele D'Alto, PhD, (1)
Carmine Dario Vizza, MD, (2)
Emanuele Romeo, MD, (1)
Roberto Badagliacca, MD, (2)
Giuseppe Santoro, MD, (1)
Roberto Poscia, MD, (2)
Berardo Sarubbi, PhD, (1)
Massimo Mancone, MD (2)
Paola Argiento, MD, (1)
Fabio Ferrante, MD, (2)
Maria Giovanna Russo, MD, (1)
Francesco Fedele, professor (2)
Raffaele Calabrò, professor (1)

First and second authors equally contributed to the study.

Affiliations
(1) = *Chair of Cardiology, Second University of Naples, A.O.Monaldi, Naples. Italy.*
(2) = *Department of Cardiovascular and Respiratory Sciences, University La Sapienza, Rome. Italy.*

Key words: pulmonary hypertension, congenital heart defects, Eisenmenger syndrome, bosentan.

Word count: 2352 words.

Abstract

Background: Oral bosentan is an established treatment for pulmonary arterial hypertension (PAH).

Objective: The aim of the present study was to evaluate safety, tolerability, and clinical and haemodynamic impact of bosentan in patients with PAH related to congenital heart disease (CHD).

Patients: Twenty-two patients with PAH CHD-related (8M, 14F, mean age 38±10) were treated with oral bosentan (62.5 mg x 2/die for the first month and then 125 mg x 2/die).

Main outcome measures: clinical status, liver enzymes, WHO functional class, resting oxygen saturations and 6-min walk test (6MWT) were assessed at baseline and at 1, 3, 6 and 12 month. Haemodynamic evaluation with cardiac catheterization was performed at baseline and at 12-month follow-up.

Results: Twelve patients had ventricular septal defect, 5 atrio-ventricular canal, 4 single ventricle and one atrial septal defect. All patients tolerated bosentan well. No major side effects were observed. After a year of therapy we observed an improvement in WHO functional class (2.5±0.7 vs 3.1±0.7; $p<0.05$), oxygen saturation at rest (87±6% vs 81±9; $p<0.001$), heart rate at rest (81±10 vs 87±14 bpm; $p<0.05$), distance travelled in the 6MWT (394±73 vs 320±108 m; $p<0.001$), oxygen saturation at the end of 6MWT (71±14 vs 63±17%; $p<0.05$), Borg's index (5.3±1.8 vs 6.5±1.3; $p<0.001$), pulmonary vascular resistances index (PVRi: 14±9 vs 22±12 WU.m^2; $p<0.001$), systemic vascular resistances index (SVRi: 23±11 vs 27±10 WU.m^2; $p<0.01$), PVRi/SVRi (0.6±0.5 vs 0.9±0.6; $p<0.05$); pulmonary (4.0±1.3 vs 2.8±0.9 l/min/m2; $p<0.001$) and systemic cardiac output (4.2±1.4 vs 3.4±1.1 l/min/m2; $p<0.05$).

Conclusions: Bosentan was safe and well tolerated in adults with PAH CHD-related during 12 months of treatment. Clinical status, exercise tolerance and pulmonary haemodynamics significantly improved.

Background.
Congenital heart diseases (CHD) are the most common congenital malformations and account for about 8 cases per 1000 births [1]. Eisenmenger syndrome (ES) is defined as a congenital heart defect that initially causes chronic large left-to-right shunt that induces severe pulmonary vascular disease and pulmonary arterial hypertension (PAH), with resultant reversal of the direction of shunting [2]. ES patients experience a poor quality of life but in most cases the disease progresses very slowly [3-5] so they have a considerably longer life expectancy compared with those with idiopathic pulmonary arterial hypertension (IPAH) and comparable functional class [6]. In fact, because of its unique hemodynamics, the right ventricle maintains its characteristics from fetal life (i.e., regression of wall thickness does not occur) and it is able to sustain an increased after load. This is likely the reason why patients with ES have a better prognosis than patients with other forms of pulmonary arterial hypertension [7].
In recent years, new treatment strategies have largely improved the clinical status and the life expectancy of patients with PAH [5-6]. Bosentan, an oral dual endothelin (ET-A /ET-B) receptor antagonist has been shown to be effective in patients with idiopathic PAH and PAH related to connective tissue disease, improving long-term quality of life [8-14]. Over the last few years, small open-label studies have suggested that bosentan is safe and well tolerated in adults with congenital heart disease (grown-up congenital heart disease - GUCH - patients) and improves functional status and exercise capacity [15,16]. These pilot studies triggered the first large randomized controlled trial on bosentan in patients with Eisenmenger syndrome, demonstrating a beneficial short-term effect of bosentan on exercise capacity and hemodynamics without compromising systemic oxygen saturation [17].

