Combination of bosentan with epoprostenol in pulmonary arterial hypertension: BREATHE-2

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ABSTRACT: The efficacy and safety of combining bosentan, an orally active dual endothelin receptor antagonist and epoprostenol, a continuously infused prostaglandin, in the treatment of pulmonary arterial hypertension (PAH) was investigated.

In this double-blind, placebo-controlled prospective study, 33 patients with PAH started epoprostenol treatment (2 ng·kg^{-1}min^{-1} starting dose, up to 14±2 ng·kg^{-1}min^{-1} at week 16) and were randomised for 16 weeks in a 2:1 ratio to bosentan (62.5 mg *b.i.d* for 4 weeks then 125 mg *b.i.d*) or placebo.

Haemodynamics, exercise capacity and functional class improved in both groups at week 16. In the combination treatment group, there was a trend for a greater (although nonsignificant) improvement in all measured haemodynamic parameters. There were four withdrawals in the bosentan/epoprostenol group (two deaths due to cardiopulmonary failure, one clinical worsening, and one adverse event) and one withdrawal in the placebo/epoprostenol group (adverse event).

This study showed a trend but no statistical significance towards haemodynamics or clinical improvement due to the combination of bosentan and epoprostenol therapy in patients with pulmonary arterial hypertension. Several cases of early and late major complications were reported. Additional information is needed to evaluate the risk/benefit ratio of combined bosentan-epoprostenol therapy in pulmonary arterial hypertension.
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Pulmonary arterial hypertension (PAH) is an uncommon disease characterised by a progressive increase in pulmonary vascular resistance leading to right ventricular failure and death [1]. PAH can be idiopathic (referred to as primary pulmonary hypertension-PPH), or occur as a complication of various conditions, including scleroderma [2] or systemic lupus erythematosus [3]. The pathogenesis of PAH involves multiple and complex mechanisms triggered by endothelial dysfunction in the pulmonary bed, resulting in pulmonary vasoconstriction and vascular remodelling. An imbalance between vasoconstrictor/vasodilator activities in favour of vasoconstriction could be responsible for altered pulmonary vascular tone and structure [4]. In PAH patients, relaxing factors such as prostacyclin [5] are decreased and nitric oxide synthesis is impaired [6], whereas constricting factors including thromboxane [7], serotonin [8], and endothelin [9] are increased. Restoration of this imbalance by targeted therapies such as prostacyclin and endothelin receptor antagonists should further improve treatment options for the management of PAH.

Prostacyclin (epoprostenol), a potent pulmonary vasodilator, decreases pulmonary vascular resistance and improves the survival of patients with severe PAH [10–15]. Despite major improvements in prognosis, mortality in patients with severe PAH treated with epoprostenol is still high, emphasising the need for novel therapeutic approaches in this patient population. In addition, epoprostenol is associated with dose-related side-effects (*e.g.* diarrhoea, flushing, headache, jaw pain, hypotension) caused by systemic vasodilation and carries the risk of line sepsis and rebound PAH from inadvertent interruption of infusion [10]. It has been recently demonstrated that predictors of survival in epoprostenol-treated patients include, among others, improvements in haemodynamic variables on epoprostenol. The present authors hypothesised that addition of bosentan, an orally active dual endothelin receptor antagonist [16–18], could further improve haemodynamics in patients initiated on intravenous epoprostenol, and by inference improve long-term results. Additional bosentan therapy may also reduce the need to up-titrate the epoprostenol dose and, therefore, potentially decrease epoprostenol dose-related side-effects.

The objectives of the Bosentan Randomized trial of Endothelin Antagonist Therapy for PAH (BREATHE-2) were to investigate the efficacy and safety of the combination of bosentan and epoprostenol in the treatment of patients with severe PAH.

Patients and methods

Patient selection

Enrolled patients had severe PAH in modified New York Heart Association (NYHA) functional classes [19] III or IV

and were scheduled for epoprostenol therapy within 2 weeks of screening. PAH was either primary or associated with connective tissue disease. Patients were excluded if they had moderate to severe interstitial lung disease (*i.e.* total lung capacity <60% or high resolution computed tomography scan total score >2 [20]), if they had started or stopped any PAH treatment within 1 month of screening, or were receiving glibenclamide (glyburide), cyclosporine-A, and/or tacrolimus.

