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Safety and tolerability of bosentan in Idiopathic Pulmonary Fibrosis: open label study

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Abstract

Idiopathic Pulmonary Fibrosis (IPF) is a fatal disease, for which no effective treatment exists. Twelve IPF patients underwent analysis of gas exchange properties by the multiple inert gas elimination technique (MIGET) on day 1 before and after 125mg bosentan, a dual endothelin antagonist, followed by chronic administration for 12 weeks (62.5mg bid week 1, 125mg bid thereafter). Primary objective was the effect of bosentan on gas exchange (day 1) and on oxygen saturation and minute ventilation (week 2).

With one exception, where redistribution of total pulmonary blood flow from normal ventilation/perfusion (V_A/Q) areas (93% pre, 72% post) to low V_A/Q areas (0% pre, 22.2% post) was encountered, no patient showed any change in gas exchange (shunt flow 8.5±3.4% before, 6.1±2.3% after bosentan [% of cardiac output, mean ±SD]; day1) or oxygen saturation and minute ventilation (week 2). Similarly, none of the secondary parameters was significantly changed at week 2 and at the end of the study period (week 12). 5 patients developed respiratory infections and 2 died because of pneumonia and this was judged as being unrelated to bosentan intake. In conclusion, bosentan administration seems not to induce clinically relevant gas exchange abnormalities in IPF patients.

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Key Words: Bosentan– endothelin antagonism – idiopathic pulmonary fibrosis – respiratory function test - MIGET

Introduction

Idiopathic Pulmonary Fibrosis (IPF) is a progressive, life-threatening lung disease of unknown aetiology [1]. Increased production of endothelin (ET) [2] has been suggested to contribute to the development of IPF and such assumption is underscored by experimental data. In detail, it had been observed that the paracrine lung ET system is activated in animal models of pulmonary fibrosis [3, 4], and ET-1 is increased in airway epithelium and type-II pneumocytes in patients with IPF compared with control subjects and patients with nonspecific fibrosis [2]. Finally, studies employing the ET-1 transgenic mouse suggested an etiologic role for ET-1 in pulmonary fibrosis [5], since these overexpressing mice develop lung fibrosis spontaneously within 9-12 months of age.

Bosentan, a non-peptide, dual receptor antagonist, is approved for the treatment of pulmonary arterial hypertension in many countries worldwide [6]. Bosentan has a high affinity to both, ET_A and ET_B receptors [7] and blocks the binding of ET-1 comprehensively. In experimental models of pulmonary fibrosis, bosentan prevented pulmonary scarring, as demonstrated by a reduction of the volume fraction of connective tissue and an increase in the volume fraction of air [3]. Bosentan, however, exerts vasodilatation to the pulmonary circulation. As systemically active vasodilators have been shown to interfere with ventilation/perfusion (V_A/Q) matching in patients with fibrotic lung disease [8], there was some concern that bosentan may have similar disadvantageous effects.

Against this background the current study was performed to investigate the safety and the tolerability of an oral administration of bosentan in 12 patients with IPF. At the beginning of the study the acute effects of bosentan on gas exchange properties were assessed by means of the multiple inert gas elimination technique (MIGET). Thereafter patients were treated for 3 months and gas exchange, lung function, exercise capacity and quality of life were assessed

and compared to baseline. We found that bosentan applicatin was not followed by induction of V_A/Q mismatch and that it could be safely administered in IPF subjects.

Materials and Methods

Patient selection

12 patients were enrolled into this open-label, single centre, non-comparative study at the Medical Clinic of the Justus-Liebig-University Giessen between February 2002 and January 2003. The study was approved by the local ethics committee and informed consent was obtained from all participants prior to inclusion. The following inclusion criteria applied: diagnosis of IPF according to the ATS/ERS Consensus Criteria for diagnosis of IPF [1], ≥18 years of age, forced vital capacity (FVC) between 50 and 90% of predicted, decline of FVC values by ≥ 10% in the previous year despite steroid/immunosuppressive treatment, diffusion capacity for carbon monoxide (DL_{CO}) > 35% of predicted, partial pressure of arterial oxygen (PaO_2) > 55mmHg and systolic blood pressure ≥ 85mmHg. Major exclusion criteria included pregnancy, breast feeding, underlying severe liver or systemic collagen/vascular disease and current treatment with glibenclamid (glyburide), tacrolimus or cyclosporine A, the latter three because of known interference with bosentan.

Study protocol

The study consisted of a screening phase (maximum of 2 weeks), a 3-part treatment phase (maximum of 12 weeks in total) and an optional extension phase (see Figure 1).

Oral bosentan (Tracleer®, Actelion Pharmaceuticals, Allschwil, Switzerland) at 62.5mg was given twice a day during the first week and escalated to 125mg given twice a day with the beginning of the second week. Treatment was continued until week 12, when screening and baseline investigations were repeated. Patients could then be enrolled into an optional study extension phase, in which visits were performed every 3 months.