Objective: The aim of this open-label, single-arm, prospective study was to evaluate the safety and tolerability of oral bosentan therapy in 24 consecutive adult patients with Eisenmenger physiology by assessing its long-term effects on clinical status, exercise capacity and cardiopulmonary haemodynamics.

Methods
Patients with pulmonary arterial hypertension due to unoperated non-restrictive intracardiac communication with a bidirectional or a right-to-left shunt (Eisenmenger pathophysiology) were enrolled.
Concomitant causes of pulmonary hypertension such as lung or liver disease were excluded using mandatory chest X-ray, respiratory function, perfusion lung scan, HRCT scan, spiral CT-scan, abdominal ultrasound.
No patient was on calcium channel blockers as they were unresponsive to acute vasodilator testing in prior haemodynamic evaluation. No other drugs effective in PAH were used during the treatment period.
Informed patient's consent was obtained prior to entering the study, and the protocol was approved by the institutional ethics committee. Enrolled patients weighed >40 kg, had been in a stable condition for at least 3 months prior to entering the study and were in WHO functional class II-IV. In addition, enrolled patients had resting systemic arterial oxygen saturations of <90% and >60% at rest in room air and were not pregnant.
Study design. This was an open-label, single-arm, prospective study. Bosentan (Tracleer®; Actelion Pharmaceuticals, Allschwil, Switzerland) was started at an oral dose of 62.5 mg twice a day and increased to the target dose of 125 mg twice a day after 4 weeks.
Clinical assessment and laboratory tests, including haemoglobin, haematocrit and liver function tests (AST/ALT) were performed at baseline (prior to initiating bosentan therapy) and monthly, or whenever

the patients' clinical status required. Exercise tolerance evaluation with six minute walk test (6MWT) [18] was performed at baseline and at 1, 3, 6 and 12-month follow-up evaluation; cardiac catheterization was performed at baseline and at the 12-month follow up evaluation.

Clinical assessment and laboratory tests. Clinical assessment included medical examination with WHO classification and measurement of systemic arterial pressure, transcutaneous oxygen saturation and heart rate. Resting systemic arterial oxygen saturation was measured indirectly by non-invasive finger pulse oximetry after 5 min absolute rest in the sitting position, and the mean of 3 consecutive readings was recorded for analysis. Clinical findings such as pretibial edema, jugular venous pulse and hepatomegaly were observed.

Monitoring also included monthly measurements of liver enzymes, with a focus on alanine aminotransferase and aspartate aminotransferase and 3 monthly measurements of haematocrit, hemoglobin and serum iron.

Effort tolerance. Exercise capacity was evaluated with an non-encouraged 6-minute walking test [18] (at baseline 6-min walking distance was calculated as the best distance covered on 2 consecutive tests performed after 60-90 minutes). Heart rate, SpO2 were recorded at rest and at the end of exercise, Borg dyspnoea index was evaluated at completion of the test. All 6-minute walking tests were performed in a 25-meter-long corridor in the same environmental conditions and at about the same time of day (± 2 hours).

Heart catheterization. Hemodynamic assessment was performed at baseline and after 12 months of treatment by right heart catheterization. Pulmonary arterial, right atrial and pulmonary capillary wedge pressures and systemic pressures were recorded at the end of a quiet respiratory cycle. Oxygen saturations in the superior vena cava, inferior vena cava, pulmonary artery and femoral artery were obtained in triplicate.

Pulmonary vein saturation was assumed at 98%. Pulmonary (Qp) and systemic (Qs) flows were obtained by the Fick principle using table-derived oxygen consumption values and calculated oxygen content at the correspondent different sites [19]. The transpulmonary pressure gradient was defined as the difference between mean pulmonary artery pressure and mean pulmonary capillary wedge. Pulmonary and systemic vascular resistance indices were calculated using the standard formula.

Statistical analysis.

All values are presented as mean ± SD except when differently indicated. Changes from baseline to month 12 were evaluated with a paired *t* test for continuous variables and with Wilcoxon's rank-sum test for categorical variable. A p value of <0.05 was considered to be statistically significant. All the reported p values were two-tailed.

Results.

Twenty-four consecutive adult patients with pulmonary hypertension from congenital heart disease were enrolled. After 1-2 weeks of treatment 2 patients (8.3%) withdrew from drug treatment because of severe leg edema despite an increased diuretic dose and were not considered in the analysis.