The study was conducted according to the Helsinki Declaration of 1975 and amendments, in adherence to the International Conference of Harmonization Good Clinical Practice Guidelines and to the US Federal Register (1997). The protocol was approved by the local ethics review committees and written informed consent was obtained.

Study design

The study was a double-blind, randomised, placebo-controlled trial and was conducted in four centres in the USA and three centres in Europe (France, Italy, and the Netherlands) (fig. 1). A total of 33 enrolled patients started epoprostenol treatment (2 ng·kg^{-1}min^{-1}). Two days later they were randomised to receive bosentan or placebo (2:1 ratio) and another 2 days later the epoprostenol dose was increased to 4 ng·kg^{-1}min^{-1}. The subsequent maximum dose increase of epoprostenol was 2 ng·kg^{-1}min^{-1} at 2-week intervals to reach a target dose of 12–16 ng·kg^{-1}min^{-1} between week 14 and 16. Upon randomisation, patients received either bosentan 62.5 mg *b.i.d* for 4 weeks followed by the target dose (125 mg *b.i.d*) or placebo. The double-blind study duration was 16 weeks.

Outcome measures

Patients were evaluated after 1, 4, 6 (European centres only), 8, 12, and 16 weeks of therapy. The primary efficacy parameter was change from baseline to week 16 in total pulmonary resistance (TPR), determined by right heart
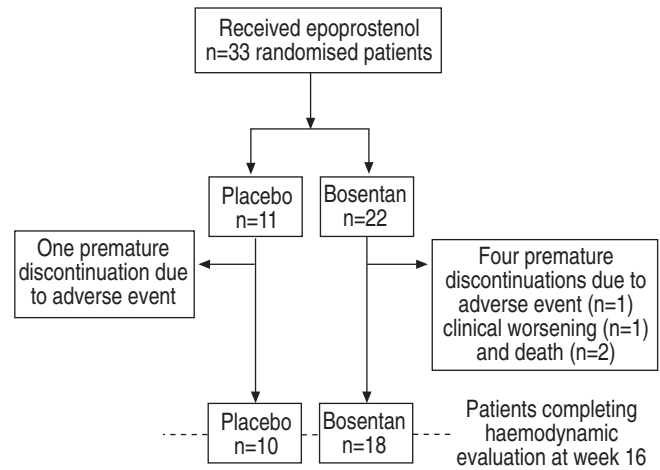

Fig. 1. – Study profile.

catheterisation. TPR is expected to provide prognostic information. A significant fall in TPR (30% relative to baseline value) has been reported to be predictive of improved survival after 3 months of epoprostenol therapy in PPH patients [11]. Secondary efficacy parameters included the change in cardiac index (CI), pulmonary vascular resistance (PVR), mean pulmonary artery pressure (mPAP), and mean right atrial pressure (mRAP). Secondary measures of efficacy also included the 6-min walk distance [21], the dyspnoea-fatigue rating [22] and modified NYHA functional class of PAH [19]. Safety was assessed by adverse event recording, laboratory assessment and electrocardiogram.

Statistical analysis

A sample size of 20 patients on bosentan and 10 on placebo was required to detect a treatment difference in the mean

Table 1. – Demographic characteristics at baseline in the placebo/epoprostenol and bosentan/epoprostenol groups (intent-to-treat population)

Characteristic	Placebo/epoprostenol	Bosentan/epoprostenol
Subjects n	11	22
M:F	5 (45):6 (55)	5 (23):17 (77)
Age yrs	47±19 (15–68)	45±17 (16–69)
Weight kg	78±16 (53–103)	70±21 (40–109)
Ethnic group		
Caucasian/White	10 (91)	18 (82)
Black	1 (9)	1 (5)
Asian		1 (5)
Other		2 (9)
Aetiology of PAH		
Primary	10 (91)	17 (77)
Scleroderma	1 (9)	4 (18)
Systemic lupus erythematosus		1 (5)
Modified NYHA functional class		
III	8 (73)	17 (77)
IV	3 (27)	5 (23)
Clinical signs of heart failure	4 (36)	10 (45)
Concomitant PAH medications (only when >4 in at least one group)		
Antithrombotic agents	10 (91)	19 (86)
High-ceiling diuretics	10 (91)	19 (86)
Potassium sparing agents	5 (45)	14 (64)
Cardiac glycosides	2 (18)	7 (32)
Calcium channel blockers	3 (27)	6 (27)
Use of supplemental oxygen	4 (36)	6 (27)
Time since diagnosis months	15±21 (1–61)	13±30 (1–138)

