

Articles

Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomised placebo-controlled study

Richard N Channick, Gérald Simonneau, Olivier Sitbon, Ivan M Robbins, Adaani Frost, Victor F Tapson, David B Badesch, Sébastien Roux, Maurizio Rainisio, Frédéric Bodin, Lewis J Rubin

Summary

Background Endothelin 1, a powerful endogenous vasoconstrictor and mitogen, might be a cause of pulmonary hypertension. We describe the efficacy and safety of bosentan, a dual endothelin-receptor antagonist that can be taken orally, in patients with severe pulmonary hypertension.

Methods In this double-blind, placebo-controlled study, 32 patients with pulmonary hypertension (primary or associated with scleroderma) were randomly assigned to bosentan (62.5 mg taken twice daily for 4 weeks then 125 mg twice daily) or placebo for a minimum of 12 weeks. The primary endpoint was change in exercise capacity. Secondary endpoints included changes in cardiopulmonary haemodynamics, Borg dyspnoea index, WHO functional class, and withdrawal due to clinical worsening. Analysis was by intention to treat.

Findings In patients given bosentan, the distance walked in 6 min improved by 70 m at 12 weeks compared with baseline, whereas it worsened by 6 m in those on placebo (difference 76 m [95% CI 12–139], $p=0.021$). The improvement was maintained for at least 20 weeks. The cardiac index was 1.0 L $\text{min}^{-1} \text{m}^{-2}$ (95% CI 0.6–1.4, $p<0.0001$) greater in patients given bosentan than in those given placebo. Pulmonary vascular resistance decreased by 223 dyn s cm^{-5} with bosentan, but increased by 191 dyn s cm^{-5} with placebo (difference –415 [–608 to –221], $p=0.0002$). Patients given bosentan had a reduced Borg dyspnoea index and an improved WHO functional class. All three withdrawals from clinical worsening were in the placebo group ($p=0.033$). The number and nature of adverse events did not differ between the two groups.

Interpretation Bosentan increases exercise capacity and improves haemodynamics in patients with pulmonary hypertension, suggesting that endothelin has an important role in pulmonary hypertension.

Lancet 2001; **358**: 1119–23

See Commentary page 1113

Division of Pulmonary and Critical Care Medicine, University of California San Diego, CA, USA (R N Channick MD, Prof L J Rubin MD); **University of Colorado Health Sciences Center, Denver, CO** (Prof D B Badesch MD); **Baylor College of Medicine and the Methodist Hospital, Houston, TX** (Prof A Frost MD); **Vanderbilt University School of Medicine, Nashville, TN** (I M Robbins MD); **Hôpital Antoine Bécélère, Clamart, France** (Prof G Simonneau MD, O Sitbon MD); **Duke University Medical Center, Durham, NC** (V F Tapson MD); **Actelion Ltd, Allschwil, Switzerland** (S Roux MD, F Bodin MD); and **Statistics for Research Ltd, Basel, Switzerland** (M Rainisio PhD).

Correspondence to: Dr Richard Channick, Division of Pulmonary and Critical Care Medicine, University of California San Diego, 9300 Campus Point Drive, 7381 7372, La Jolla, CA 92037-1300, USA (e-mail: rchannick@ucsd.edu)

Introduction

Primary pulmonary hypertension is a disease resulting in progressive deterioration that is characterised by an increase in pulmonary vascular resistance leading to right ventricular failure and death.¹ Pulmonary hypertension can arise in isolation (primary pulmonary hypertension), or as a complication of systemic diseases (eg, systemic sclerosis or scleroderma).² Conventional therapy with vasodilators^{3,4} and anticoagulants⁵ is effective for only a few patients. The US National Institutes of Health (NIH) registry of patients with primary pulmonary hypertension records a median life expectancy of 2.8 years from diagnosis.⁵ Similarly, patients with pulmonary hypertension associated with scleroderma have a survival of 40–55% at 2 years.² Epoprostenol has been shown to be more effective than conventional treatment and has greatly improved the life expectancy of patients with severe pulmonary hypertension.⁶ However, epoprostenol requires permanent intravenous access and is associated with many side-effects and complications.^{6,7} The success of long-term intravenous epoprostenol prompted development of analogue molecules that can be inhaled (eg, iloprost⁸) or taken orally (eg, beraprost⁹). However, no randomised, placebo-controlled trials have been done with these new treatments.