In 22 participating patients, conventional therapy with oxygen, diuretics, ACE-inhibitors, digoxin or warfarin was continued during the study without significant changes. No patient used calcium channel blockers. (Tables I, II).

Table I: concomitant therapy

Drug	Patients on treatment	Daily dose
Digoxin	12	0.125-0.25 mg
Furosemide	18	25-125 mg
Warfarin	15	2.5-6 mg
Aldactone	13	25-100 mg
Enalapril	3	10-20 mg
Nifedipine	0	-
Nitrates	0	-
Amiodarone	0	-

Table II

Demography and diagnosis in adult patients with CHD.

Patients	22
Men:women	8:14
Age (y)	38±10
Follow-up (months)	12±3
Diagnosis:	
- VSD	12
- AVC	5
- ASD	1
- Single ventricle (complex)	4

VSD, ventricular septal defect; AVC, atrio-ventricular canal; ASD, atrial septal defect.

Mean treatment duration was 12.3 ± 3.3 months (range, 9 to 16 months). Bosentan treatment was generally well tolerated. No deaths or any serious adverse drug reactions were noted.

Aspartate aminotransferase and alanine aminotransferase plasma levels remained <3-fold the upper limit of normal (ULN) in all but 3 patients throughout the observation period. In all these patients, showing an aminotransferase level elevation 4 times the ULN, the dose of bosentan at 2 months of observation was reduced from 125 mg twice a day to 62.5 mg twice a day with a complete normalization of the aminotransferase level. These 3 patients continued receiving 62.5 mg twice a day during the follow-up. One patient required a bosentan dose reduction from 125 mg twice a day to 62.5 mg twice a day due to the development of moderate leg edema. Finally, 18/22 patients received bosentan 125 mg twice a day and 4/22 received bosentan 62.5 mg twice a day during the follow-up. The changes from baseline to 1 year of bosentan treatment in clinical, pulse oximetry exercise capacity and haemodynamic status are shown in Table III and in Figures 1 and 2.

Table III - Clinical, hematological and haemodynamic variables before and after oral bosentan treatment.

	Basal	**End of observation**	***P***
Clinical status			
Sat art O2 (%)	81±9	87±6	*<0.001*
HR (bpm)	87±14	81±10	*<0.05*
WHO functional class	3.1±0.7	2.5±0.7	*<0.05*
Exercise tolerance: 6MWT			
Travelled distance (m)	320±108	394±73	*<0.001*
HR at the end (bpm)	119±17	112±24	*ns*
Sat art O2 at the end (%)	63±17	71±14	*<0.05*
Borg Index	6.5±1.3	5.3±1.8	*<0.001*
Heart catheterization			
▪ *Pressure*			
RA (mmHg)	12±4	11±3	*ns*
sPA (mmHg)	106±28	105±37	*ns*
dPA (mmHg)	52±8	49±16	*ns*
mPA (mmHg)	73±18	71±22	*ns*
mCWP (mmHg)	12±3	12±4	*ns*
mSA (mmHg)	84±14	83±18	*ns*
▪ *Blood flow*			
QP (l/m/m^2)	2.8±0.9	4.0±1.3	*<0.001*
QS (l/m/m^2)	3.4±1.1	4.2±1.4	*<0.05*
QP/QS	0.9±0.3	1.0±0.3	*ns*
▪ *Vascular Resistances*			
PVRi (WU.m^2)	22±12	14±9	*<0.001*
SVRi (WU.m^2)	27±10	23±11	*<0.01*
PVRi/SVRi	0.9±0.6	0.6±0.5	*<0.05*
Biochemistry			
HTC (%)	57 ± 7	55 ± 7	*ns*
Hb (mg/dL)	18 ± 4	17 ± 6	*ns*
PTls (1000/μL)	198 ± 75	179 ± 67	*ns*
WCC (1000/μL)	7.4 ± 2.3	7.2 ± 2.8	*ns*
Aspartate aminotraferase (U/L)	24 ± 9	28 ± 12	*ns*
Alanine aminotraferase (U/L)	31 ± 21	31 ± 19	*ns*
pH	7.37 ± 0.05	7.38 ± 0.07	*ns*
Pa O_2 (mmHg)	45.5 ± 5.2	50.3 ± 10.1	*ns*
Pa CO_2 (mmHg)	39.3 ± 8.6	38.9 ± 7.3	*ns*