Data are presented as n (%) of patients or mean±SD (range). M: male; F: female; PAH: pulmonary arterial hypertension; NYHA: New York Heart Association.
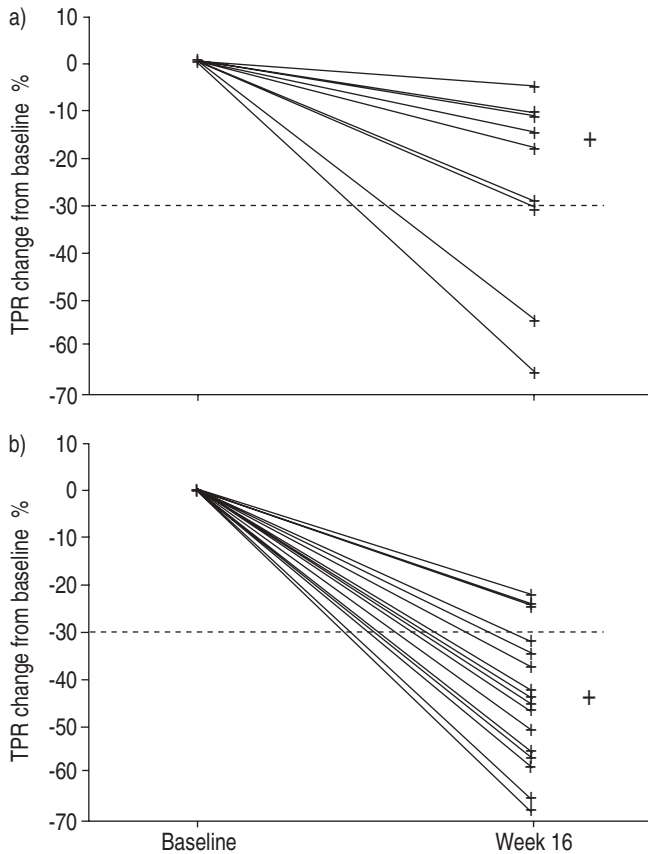
Fig. 2. – Total pulmonary resistance (TPR) change from baseline for patients on a) placebo/epoprostenol (n=10) and on b) bosentan/epoprostenol (n=19). +: median values for each group at week 16. Results presented are for patients who completed the 16-week study. The dashed line indicates a 30% reduction in TPR.

percentage change in TPR of 28% (α-probability=0.05; type-II error β=0.20, 80% power). Patients who discontinued study medication due to an adverse event, lung transplantation or death were analysed using the assessment recorded at the time of premature withdrawal. In the event that no assessment was recorded, patients were assigned the worst rank value. All other patients without a week 16 assessment were assigned a zero change from baseline for haemodynamic parameters and had their last 6-min walk distance, dyspnoea-fatigue rating, and modified NYHA functional class carried forward.

The significance levels of the differences between treatment groups were evaluated with the two-samples Student's t-test and, additionally, the Mann-Whitney U-test. Data are expressed as median or mean±SEM and demographic data as mean±SD.

Results

A total of 33 patients were included in the study (22 patients received bosentan/epoprostenol and 11 received placebo/epoprostenol) (fig. 1). One code break due to PAH worsening occurred before the week 16 assessment for a combined treatment patient who, thereafter, continued unblinded combined treatment.

Demographics and baseline characteristics

The study population was mostly composed of Caucasian PPH patients (table 1). All patients were in Class III (76%) or IV (24%) at baseline. Patients were taking at least one medication for PAH (mostly diuretics and anticoagulant agents). The bosentan/epoprostenol group included more women and more patients with scleroderma and clinical signs of heart failure.