That endothelin 1 has a pathogenic role in pulmonary hypertension has been documented.¹⁰ It is both a potent vasoconstrictor and a smooth-muscle mitogen, and might therefore contribute to the increase in vascular tone and the pulmonary vascular hypertrophy associated with pulmonary hypertension. Patients with primary pulmonary hypertension¹¹ or diffuse scleroderma¹² have high concentrations of endothelin 1 in plasma, which are inversely correlated with outlook.¹¹ High concentrations of endothelin 1 have also been recorded in the lungs of patients with pulmonary hypertension,¹³ idiopathic pulmonary fibrosis,¹⁴ or postobstructive pulmonary vasculopathy,¹⁵ suggesting that new treatments for pulmonary hypertension could act by blocking endothelin receptors.

Bosentan (Ro 47-0203), an orally active non-peptide antagonist of both endothelin receptor subtypes (ET_A and ET_B), has been shown to decrease inflammatory reactions, prevent increase in permeability of pulmonary vessels, and prevent development of fibrosis in animals with pulmonary inflammation.^{16,17} In rats with chronic pulmonary hypertension, bosentan reduces pulmonary arterial pressure, pulmonary vascular hypertrophy, and right ventricular hypertrophy, without inducing systemic vasodilatation.¹⁸ In a pilot study¹⁹ of acute administration of high doses of bosentan to patients with pulmonary hypertension, pulmonary and systemic resistance decreased, suggesting that chronic doses might be necessary for a significant and selective effect. Thus, the clinical effects of bosentan as a long-term oral treatment still need to be assessed. We assessed the effects of

bosentan on exercise capacity and cardiopulmonary haemodynamics, and the safety and tolerability of bosentan in patients with pulmonary arterial hypertension.

Methods

Patients

We recruited patients who had symptomatic, severe, primary pulmonary hypertension or pulmonary hypertension due to scleroderma (in functional classes III–IV according to 1998 WHO classification²⁰), despite previous treatment with vasodilators, anticoagulants, diuretics, cardiac glycosides, or supplemental oxygen. Patients were included if they had a baseline 6-min walking distance of between 150 m and 500 m, a mean pulmonary artery pressure of greater than 25 mm Hg, a pulmonary capillary wedge pressure of less than 15 mm Hg, and a pulmonary vascular resistance of greater than 240 dyn s cm⁻⁵. Patients were excluded if they were in functional class IV (since for ethical reasons, such patients were also required to have a stable clinical status), if they had started or stopped any of the above treatments within 1 month of screening, if they were receiving chronic treatment with epoprostenol, or if they had received glibenclamide or ciclosporin within 1 month of enrolment (to avoid potential drug interactions).

The study was done according to the 1983 revision of the 1975 Declaration of Helsinki, and adhered to local guidelines for good clinical practice. It was approved by a local ethics review committee, and written informed consent was obtained from all patients.

Procedures

The study was a double-blind, randomised, placebo-controlled trial that was done in five centres in the USA and one in France. 32 patients were randomly assigned 62.5 mg bosentan twice daily for the first 4 weeks followed by the target dose (125 mg twice daily) unless drug-related adverse events arose (eg, hypotension), or matching doses of placebo. Randomisation was computer generated using the Almedica Drug Labelling System, with a block size of three. The 2/1 randomisation ratio (bosentan/placebo) was about the same for each centre. All patients remained on study medication until study end, which was defined as the day the last enrolled patient completed the week 12 assessment. Consequently, the study was composed of 2 periods: period 1, which was mandatory for all patients, was a fixed duration of 12 weeks, whereas period 2 varied between 0 and 16 weeks. At study end, all patients were eligible to enter an open-label study of bosentan.