Sat art 02%, transcutaneous oxygen saturation in percent; HR, heart rate; 6-MWT, 6-minute walking test; RA, right atrial pressure; sPAP, systolic pulmonary arterial pressure; dPAP, diastolic pulmonary arterial pressure; mPAP, mean pulmonary arterial pressure; mCWP, mean capillary wedge pressure; mSAP, mean systemic arterial pressure; QP: pulmonary cardiac output; QS: systemic cardiac output; QP/QS, pulmonary to systemic cardiac output ratio; PVRi, pulmonary vascular resistances index; SVRi, systemic vascular resistances index; PVRi/SVRi, pulmonary to systemic vascular resistances ratio; HTC: haematocrit; Hb, hemoglobin; Ptl, Platelets; WCC, white cell count; pH, systemic arterial pH; Pa O_2, systemic arterial O_2; Pa CO_2, systemic arterial CO_2; ns, not significant.

At baseline, 4 patients were in WHO class II, 12 in class III and 6 in class IV. After 12 months of treatment WHO class improved by at least one class in 14 and remained unchanged in 8 patients, resulting in a statistically significant improvement.
Similarly, effort tolerance improved from baseline as shown by the significant increase in 6-minute walk distance (67.3±75 m, $p<0.001$) (Fig 1) with a reduction in Borg dyspnea index (-1.5±2, $p=0.001$). After a year of treatment pulse oxymetry significantly increased at rest (+5.3+5.1 %, $p=0.001$) as well as at the end of the walking test (4.9+9.6 %, $p<0.05$). Regarding the hemodynamic status, compared to baseline, we observed a significant reduction in pulmonary vascular resistances index (PVRi: -9.7±1 WU, $p=0.001$) and systemic vascular resistances index (SVRi: -4.5±1; $p<0.01$) with a concomitant reduction of PVRi/SVRi (-0.25±0.1; $p<0.05$). Moreover, we observed an increase in Qp (1.1±1.1 l/min, $p=0.001$) and Qs (0.6±1.1 l/min, $p=0.05$) with a small, but non significant modification in pulmonary and systemic pressure.

Discussion

This study is the first prospective relatively long-term hemodynamic evaluation of Bosentan therapy in CHD-related PAH patients. Bosentan treatment was investigated in several small, uncontrolled case series in the present indications. Christensen et al [20] treated 9 patients with CHD-related PAH with oral Bosentan. Clinical and non-invasive evaluation showed a significant improvement of oxygenation and functional status with minimal side effects at a 9.5 month follow-up. Gatzoulis et al [15] reported a substantial improvement in clinical status, 6-minute walking distance and haemodynamics in 10 adult patients with Eisenmenger physiology after a short-term (3-month) treatment. Recently, an open label prospective multicentered study, Schultze-Neick et al [16] observed that bosentan treatment was well tolerated and improved functional status as well as exercise capacity in 33 adult patients with CHD-related PAH at 2.1 year follow-up. In this study only 27/33 patients at baseline and 17/33 during the follow-up underwent heart catheterization: no statistically significant haemodynamic modification was observed, despite slight trends in improvements in PVRi and right ventricular systolic pressure. Anastopopulos et al [21], studying an eterogeneous population of 21 patients with PAH due to different operated or unoperated CHD, observed a short-term (16 week treatment) improvement of clinical, exercise tolerance and haemodynamics. These favourable findings in adults are further supported by data from a subset in an open, prospective trial of 9 children with PAH-CHD in functional class II or III, who all stabilized or improved during 3-month bosentan treatment. [22, 23].
The Bosentan Randomized Trial of Endothelin Antagonist Therapy-5 (BREATHE-5) [17], a 16-week, multicenter, randomized, double-blind, placebo-controlled study, recently evaluated the effect of bosentan in 54 patients with WHO functional class III Eisenmenger syndrome. The placebo-corrected effect on systemic pulse oximetry demonstrated that bosentan did not worsen oxygen saturation. Moreover, compared with placebo, bosentan improved exercise capacity and haemodynamics: treatment effect on 6MWT of 53.1 m, and significant reduction of PVRi, mPAP, SVRi and mSAP. Four patients discontinued as a result of adverse events, 2 (5%) in the bosentan group and 2 (12%) in the placebo group.
Our results confirm these previous observations suggesting that patients with CHD-related PAH have a sustained benefit from long-term bosentan therapy. Our findings agree with previous observations [15-23] ruling out the hypothesis that bosentan has a greater effect on systemic circulation than the obstructed pulmonary vascular bed of ES patients, and could worsen systemic hypoxemia as a result of increased in right-to-left shunt.
After a year of treatment, we observed a significant improvement in WHO functional class, 6-minute walking with a concomitant increase in Qp, Qs and reduction in PVRi, SVRi, PVRi/SVRi ratio. In our series, the significant reduction of PVRi/SVRi ratio suggests a greater effect of bosentan on pulmonary rather than on systemic circulation. Although in patients with Eisenmenger syndrome small pulmonary