Cardiopulmonary haemodynamics

TPR decreased from baseline to week 16 in both the bosentan/epoprostenol and the placebo/epoprostenol groups (fig. 2, table 2). The decrease in TPR was greater in the bosentan/epoprostenol group (-36.3±4.3%, mean±SEM) than in the placebo/epoprostenol group (-22.6±6.2%), although the treatment group difference was not statistically significant (p=0.08 with Student's t-test and Mann-Whitney U-test) (table 2). Due to the substitution rules for missing data and the small number of patients, the median may be a better indication of the potential difference between treatment groups. A greater treatment difference was observed in the median change (-648 dyn·s^{-1}cm^5 (-39.6%) for bosentan/epoprostenol versus -191 dyn·s^{-1}cm^5 (-14.3%) for placebo/epoprostenol).

Other haemodynamic parameters (CI, PVR, mPAP, and mRAP) improved from baseline in both treatment groups (table 2) and there were nonsignificant trends in favour of the bosentan/epoprostenol group.

Exercise capacity

Both treatment groups attained clinically relevant increases in the 6-min walk distance (68 m (median) in the

Table 2. – Haemodynamics for the placebo/epoprostenol and bosentan/epoprostenol groups at baseline and week 16 (intent-to-treat population)

Haemodynamic parameter	Placebo/epoprostenol			Bosentan/epoprostenol			p-value
	Baseline	Week 16	% change	Baseline	Week 16	% change	
TPR dyn·s^{-1}cm^5	1628±154	1242±153	-22.6±6.2	1697±142	1016±78	-36.3±4.3	0.08
CI L·min^{-1}m^2	1.7±0.2	2.3±0.2	37.9±13.3	1.7±0.1	2.5±0.1	48.7±11.0	0.6
PVR[¶] dyn·s^{-1}cm^5	1426±140	1050±154	-25.7±7.2	1511±129	947±104	-35.2±5.4	0.3
mPAP mmHg	60.9±2.9	59.2±3.2	-2.2±3.6	59.2±4.0	52.5±2.4	-9.0±6.0	0.3
mRAP mmHg	11.9±2.2	12.2±1.8	0.3±1.3[#]	11.9±1.1	10.0±1.2	-1.9±1.4[#]	0.7

Data are presented as mean±SEM. TPR: total pulmonary resistance; CI: cardiac index; PVR: pulmonary vascular resistance; mPAP: mean pulmonary artery pressure; mRAP: mean right atrial pressure. Note: the two bosentan patients who died and the two patients (one bosentan and one placebo) who were withdrawn were assigned the worst value observed at the 16 week time point or at withdrawal in all patients belonging to the same analysis population. [#]: absolute change in mRAP; changes in mRAP are more meaningful when expressed as absolute changes rather than per cent changes because of the small absolute values and the large variation in the per cent changes due to the substitution rules; [¶]: one placebo/epoprostenol and two bosentan/epoprostenol patients did not have a PVR calculation because of missing pulmonary artery wedge pressure. n=11 (placebo/epoprostenol) and 22 (bosentan/epoprostenol).

bosentan/epoprostenol group *versus* 74 m (median) in the placebo/epoprostenol group) (fig. 3). The median dyspnoea-fatigue ratings improved by 1.0 unit in the placebo/epoprostenol

Fig. 3. – Six-minute walk distance (6MWD) for the placebo/epoprostenol (n=10) and bosentan/epoprostenol (n=19) groups at a) baseline and b) week 16 (intent-to-treat population). Data are presented as a) mean±95% CI and b) median±95% CI. Two patients on bosentan/epoprostenol therapy were assigned a 0-m walk distance and a 0-dyspnoea-fatigue rating at week 16. Two patients (one on placebo/epoprostenol and one on bosentan/epoprostenol) were too impaired to walk and two patients on bosentan/epoprotenol did not perform the assessment. These patients were not included in the analysis.

Table 3. – Adverse events in the placebo/epoprostenol and bosentan/epoprostenol groups observed for ⩾three patients until 28 days after the end of the study (safety population)

Adverse event	Placebo/ epoprostenol	Bosentan/ epoprostenol
Subjects n	11	22
Pain in jaw	10 (91)	13 (59)
Diarrhoea	3 (27)	12 (55)
Flushing	5 (45)	6 (27)
Headache	4 (36)	6 (27)
Oedema lower limb	1 (9)	6 (27)
Limb pain	2 (18)	5 (23)
Nausea	2 (18)	4 (18)
Dermatitis	1 (9)	4 (18)
Cardiac failure	2 (18)	3 (14)
Upper respiratory tract infection	1 (9)	3 (14)
Abnormal hepatic function	2 (18)	2 (9)
Cough	1 (9)	2 (9)
Dizziness	1 (9)	2 (9)
Dyspnoea	1 (9)	2 (9)
Epistaxis	1 (9)	2 (9)
Myalgia	1 (9)	2 (9)
Worsening PHT	2 (18)	1 (5)