During period 1, patients were assessed on an outpatient basis at 4, 8, and 12 weeks of treatment. The primary endpoint was exercise capacity at week 12 and was measured by the distance a patient could walk in 6 min (6-min walk test). This standard test of exercise capacity has been described elsewhere,²¹ and is a good measure of the effects of potential treatments for pulmonary hypertension.⁶ Furthermore, walk-test performance has been predictive of mortality in patients with primary pulmonary hypertension.²² Secondary measures of efficacy included cardiopulmonary haemodynamics (pulmonary vascular resistance, cardiac index, mean pulmonary artery pressure, pulmonary capillary wedge pressure, and mean right atrial pressure) measured by right heart catheterisation (at baseline and week 12). Cardiac index (L min⁻¹ m⁻²) was cardiac output (L/min) divided by body surface area (m²); pulmonary vascular resistance (dyn s cm⁻⁵) was calculated by (mean

pulmonary artery pressure [mm Hg] – pulmonary capillary wedge pressure [mm Hg])/cardiac output (L/min) × 80. Secondary measures of efficacy also included Borg dyspnoea index,²³ which was obtained immediately after completion of the 6-min walk test, WHO functional class of pulmonary hypertension, and withdrawal because of clinical worsening. Safety was assessed by number of adverse events, laboratory assessment, and electrocardiogram. In period 2, patients were assessed on an outpatient basis at 20 and 28 weeks of therapy. Efficacy during this period was also measured by the 6-min walk test.

Statistical analysis

A sample size of 30 patients was calculated to detect a mean difference of 50 m (SD 50) in the 6-min walk test from baseline to week 12, with 80% power, and at a one-sided α level of 0.05 by Student's *t* test. To keep bias to a minimum, missing data at the week-12 assessment were derived from predefined replacement rules. Discontinuation of study medication because of clinical worsening was analysed with the patient's assessment at the time of premature withdrawal (in patients who died or had lung transplantation, the same rule would have applied). If no assessment was recorded, these patients were assigned the worst rank value—0 m for the 6-min walk test; a score of 10 for the Borg dyspnoea index; class IV for WHO functional class; the highest pulmonary artery pressure recorded in the same patient population, the highest pulmonary capillary wedge pressure, the highest right atrial pressure, the highest pulmonary vascular resistance, and the greatest decrease from baseline cardiac index to week 12 from the same group. All other patients without an assessment at week 12 had their last 6-min walking distance, Borg dyspnoea index, and WHO functional class carried forward, but were excluded from the haemodynamic analysis. By design, the analysis of the 6-min walking distance at week 20 and 28 could only be exploratory. For patients without a week-20 assessment, we used their last 6-min walking distance.

We calculated the significance of the differences from baseline to week 12 between treatment groups for the 6-min walk test, the Borg dyspnoea index, and the cardiopulmonary haemodynamic measurements with the two-samples Student's *t* test (these were verified with the Wilcoxon's rank-sum test). Change from baseline to week 12 of the WHO functional class was analysed with Wilcoxon's rank-sum test. The proportion of patients who withdrew because of clinical worsening was analysed with Fisher's exact test. With the exception of the a priori planned analysis on the 6-min walk test, which was confirmatory, all other analyses were exploratory by their nature. Statistical analyses were done on an intention-to-treat basis. All *p* values were two-tailed; 95% CIs were calculated for differences within and between treatment groups.

Results

Of 36 patients recruited, 32 were included in the study; 21 were assigned to bosentan and 11 placebo (figure 1). We excluded four patients because they did not meet all the entry criteria. All patients remained in the study until the last patient had completed the week 12 assessments, unless they were withdrawn because of clinical worsening. As a result, the total length of treatment varied from 83 to 202 days. No code break took place before the week 12 assessment.