arteries are affected by fixed obstructive pathological changes, it has been suggested that there may be reverse remodeling of pulmonary vascular changes with endothelin receptor antagonists on the basis of their antiproliferative properties [8, 11].
The decrease in pulmonary vascular resistance observed in our study is in the range of 20-25% from the baseline, similar to what was obtained in randomized controlled studies conducted in patients with idiopathic and CHD-related PAH [8,14,17].
Experimental data demonstrated that ET-1 not only modulates vascular smooth muscle tone [24], but also promotes cellular proliferation [25], initiates cardiac myocyte [26], and nonmyocyte [27] hypertrophy, and regulates secretion of neurohormonal mediators of cardiac and vascular hypertrophy [28]. Bosentan, an oral ET-A/ET-B inhibitor, not only prevents the development of pulmonary hypertension but can also reverse established pulmonary hypertension and pulmonary vascular remodeling induced by chronic hypoxia [29]. These experimental observations could explain a long-term efficacy of oral bosentan in patients with Eisenmenger physiology as a result of its favourable effect on pulmonary vessel remodelling.
In patients with ES there remains a theoretical point of concern using a non selective pulmonary vasodilator: the possible increase in right-to-left shunt. However, in our population during bosentan therapy we saw no reduction in resting or exercise SpO2 compared to baseline.
One year of bosentan treatment caused a greater reduction in right ventricular than in the left ventricular afterload, causing a reduction in the right-to-left shunt, an improvement in pulmonary blood flow and ultimately in systemic oxygen delivery. This pathophysiological mechanism explains the significant improvement in clinical status and effort tolerance seen during the follow-up.

Study limitation.
The present results needs to be viewed considering the background of several potential limitations.
First, this study was not controlled, and therefore, the contribution of a placebo effect is unknown. However, an attempt was made to exclude this effect by allowing no less than a 9-month treatment. Placebo effects disappear after 1 to 2 months in randomized controlled studies [14]. Secondly, in the absence of randomization procedures, the influence of unknown bias, for example through patient selection, cannot be determined. Finally, the specific type of intracardiac abnormality largely determines the natural history and therefore, ideally, the response to any therapy should be compared with the long-term outcome in that particular abnormality.

Conclusion
Our study, in agreement with previous observations, suggests that long-term bosentan treatment is safe, well tolerated and effective in patients with pulmonary hypertension related to congenital heart disease. Careful patient management is recommended in the current monitoring schedule and flowchart for modifying the dosing schedule in case of elevated liver function tests or significant side effects (i.e., leg edema). This is especially relevant in patients with Eisenmenger physiology who usually have multiorgan involvement and thus may be at greater risk for liver or other organ dysfunction [15]. Our safety experience is consistent with the results of the controlled clinical trials [8,14] and the wide experience from the bosentan postmarketing surveillance database, in which a large number of patients with PAH-CHD are included [30]. A recent larger multicenter, randomized, double-blind, placebo-controlled study with bosentan in patients with Eisenmenger syndrome confirms these results [17].

No author has conflict of interest.

Abbreviations:
6-min walk test (6MWT)
Computed tomography (CT)
Congenital heart disease (CHD)
Eisenmenger syndrome (ES)
Endothelin-1 (ET-1)
Grown-up congenital heart (GUCH)
High resolution computed tomography (HRCT)
Idiopathic pulmonary arterial hypertension (IPAH)
Pulmonary arterial hypertension (PAH)
Pulmonary flow (Qp)
Pulmonary vascular resistances (PVR)
Systemic flow (Qs)
Upper limit of normal (ULN)

Figure legends:

Figure 1

A) Travelled distance at 6MWT.
B) Heart rate at the end of 6MWT.
Open bars: basal condition; black bars: one year follow-up.

Figure 2

A) Oxygen saturation at the end of 6MWT.
B) Borg index at 6MWT.
Open bars: basal condition; black bars: one year follow-up.