Methods

On the first day, right heart catheterisation was undertaken, employing a Swan-Ganz Catheter (Baxter Health care, Irvine, California) and conventional thermodilution technique [8]. During this right heart catheterisation pulmonary vascular response was evaluated by a short term inhalative nitric oxide (NO) run (started at 5 ppm, with stepwise increase to 10-15 ppm) and 4 hours after application of a single dose of bosentan 125mg. Moreover, Va/Q matching was assessed using the multiple inert gas elimination technique (MIGET) as previously described [8]. Parameters obtained by these techniques included: cardiac output (CO), cardiac index (CI), central venous pressure (CVP), pulmonary vascular resistance (PVR), systemic arterial resistance (SAR), pulmonary capillary wedge pressure (PCWP), heart rate (HR), mean pulmonary artery pressure (MPAP), mean systemic arterial pressure (MSAP), oxygen saturation (O_2 Sat), minute ventilation (MV), pulmonary shunt flow, perfusion of low V_A/Q, normal V_A/Q and high V_A/Q areas (in % of total perfusion), arterial blood gas analysis including PaO_2, $PaCO_2$, pH, base excess (BE).

Treatment was continued until week 12. At day 1 and week 12, body weight, lab tests, vital signs, pulmonary function and oxygen saturation were recorded. Concomitant medication was recorded and is indicated in the online data supplement.

Endpoints

The primary safety endpoint was a combined one assessed at day 1 and at the end of week 2. Bosentan was considered as well tolerated if the following criteria were NOT met: (1) The arterial PaO_2 decreased by > 10% or the cardiac output decreased by > 20% below their respective baseline values (day 1). Prior to the first amendment of this study, also an increase in perfusion of low V_A/Q areas or shunt flow by more than 20% was defined as criterion, but later omitted in order not to needlessly discontinue patients who had variable MIGET

measurements but no consistent evidence for clinically relevant V_A/Q mismatch. (2) The minute ventilation increased by > 20% from the baseline value (within first 2 weeks). (3) The O_2 saturation at rest decreased by > 5% from the baseline value (within first 2 weeks).

Endpoints assessing additional safety parameters included changes in the MIGET data obtained during right heart catheter testing on day 1 (% shunt perfusion, % perfusion of low V_A/Q areas, standard deviation of V_A and Q), changes in MV, O_2 Sat, heart rate (HR) and blood pressure (BP), body weight and incidence of adverse events (AEs) and serious adverse events (SAE) and laboratory abnormalities. In addition aspartate and alanine aminotransferase (AST and ALT) had to be closely monitored according to the protocol, which was in line with the guidelines in the summary of product characteristics.

Endpoints assessing efficacy included changes in lung function (TLC, VC, FVC, FEV1, RV), CO diffusion capacity, 6-minute walk distance and Borg dyspnea score (exercise capacity) as well as quality of life (measured with the EQ 5D questionnaire and Mahler index [9, 10]).

For determination of SP-D, a surrogate parameter for severity of disease, blood was collected from the antecubital vein at baseline and after 12weeks and serum was aliquoted and stored at -80°C until assay performance. SP-D in serum was quantified using a sandwich type ELISA system with two monoclonal antibodies (IE11, VI F11-Biotin, BMA, Heidelberg, Germany). The detection limit was 100 pg SP-D per ml serum. Assays were performed in duplicate for each sample.

Statistical analysis

The sample size of 12 patients was based on empirical considerations and on previous publications in the field [11]. Intention to treat analysis was performed. For this purpose, quantitative secondary safety and efficacy endpoints were summarized using location and scale statistics together with 95% confidence limits (CL) for the mean. Qualitative variables

were summarized using frequency counts and proportions. Exploratory p-values were provided using the paired signed rank test. Observed p-values were compared to the standard nominal two-sided 0.05 alpha level with no corrections for multiplicity of endpoints due to the exploratory nature of the testing. Statistical Analysis System software (SAS Institute, Cary, NC, USA) was used for the analysis of the efficacy and safety data. For more details see online data supplement..

Results

Twelve patients were included into the study, with a mean age of 60.6 ± 9.5 years and an IPF diagnosis based on either open lung biopsy (n=5) or on clinical grounds (n=7). Patients had a mean FVC value of 56% of predicted and a mean DL_{CO} value of 37% of predicted (see table 1) and the mean loss of FVC ranged at 11.2% per year (see table 1).