Treatment groups were well matched with respect to baseline characteristics (table 1). All patients were in

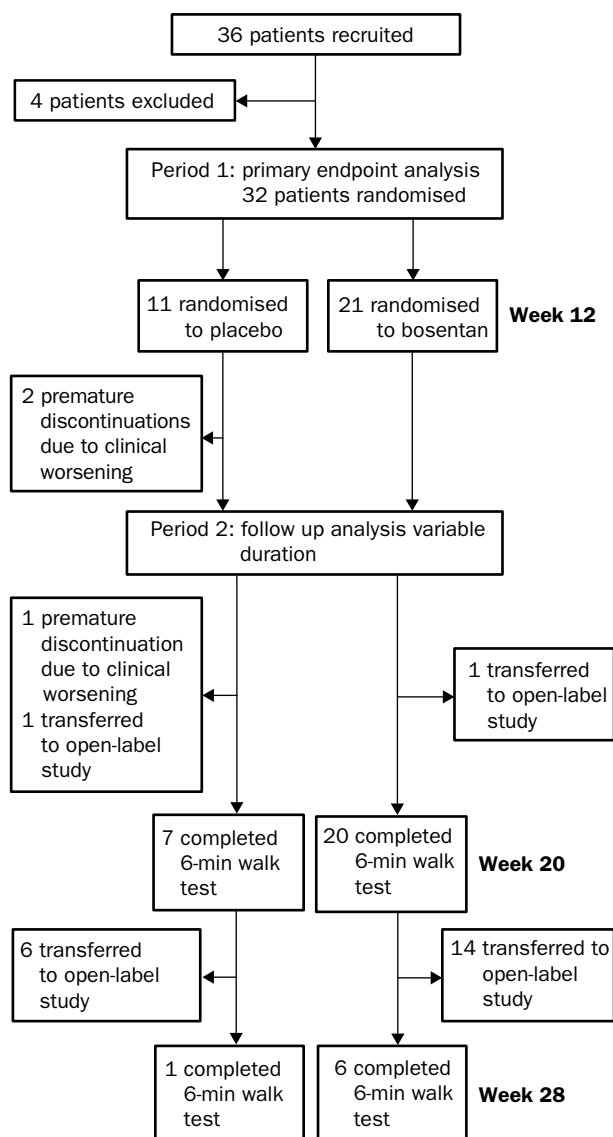


Figure 1: Trial profile

WHO functional class III at baseline. In both groups, more patients had primary pulmonary hypertension than pulmonary hypertension due to scleroderma (table 1). Use of concomitant medication (including anticoagulants and vasodilators) did not differ between groups. The main difference between groups was the longer duration of disease before the trial in patients assigned placebo than those assigned bosentan ($p=0.1058$).

In patients given bosentan, the distance walked in 6 min after 12 weeks of treatment lengthened from baseline by 70 m (from 360 m [SE 19] at baseline to 430 m [14] at week 12, $p<0.05$), whereas no change was seen in those given placebo (355 m [25] vs 349 m [44]) (figure 2). The median distance walked in 6 min increased by 51 m in patients given bosentan and decreased by 6 m in those given placebo; the mean change was 76 m (95% CI 12–139, $p=0.021$) further for patients given bosentan than those given placebo. This difference was already evident at week 8 (figure 2).

The Borg dyspnoea index at week 12 was 1.6 (95% CI 0.0–3.1) lower in patients given bosentan than those given placebo. One placebo-treated patient could not perform the week 12 assessment because of clinical

	Placebo (n=11)	Bosentan (n=21)
Demographic variables		
Sex		
Men	0	4 (19%)
Women	11 (100%)	17 (81%)
Age (mean [SD], years)	47.4 (14.0)	52.2 (12.2)
Weight (mean [SD], kg)	87.1 (17.7)	85.9 (22.8)
Ethnic group		
Black	2 (18%)	3 (14%)
White	9 (82%)	16 (76%)
Other	0	2 (10%)
Cause of pulmonary hypertension		
Primary	10 (91%)	17 (81%)
Secondary to scleroderma	1 (9%)	4 (19%)
WHO functional class		
III	11 (100%)	21 (100%)
IV	0	0
6-min walking distance (mean [SD], m)	355 (82)	360 (86)
Dyspnoea index (mean [SD], Borg scale)	4.18 (1.94)	4.38 (1.80)
Oral anticoagulants		
Warfarin	8 (73%)	15 (71%)
Oral vasodilators		
Diltiazem	2 (18%)	6 (29%)
Amlodipine	4 (36%)	3 (14%)
Time since diagnosis (mean [SD], months)	36.4 (34.4)	21.1 (17.6)
Haemodynamic variables		
Cardiac index (mean [SD], L min ⁻¹ m ⁻²)	2.5 (1.0)†	2.4 (0.7)‡
Pulmonary vascular resistance (mean [SD], dyn s cm ⁻⁵)	942 (430)†	896 (425)*
Pulmonary artery pressure (mean [SD], mm Hg)	56 (10)†	54 (13)‡
Pulmonary capillary wedge pressure (mean [SD], mm Hg)	8.3 (3.3)†	9.3 (2.4)*
Mean right atrial pressure (mean [SD], mm Hg)	9.9 (4.1)†	9.7 (5.6)*
Blood pressure† (mean [SD], mm Hg)	92 (15)	94 (12)‡
Heart rate (beats/min)†	88 (12)	83 (16)‡