Data are presented as n (%) of patients. PHT: worsening pulmonary hypertension.

group and did not change in the bosentan/epoprostenol group. The treatment group differences for the walk test and dyspnoea-fatigue ratings were not statistically significant. These results were obtained after assigning a 0-m walk distance and a 0-dyspnoea-fatigue rating at week 16 to two patients on bosentan/epoprostenol therapy, which decreased the mean walk performance of the bosentan/epoprostenol group.
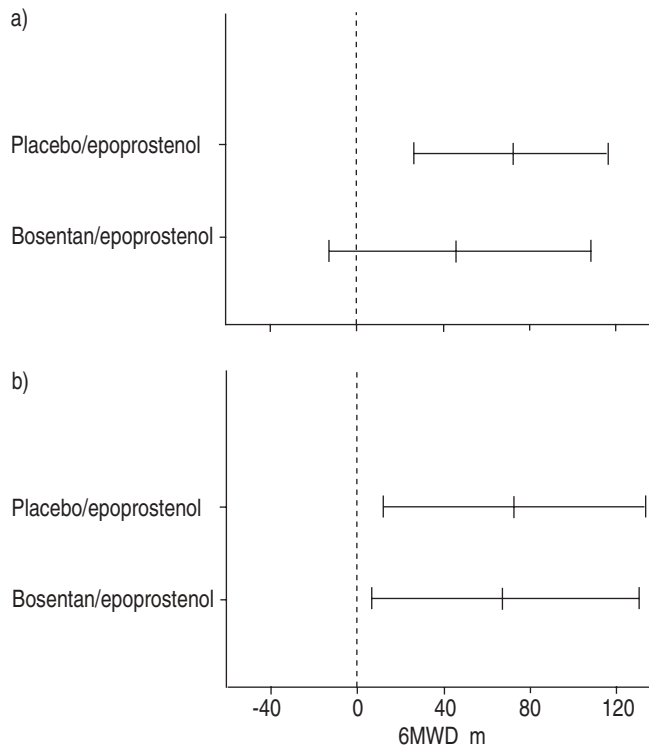
Modified NYHA functional class

Functional class of PAH improved from baseline to week 16 for 13 patients (59%) in the bosentan/epoprostenol group and for five patients (45%) in the placebo/epoprostenol groups (fig. 4). Among these improved patients, four bosentan/epoprostenol patients and one placebo/epoprostenol patient were initially in Class IV. The treatment group difference was not statistically significant.

Safety and tolerability

The most frequently reported adverse events were those known to be associated with epoprostenol therapy (jaw pain, diarrhoea, flushing, and headache) (table 3). Except for diarrhoea, these adverse events were more frequent in the placebo/epoprostenol group. The only adverse event associated with bosentan therapy that occurred more frequently in patients treated with bosentan/epoprostenol than in those on epoprostenol alone was leg oedema (27% *versus* 9%). A clinically relevant decrease in haemoglobin concentration was observed in one patient receiving placebo/epoprostenol. Abnormal hepatic function (asymptomatic increases in the level of hepatic transaminases), which has been reported in previous trials with bosentan, was more frequent in the present study in the placebo/epoprostenol (18%) group than in the bosentan/epoprostenol group (9%). Two patients, one in each treatment group, were withdrawn from the study because of increases in hepatic transaminases.

The number of serious adverse events associated with PAH (cardiopulmonary failure) was similar in the two treatments groups (14% in the bosentan/epoprostenol group *versus* 18% in the placebo/epoprostenol group).