References

1. MacMahon B, McKeown T, Record RG. The incidence and life expectation of children with heart disease. *Br Heart J* 1953;15:121.
2. Wood P. The Eisenmenger syndrome or pulmonary hypertension with reversed central shunt. *Br Med J* 1958;46:755-62.
3. Farber HW, Loscalzo J. Pulmonary arterial hypertension. *N Engl J Med* 2004;351:1655-65.
4. Daliento L, Somerville J, Presbitero P, et al. Eisenmenger syndrome. Factors relating to deterioration and death. *Eur Heart J* 1998;19:1845-55.
5. Guidelines on diagnosis and treatment of PAH. The Task Force on Diagnosis and Treatment of Pulmonary Arterial Hypertension of the European Society of Cardiology. *Eur Heart J* 2004;25:2243-78.
6. Hopkins WE, Ochoa LL, Richardson GW, Trulock EP. Comparison of thehemodynamics and survival of adults with severe primary pulmonary hypertension or Eisenmenger syndrome. *J Heart Lung Transplant* 1996;15:100–105.
7. Berman EB, Barst RJ. Eisenmenger's syndrome: current management. *Prog Cardiovasc Dis* 2002;45:129 -38.
8. Rubin LJ, Badesch DB, Barst RJ, et al. Bosentan therapy for pulmonary arterial hypertension. *N Engl J Med* 2002;346:896-903.
9. N, Seeger W, Naeije R, et al. Comparative analysis of clinical trials and evidence-based treatment algorithm in pulmonary arterial hypertension. *J Am Coll Cardiol* 2004;43:81S-88S.
10. McLaughlin VV, Sitbon O, Badesch DB, et al. Survival with first-line bosentan in patients with primary pulmonary hypertension. *Eur Respir J* 2005;25:244-9.
11. Galie N, Manes A, Branzi A. The endothelin system in pulmonary arterial hypertension. *Cardiovasc Res* 2004;61:227-37.
12. Tutar HE, Imamoglu A, Atalay S, et al. Plasma endothelin-1 levels in patients with left to-right shunt with or without pulmonary hypertension. *Int J Cardiol* 1999;70:57-62.
13. Adatia I, Haworth SG. Circulating endothelin in children with congenital heart disease. *Br Heart J* 1993;69:233-6.
14. 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.
15. Gatzoulis MA, Rogers P, Wei L, et al. Safety and tolerability of bosentan in adults with Eisenmenger physiology. *Int J Cardiol* 2005;98:147-51.
16. Schulze-Neick I, Gilbert N, Ewert R, et al. Adult patients with congenital heart disease and pulmonary hypertension: First open prospective multicenter study of bosentan therapy. *Am Heart J* 2005;150:716e7-716e12.
17. Galiè N, Beghetti M, Gatzoulis MA et al. Bosentan Therapy in Patients With Eisenmenger Syndrome. A Multicenter, Double-Blind, Randomized, Placebo-Controlled Study. *Circulation* 2006;114:48-54.
18. Guyatt GH, Sullivan MJ, Thompson PJ, et al. The 6 minute walk: a new measure of exercise capacity in patients with chronic heart failure. *Can Med Assoc J* 1985;132(8):919-23.
19. Grossman W. Clinical measurement of vascular resistance and assessment of vasodilator drugs. In: Grossman W, ed. *Cardiac Catheterization and Angiography*. Philadelphia, PA: Lea and Febiger; 1991:143-151.
20. Christensen DD, McConnell ME, Book WM, et al. Initial Experience With Bosentan Therapy in Patients With the Eisenmenger Syndrome. *Am J Cardiol* 2004;94:261-263.