*n=19. †n=10. ‡n=20.

Table 1: Demographic and haemodynamic characteristics

worsening (decompensated right heart failure); consequently his walking distance was set to 0 and his Borg dyspnoea index to 10, as per protocol.

Bosentan increased the mean 6-min walking distance from baseline to week 20 (from 360 m to 437 m, $p<0.0001$), whereas the distance was decreased in the placebo group (from 355 m to 340 m, $p=0.6846$) (figure 2). The effect of bosentan on 6-min walking distance was significantly better than that of placebo ($p=0.0097$). For patients who completed the week-28 assessments (bosentan n=6, placebo 1) the treatment effect on the 6-min walking distance was maintained (data not shown because of insufficient patients).

Treatment with bosentan significantly improved cardiopulmonary haemodynamics from baseline to week 12 compared with placebo (table 2). Pulmonary vascular resistance fell significantly from baseline in patients given bosentan, and rose in those given placebo. Treatment with bosentan decreased the mean pulmonary artery pressure, the pulmonary capillary wedge pressure, and the mean right atrial pressure. In contrast, all three measures increased in the placebo group. One patient in each treatment group had no week-12 assessment and were thus excluded from haemodynamic assessment.

The improvements in haemodynamics seen with bosentan were not associated with a change in heart rate

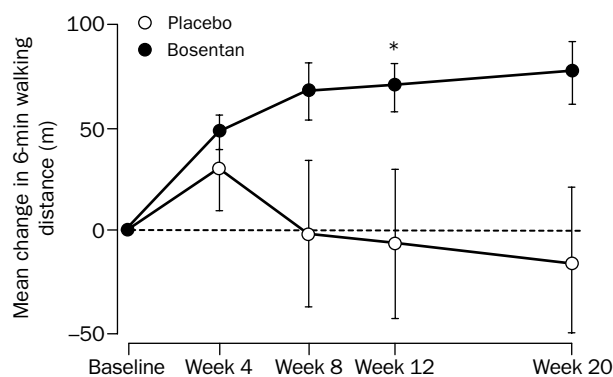


Figure 2: Change in 6-min walking distance from baseline to week 20

Points=mean, bars=SE. Patients who did not complete week-20 assessments (bosentan n=1, placebo 4) had their last observed value carried forward. * $p < 0.05$ versus baseline, $p = 0.021$ versus placebo.

(baseline 83 beats/min [SD 16], end of study 82 beats/min [14]) nor with the mean arterial blood pressure (baseline 94 mm Hg [12], end of study 85 mm Hg [9]).

Functional class of pulmonary hypertension improved in patients given bosentan. At baseline, all patients in both treatment groups were in functional class III. After 12 weeks of treatment with bosentan, nine of 21 patients (43%) improved to class II, 12 (57%) remained in class III, and none deteriorated to class IV ($p = 0.0039$). With placebo, only one of 11 patients (9%) improved to class II, eight (73%) remained in class III, and two (18%) deteriorated to class IV ($p = 1.0000$). Bosentan significantly improved the functional class of patients compared with placebo ($p = 0.019$).

Treatment with bosentan significantly increased the time to clinical worsening compared with placebo ($p = 0.033$). Clinical worsening (right ventricular heart failure or aggravated pulmonary hypertension) was seen in three patients on treatment days 51, 58, and 84. All three patients were in the placebo group and discontinued study medication. No clinical worsening was reported in patients given bosentan.