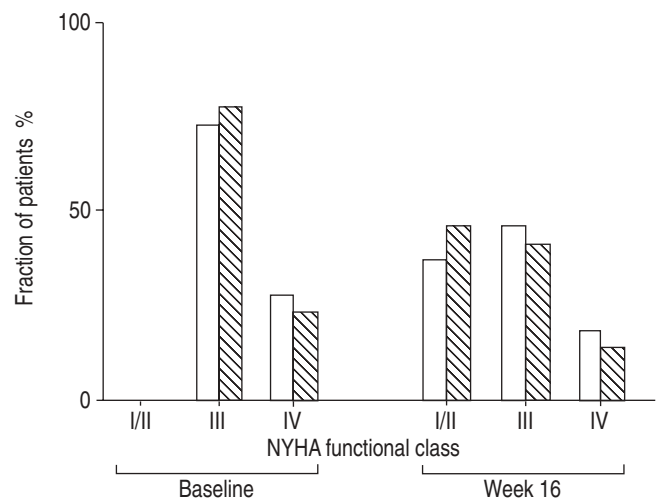Two patients receiving bosentan/epoprostenol treatment

Fig. 4. – Modified New York Heart Association (NYHA) functional class for the placebo/epoprostenol (□) and bosentan/epoprostenol (▨) groups at baseline and week 16 (intent-to-treat population).

Table 4. – Vital signs for the placebo/epoprostenol and bosentan/epoprostenol groups at baseline and week 16 (safety population)

Vital signs	Placebo/epoprostenol			Bosentan/epoprostenol		
	Baseline	End of treatment	Absolute change	Baseline	End of treatment	Absolute change
Heart rate bpm	85.2±4.5	91.8±3.3	6.6±3.6	83.8±2.1	83.3±1.8	-0.5±3.0
Systolic BP mmHg	120.4±4.8	110.5±4.9	-9.8±3.3	110.2±2.7	106.3±2.6	-3.9±3.1
Diastolic BP mmHg	75.9±4.6	70.0±3.3	-5.9±2.8	71.9±2.3	65.9±2.3	-6.0±2.7

Data are presented as mean±SEM. BP: blood pressure; bpm: beats per minute. n=11 (placebo/epoprostenol) and 22 (bosentan/epoprostenol).

died during the study; one class IV patient with PAH due to systemic sclerosis died from acute cardiopulmonary failure, the other patient (baseline class III PPH) became anaemic and subsequently developed pneumonia with rapidly progressing right heart failure. A third patient receiving bosentan/epoprostenol treatment died after being withdrawn from the study for PAH worsening. These deaths were not considered related to study treatment by the clinical investigators, but rather reflected the severity and progressive nature of PAH.

Although the two groups received similar doses of epoprostenol, the haemodynamic improvement in the placebo/epoprostenol group was associated with an increase in heart rate (6.6±3.6 mean±SEM), while in the bosentan/epoprostenol group a similar improvement was achieved with no increase in heart rate (-0.5±3.0 mean±SEM) (table 4). Decreases in blood pressures were observed in both groups (table 4) although the decrease in systolic blood pressure was less in the bosentan/epoprostenol group than in the placebo/epoprostenol group. Hypotension was reported for two patients both of whom were in the placebo/epoprostenol group.

Discussion

This is the first double-blind, randomised, placebo-controlled study combining an endothelin receptor antagonist with intravenous epoprostenol as initial therapy in NYHA class III and IV PAH patients. As expected from previous studies with epoprostenol [10–15] and with bosentan [16–18], both epoprostenol and combined bosentan/epoprostenol treatments improved haemodynamics, exercise capacity and functional class at week 16 in patients with severe PAH. The combination of bosentan and epoprostenol resulted in nonstatistically significant trends toward improvement in all haemodynamic variables compared to treatment with epoprostenol/placebo. In addition, no significant difference could be established between treatment groups in exercise capacity (walk distance and dyspnoea-fatigue rating) or NYHA functional class. The study was not powered to detect such changes. The absence of significant differences regarding primary and secondary endpoints could possibly be explained by the small sample size. Treatment efficacy may also have been lessened in the bosentan/epoprostenol group compared to the placebo/epoprostenol group due to the presence of a larger percentage of scleroderma patients (18% versus 9%, respectively) for which morbidity and mortality remains quite high [23]. Previous studies have shown that, while bosentan improves the exercise capacity of PPH patients, it prevents the rapid deterioration of scleroderma patients [18]. Scleroderma patients have a worse prognosis than PPH patients even with similar baseline haemodynamics [24]. In the present study, one death and one withdrawal due to PAH worsening occurred in scleroderma patients, which may reflect the poor prognosis of these patients.