21. Apostolopoulou SC, Manginas A, Cokkinos DV, et al. Effect of the oral endothelin antagonist bosentan on the clinical, exercise and haemodynamic status of patients with pulmonary arterial hypertension related to congenital heart disease. *Heart.* 2005;91(11):1447-52.
22. Barst RJ, Ivy D, Dingemanse J, et al. Pharmacokinetics, safety, and efficacy of bosentan in pediatric patients with pulmonary arterial hypertension. *Clin Pharmacol Ther* 2003;73:372-82.
23. Rosenzweig E, Ivy D, Widlitz A, et al. Effects of long-term bosentan in children with pulmonary arterial hypertension. *J Am Coll Cardiol* 2005;46(4):697-704.
24. Ohlstein EH, Douglas SA. Endothelin-1 modulates vascular smooth muscle structure and vasomotion: implications in cardiovascular pathology. *Drug Dev Res* 1993;29:108-128.
25. Morbidelli L, Orlando C, Maggi CA, et al. Proliferation and migration of endothelial cells is promoted by endothelins via activation of ETB receptors. *Am J Physiol* 1995; 269:686-695.
26. Ito H, Hirata Y, Hiroe M, et al. Endothelin-1 induces hypertrophy with enhanced expression of muscle-specific genes in cultured neonatal rat cardiomyocytes. *Circ Res* 199169:209-215.
27. Takanashi M, Endoh M. Characterization of positive inotropic effect of endothelin on mammalian ventricular myocardium. *Am J Physiol* 1991;261:611-619.
28. Otsuka A, Mikami H, Katahira K, et al. Changes in plasma renin activity and aldosterone concentration in response to endothelin injection in dogs. *Acta Endocrinol* 1989;121:361-364.
29. Chen SJ, Chen YF, Meng QC, et al. Endothelin-receptor antagonist bosentan prevents and reverses hypoxic pulmonary hypertension in rats. *J Appl Physiol* 1995;79:2122–2131.
30. Humbert M, van Lierop C, Kiely DG, et al. Long term safety profile of bosentan in patients with pulmonary arterial hypertension: results from the European surveillance program. Data on file. Allschwil (Switzerland): *Actelion Pharmaceuticals*, Ltd; 2004.