No patient had lung transplantation or died during the course of the study. During the first 12 weeks of treatment, adverse events were transient, and close in frequency and nature between groups (seven of 11 patients taking placebo and nine of 21 taking bosentan). No hypotension or clinically significant changes in haematological or biochemical measures were seen in either group. Increases in the concentration of hepatic aminotransferases were seen in two patients assigned bosentan, but these increases were not associated with symptoms, and the concentrations returned to normal without discontinuation or change of dose. The overall tolerability profile was close in both groups.

A p value of less than or equal to 0.05 was judged significant in the 6-min walk test. Patients given bosentan had a significantly greater improvement in 6-min walking

distance than those given placebo ($p = 0.021$). Further confirmation was obtained by modification of the replacement rules: a patient in the placebo group who did not complete a 6-min walking distance assessment at week 12 had his last measured result carried forward instead of being assigned a value of zero, the treatment effect was still 53 m (SE 25) ($p = 0.041$).

A subgroup analysis was done to investigate the effect of the time from diagnosis to randomisation on the change in walking distance. Irrespective of whether patients were randomly assigned to treatment or placebo before or after the overall median value, they consistently showed a greater improvement with bosentan than with placebo. We showed no significant treatment-by-centre interaction.

Discussion

Our results show that chronic oral administration of a dual endothelin-receptor antagonist significantly improved exercise capacity and cardiopulmonary haemodynamics in patients with primary pulmonary hypertension or pulmonary hypertension due to scleroderma. Moreover, bosentan consistently improved all endpoints studied. Exercise capacity and haemodynamic function either deteriorated or remained unchanged in patients given placebo, as would be expected from clinical experience. The effect of bosentan on pulmonary haemodynamics included a decrease in pulmonary vascular resistance, mean pulmonary artery pressure, and mean right atrial pressure. The decrease in pulmonary vascular resistance probably accounted for the increase in cardiac index and thus for the improved 6-min walking distance.

We recorded a beneficial effect of chronic oral low-dose bosentan on cardiopulmonary haemodynamics, without a significant reduction in systemic arterial blood pressure. Our results contrast with those of a short-term pilot study,¹⁹ in which the effect of intravenous bosentan on cardiac index and pulmonary vascular resistance was small, despite a significant decrease in systemic vascular resistance. This discrepancy could be accounted for by differences in methods of administration, dose, or both.

The number and nature of adverse events were closely similar between patients given bosentan and those given placebo. These results are supported by clinical studies of patients with different pathologies,²⁴ which suggest that bosentan at a dose of 125 mg taken twice daily is well tolerated. Increases of hepatic enzymes (mainly aspartate aminotransferase and alanine aminotransferase), without symptoms, have previously been seen in some patients given high doses of bosentan (500 mg twice daily).²⁵ These abnormalities were reversible within 2–6 weeks of treatment discontinuation. The frequency of raised concentrations of hepatic enzymes is dose-dependent, as suggested by a long-term follow-up study²⁶ of patients with heart failure who were given 125 mg bosentan twice daily; only one of 23 patients given bosentan had an increase in concentration of enzymes in the liver.

Variable	Change from baseline		Difference between treatments	
	Placebo (n=10)	Bosentan (n=20)	Difference (95% CI)	p
Cardiac index (mean [SE], L min ⁻¹ m ⁻²)	-0.5 (0.1)	0.5 (0.1)	1.0 (0.6 to 1.4)	<0.001
Pulmonary vascular resistance (mean [SE], dyn s cm ⁻⁵)	191 (74)	-223 (56)*	-415 (608 to -221)	≤0.001
Pulmonary artery pressure (mean [SE], mm Hg)	5.1 (2.8)	-1.6 (1.2)	-6.7 (-11.9 to -1.5)	0.013
Pulmonary capillary wedge pressure (mean [SE], mm Hg)	3.9 (1.8)	0.1 (0.8)*	-3.8 (-7.3 to -0.3)	0.035
Mean right atrial pressure (mean [SE], mm Hg)	4.9 (1.5)	-1.3 (0.9)*	-6.2 (-9.6 to -2.7)	0.001

*Bosentan (n=19).