Substitution rules were applied for two patients who died on bosentan/epoprostenol during the study period, which resulted in large decreases in walk distance for these patients

and a skewed distribution for the treatment group. The first patient died at day 15 during the initial titration period and did not receive full doses of either epoprostenol or bosentan. The second one died at day 111 after several complications including severe epistaxis, anaemia, and pneumonia. Analysis of these cases by the clinical investigators concluded that the deaths were not related to the combination treatment but rather reflected the severity and progressive nature of PAH.

The most frequent adverse events (jaw pain, diarrhoea, flushing, and headache) were those known to be associated with epoprostenol therapy [10, 12]. The only adverse event associated with bosentan therapy that occurred more frequently in the combination therapy group was leg oedema, possibly related to a peripheral effect since the mean right atrial pressure tended to be reduced. Abnormal laboratory results including decreased haemoglobin concentration and elevated hepatic transaminases, have been reported in previous bosentan trials [16, 18]. Decreased haemoglobin concentration is not uncommonly observed with vasodilator therapy due to fluid retention resulting in haemodilution. The incidence of elevated hepatic transaminases was similar in the two groups (9% of bosentan/epoprostenol patients versus 18% in the placebo/epoprostenol group).

Interestingly, the combination therapy appeared to have a lower occurrence of the side-effects usually associated with epoprostenol (jaw pain, flushing, and headache). The combination therapy also induced less systemic hypotension than epoprostenol alone and had no significant effect on heart rate which, in contrast, increased with epoprostenol alone. These data suggest that bosentan may blunt activation of the sympathetic nervous system thereby reducing reflex tachycardia, a phenomenon that has also been observed with other neurohormonal antagonists such as ACE inhibitors [25] and β-blocking agents [26] and may require further investigation.

Pharmacokinetic interaction is unlikely to contribute to the profile of the combination therapy, since both drugs have different metabolic and excretion profiles [27, 28]. In addition, a recent study in children with PAH reported no significant effect of epoprostenol on the pharmacokinetics of bosentan [29], although any potential effects of bosentan on epoprostenol concentrations were not studied.

Bosentan in combination with prostanoids may be of interest in clinical practice, as recently suggested by open series where, in contrast to the present study, bosentan was added to a long-standing prostanoid treatment. Consideration of adding bosentan when the efficacy of prostanoid therapy is deemed unsatisfactory requires further investigation. However, HOEPER et al. [30] have reported improvements of 6-min walk distance and peak oxygen consumption in patients with severe PAH treated with bosentan in addition to inhaled iloprost or oral beraprost. Additionally, an additive effect may allow the reduction of the dose of epoprostenol and hence its related side-effects. However, it remains to be seen if the benefit for the patient outweighs the increase in therapeutic cost. The main limitation of the current study is the small number of patients enrolled, which may have prevented

statistically significant discrimination between the two treatment groups. In addition, a total of five patients were withdrawn from the study: there were four withdrawals in the bosentan/epoprostenol group (two deaths due to cardiopulmonary failure, one clinical worsening, one adverse event due to elevated levels of hepatic transaminases) and one withdrawal in the placebo/epoprostenol group (adverse event due to elevated levels of hepatic transaminases). The deaths and clinical worsening in the bosentan/epoprostenol group were attributed to the progression of the severe PAH. A definitive conclusion on the safety of the combination therapy could not be drawn.

The objective of the present study was to detect potential additive effects of the combination of bosentan and epoprostenol treatments. Therefore, simultaneous initiation of bosentan and epoprostenol along with controlled uptitration of both drugs was attempted for all patients. Alternatively, one treatment could have been added after stabilisation with the previous therapy. However, starting bosentan treatment for patients on stable epoprostenol therapy could have led to the enrolment of patients under a wide range of epoprostenol doses since no standardised treatment dosage is recognised.

In conclusion, the combination of bosentan and epoprostenol therapies may be a therapeutic option for the management of patients with severe pulmonary arterial hypertension. Although the findings from this randomised, placebo-controlled study indicate a nonsignificant trend for a greater improvement in haemodynamic parameters in favour of the combined therapy, the results should be interpreted with caution. Power was the major limitation of this study, which enrolled only 33 patients. Larger trials designed to address long-term efficacy and safety of bosentan plus epoprostenol in appropriately selected patients are required to confirm the value of this novel therapeutic strategy.

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