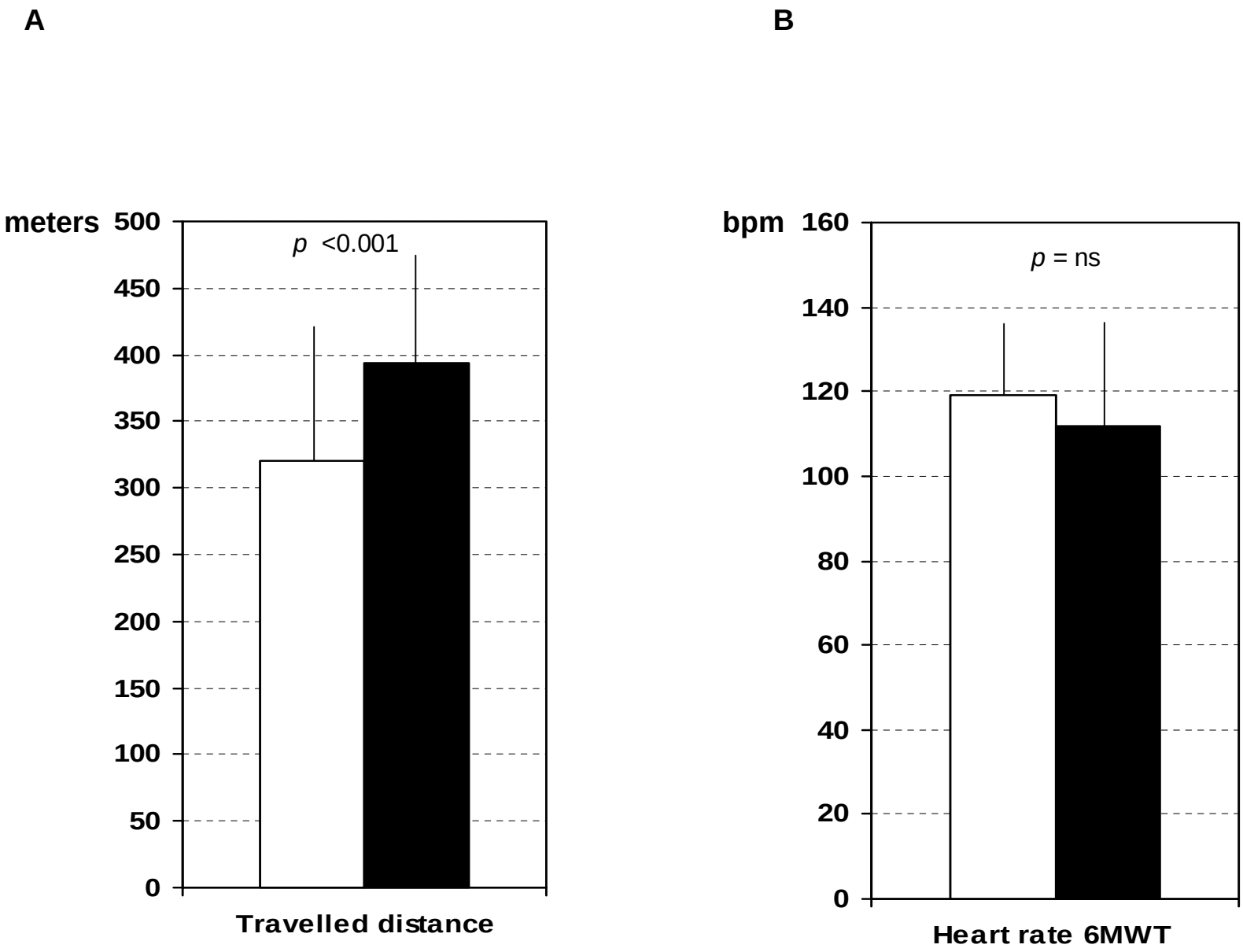

Fig 1

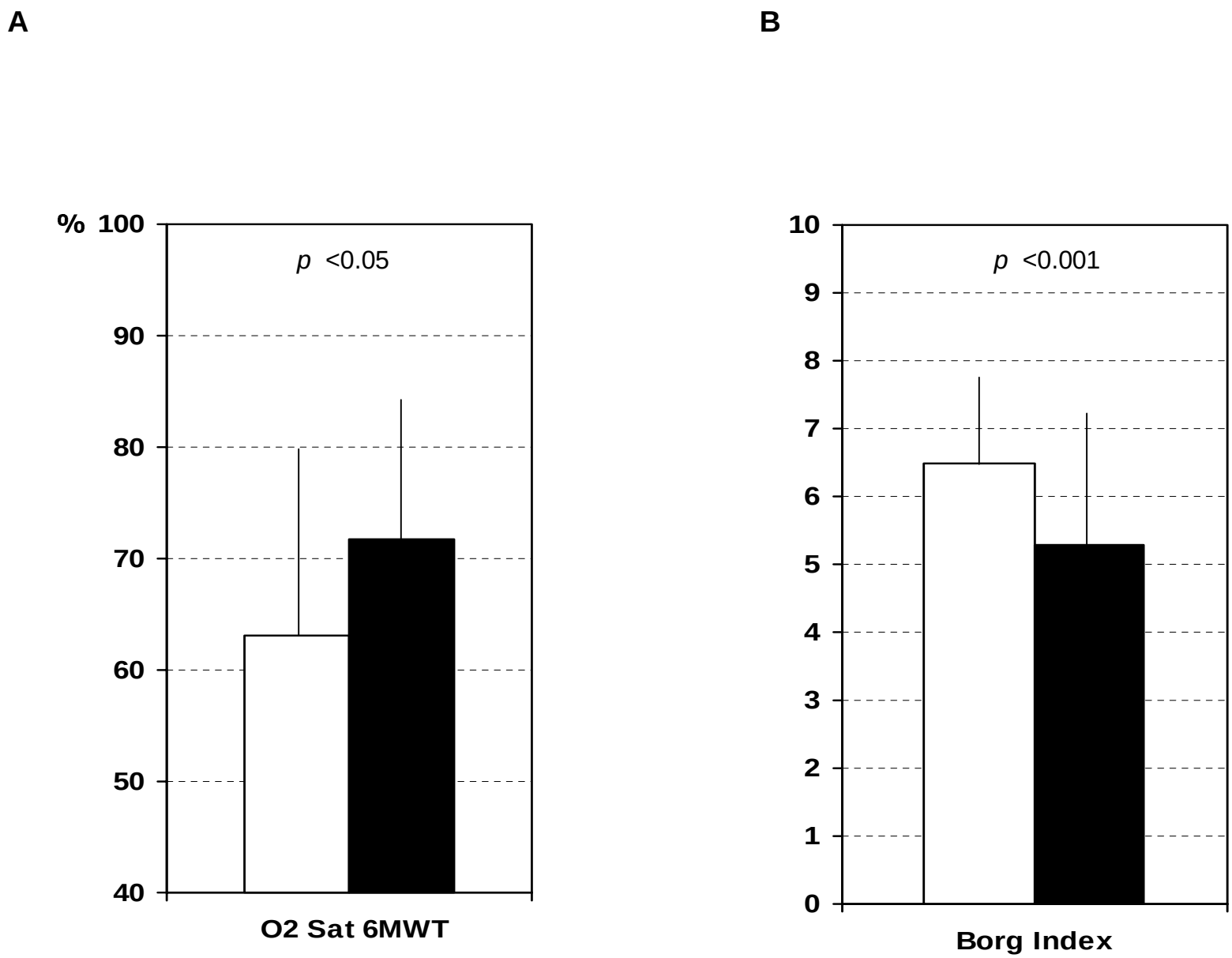

Fig 2