Table 2: Haemodynamic effects of placebo and bosentan at week 12

Similarly, in our results, only two patients given 125 mg bosentan twice daily had raised concentrations of enzymes in the liver, and in both, the concentration returned to normal without discontinuation or change of dose.

Although our results are promising, they are restricted by the small number of patients (32 treated in six centres), the large proportion of men (all in the bosentan group), the absence of patients who were functional class IV at baseline, and the predominance of patients with primary pulmonary hypertension versus scleroderma. Further studies are needed to assess the efficacy of bosentan in other causes of pulmonary hypertension (such as congenital heart disease, portal hypertension, or infection with HIV), and long-term data are needed to clarify whether development of right heart failure can also be prevented by bosentan.

Nevertheless, our results suggest that endothelin plays an important part in the pathogenesis and evolution of pulmonary hypertension. Oral use of a dual endothelin-receptor antagonist, such as bosentan, which blocks both ET_A and ET_B receptors, could be a new therapeutic approach for this disease. Both types of receptors have fundamental roles in pulmonary vasoconstriction,²⁷ increased inflammation,¹⁶ proliferation,²⁸ fibrosis,²⁹ and bronchoconstriction.³⁰ Furthermore, the ET_B receptor can be induced in disease situations,³¹ and contributes to the vasoconstricting, profibrotic, and proliferative effects of endothelin. These properties suggest that blockage of both ET_A and ET_B receptors could prevent the pathological effects of endothelin.

Contributors

Richard N Channick, G erald Simonneau, Olivier Sitbon, Ivan M Robbins, Adaani Frost, Victor F Tapson, David B Badesch, and Lewis J Rubin contributed in patient recruitment and assessments, and preparation of the report. S ebastien Roux, Maurizio Rainisio, and Fr ed eric Bodin designed the study, assisted by Lewis J Rubin, monitored clinical and laboratory assessments, analysed the data, and prepared the report.

Acknowledgments

We thank all local investigators and their staff; Katie Kinninger, Annie Pelissier; Wendi R Mason; Helena Purl; Abby Krichman and Keri White; Chris Stevens, Kris Wynne, Betty Booker, Karen Fagan, and Bertron Groves.

References

- Rubin LJ. Primary pulmonary hypertension. *N Engl J Med* 1997; **336**: 111–17.
- Stupi AM, Steen VD, Owens GR, Barnes EL, Rodnan GP, Medsger TA Jr. Pulmonary hypertension in the CREST syndrome variant of systemic sclerosis. *Arthritis Rheum* 1986; **29**: 515–24.
- Rich S, Kaufmann E, Levy PS. The effect of high doses of calcium-channel blockers on survival in primary pulmonary hypertension. *N Engl J Med* 1992; **327**: 76–81.
- Barst RJ. Pharmacologically induced pulmonary vasodilatation in children and young adults with primary pulmonary hypertension. *Chest* 1986; **89**: 497–503.
- D'Alonzo GE, Barst RJ, Ayres SM, et al. Survival in patients with primary pulmonary hypertension: results from a national prospective registry. *Ann Intern Med* 1991; **115**: 343–49.
- Barst RJ, Rubin LJ, Long WA, et al, for the Primary Pulmonary Hypertension Study Group. A comparison of continuous intravenous epoprostenol (prostacyclin) with conventional therapy for primary pulmonary hypertension. *N Engl J Med* 1996; **334**: 296–302.
- Badesch DB, Tapson VF, McGoon MD, et al. Continuous intravenous epoprostenol for pulmonary hypertension due to the scleroderma spectrum of disease: a randomized, controlled trial. *Ann Intern Med* 2000; **132**: 425–34.
- Hoepfer MM, Schwarze M, Ehlerting S. Long-term treatment of primary pulmonary hypertension with aerosolized iloprost, a prostacyclin analogue. *N Engl J Med* 2000; **342**: 1866–70.
- Okano Y, Yoshioka T, Shimouchi A, Satoh T, Kunieda T. Orally active prostacyclin analogue in primary pulmonary hypertension. *Lancet* 1997; **349**: 1365.
- MacLean MR. Endothelin-1: a mediator of pulmonary hypertension? *Pulm Pharmacol Ther* 1998; **11**: 125–32.
- Gali  N, Grigioni F, Bacchi-Reggiani L, et al. Relation of endothelin-1 to survival in patients with primary pulmonary hypertension. *Eur J Clin Invest* 1996; **26** (suppl 1): 48.
- Yamane K. Endothelin and collagen vascular disease: a review with special reference to Raynaud's phenomenon and systemic sclerosis. *Intern Med* 1994; **33**: 579–82.
- Giaid A, Yanagisawa M, Langleben D, et al. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N Engl J Med* 1993; **328**: 1732–39.
- Saleh D, Furukawa K, Tsao MS, et al. Elevated expression of endothelin-1 and endothelin-converting enzyme-1 in idiopathic pulmonary fibrosis: possible involvement of proinflammatory cytokines. *Am J Respir Cell Mol Biol* 1997; **16**: 187–93.
- Giaid A, Stewart DJ, Michel RP. Endothelin-1-like immunoreactivity in postobstructive pulmonary vasculopathy. *J Vasc Res* 1993; **30**: 333–38.
- Filep JG, Fournier A, F oldes-Filep E. Acute pro-inflammatory actions of endothelin-1 in the guinea-pig lung: involvement of ET_A and ET_B receptors. *Br J Pharmacol* 1995; **115**: 227–36.
- Park SH, Saleh D, Giaid A, Michel RP. Increased endothelin-1 in bleomycin-induced pulmonary fibrosis and the effect of an endothelin receptor antagonist. *Am J Respir Crit Care Med* 1997; **156**: 600–08.
- Chen SJ, Chen YF, Meng QC, Durand J, Dicarolo VS, Oparil S. Endothelin-receptor antagonist bosentan prevents and reverses hypoxic pulmonary hypertension in rats. *J Appl Physiol* 1995; **79**: 2122–31.
- Williamson DJ, Wallman LL, Jones R, et al. Hemodynamic effects of bosentan, an endothelin receptor antagonist, in patients with pulmonary hypertension. *Circulation* 2000; **102**: 411–18.
- Rich S. World Symposium on Primary Pulmonary Hypertension 1998, Evian, France. (www.who.int/ncd/cvd/pph.html, accessed on Sept 3, 1999).
- 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**: 919–23.
- 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.
- Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 1982; **14**: 377–81.
- Roux S, Breu V, Ertel SI, Clozel M. Endothelin antagonism with bosentan: a review of potential applications. *J Mol Med* 1999; **77**: 364–76.
- Mylona P, Cleland JG, for the Cardio.net Editorial Team. Update of REACH-1 and MERIT-HF clinical trials in heart failure. *Eur J Heart Fail* 1999; **1**: 197–200.
- Krum H, Charlton V, Widmann T, Packer M. Long-term, open-label experience with an endothelin receptor antagonist, bosentan, in patients with severe chronic heart failure. *Circulation* 1999; **100**: I-646.
- McCulloch KM, Docherty CC, Morecroft I, MacLean MR. Endothelin_B receptor-mediated contraction in human pulmonary resistance arteries. *Br J Pharmacol* 1996; **119**: 1125–30.
- Carratu P, Scuri M, Styblo JL, Wanner A, Glassberg MK. ET-1 induces mitogenesis in ovine airway smooth muscle cells via ET_A and ET_B receptors. *Am J Physiol* 1997; **272**: L1021–4.
- Katwa LC, Guarda E, Weber KT. Endothelin receptors in cultured adult rat cardiac fibroblasts. *Cardiovasc Res* 1993; **27**: 2125–29.
- Gater PR, Wasserman MA, Renzetti LM. Effects of Ro 47-0203 on endothelin-1 and sarafotoxin S6c-induced contractions of human bronchus and guinea-pig trachea. *Eur J Pharmacol* 1996; **304**: 123–28.
- Cardillo C, Kilcoyne CM, Waclawiw M, Cannon RO III, Panza JA. Role of endothelin in the increased vascular tone of patients with essential hypertension. *Hypertension* 1999; **33**: 753–58.