Right heart catheterisation on day 1

On day 1, patients underwent right heart catheterisation and MIGET analysis of gas exchange. Distribution of V_A and Q (see table 2) and pulmonary and systemic hemodynamics (see table 3) were assessed under resting conditions, after NO application and a single dose of 125mg bosentan, respectively. As depicted in table 2, IPF subjects showed a narrow distribution of ventilation and perfusion around V_A/Q ratios of 1, suggesting an almost optimum matching of perfusion to ventilation despite the advanced underlying lung disease. Shunt flow was, however, increased to ~8.5% of total perfusion, thus representing the predominant gas exchange abnormality. Sequential application of inhalative NO at a dosage of 10-15 ppm and a single dose of 125mg bosentan orally did not substantially change this V_A/Q distribution when averaging all 12 patients. In the sum of patients, some significant decrease of shunt flow was encountered upon bosentan intake (table 2), but this was paralleled by a non significant increase in perfusion of low VA/Q areas. In contrast to the overall missing effect of bosentan on ventilation/perfusion distribution, one of the first patients being studied showed a re-distribution of perfusion from normal V_A/Q areas (93% pre to 72% post) to low V_A/Q areas (0% pre, 22.2% post) after receiving the single dose of bosentan, whereas shunt flow did not change considerably (6.9% pre, 5.5% post). To some extent, these changes were already encountered under NO administration (perfusion of normal V_A/Q 86%, of low V_A/Q areas 6.2%, shunt flow 8.3%). Except from these MIGET data, the patient did not show any

relevant deterioration with regard to clinical, hemodynamic or gas exchange data. However, this patient had to be permanently withdrawn from the study because the primary safety parameter was also based on MIGET data (perfusion to shunt or low V_A/Q areas should not increase by more than 20% of total CO) in the beginning of the study.

Later on the primary safety parameter was changed with an amendment to the study protocol as described earlier. Except for this patient, no other patient showed any similar response to bosentan or NO, as also evident from the values depicted in table 2. In view of the hemodynamics, patients revealed very mild pulmonary arterial hypertension, with an average PVR value of 209 dyn*sec/cm^5 and a mean PAP value of ~22mmHg. Both NO and bosentan were able to significantly reduce PVR and PAP (see table 3). CI, pulse rate and SVR remained largely unaffected by both, NO and bosentan.

Chronic treatment

From the 12 patients enrolled in the study, 11 patients continued bosentan treatment after right heart catheterisation on day 1. For the first week, 62.5mg twice daily were applied, followed by an up-titration to 125mg twice daily after the first week. Bosentan intake did not affect pulmonary function tests including total airway resistance (data not given in detail), O2 saturation and minute ventilation at the end of week 2 (see table E3 of online data supplement) as compared to baseline.

Similarly, within the three months treatment period no significant change of lung function, exercise capacity and quality of life and dyspnoe scores was encountered in the 11 patients, with some tendency towards deterioration of FVC and DLco values (see table 4, table E3 of the online supplement).

In general, chronic bosentan treatment was well tolerated. Patients reported of leg edema in 2 cases, and mild abnormal liver function was encountered in one patient, which made

temporary discontinuation of the study drug necessary. A mild drop of haemoglobin values was observed in two patients. Asymptomatic hypotension was reported in one patient on day 11, positional vertigo on day 22 in another patient (see table 5). During the 3-month study, overall 7 patients developed respiratory tract infections with a need of antibiotic treatment, of whom 2 patients ultimately died from severe pneumonia. Detailed narratives are available in the online supplement. As can be taken from these narratives, both subjects had an already advanced course of the disease, had experienced a rapid progression prior to entering the study and were on oral steroids and immunosuppressants (table 5). Of the 11 patients initially undergoing chronic treatment, 4 entered the optional extension phase after three months. Two of these 4 patients died due to severe pneumonia on day 234 and on day 531, the other 2 patients are still on bosentan (125mg twice daily). One of these patients developed two episodes of respiratory tract infections necessitating oral antibiotic treatment within the extension phase.

Serum levels of Surfactant Protein D, assessed at baseline and at the end of the regular study period, decreased by 30.2% in 7 out of 8 patients where both samples were available ($p=0.0447$, see figure 2).

Discussion

In the current study the dual ET_A and ET_B receptor blocker, bosentan, 125mg bid, was investigated primarily for safety and tolerability in human subjects with IPF. It was found that, except of one patient, in whom re-distribution of pulmonary blood flow into low VA/Q areas in absence of any change in hemodynamics, PaO2 or clinical condition was observed, none of the other patients did show any influence of bosentan intake on gas exchange on day 1 or saturation and minute ventilation at week2. Similarly, lung function and exercise capacity remained stable over a period of 12weeks and bosentan was well tolerated. During the regular study period 5 respiratory infections occurred and 2 patients were dying from pneumonia and this was primarily found to be unrelated to bosentan intake.

Bosentan has been investigated in a variety of clinical settings and is approved for PAH in Europe, the United States and Japan and other countries. The approved dose for PAH is 125 or 250mg twice daily although doses of up to 2000mg have been investigated in different indications. Common side effects include flushing, abnormal hepatic function, leg oedema, headache and anaemia. In addition, bosentan treatment may initially result in a dose-dependent, slight reduction of systemic blood pressure.

One may anticipate that in IPF patients with an advanced course of the disease and far-reaching alterations of the lung's architecture a mismatch of ventilation and perfusion (V_A/Q mismatch) may contribute to gas exchange abnormalities. As performed in this study, such V_A/Q mismatch may best be characterized using the multiple inert gas elimination technique (MIGET), which has been used in the past in patients with ARDS [12] and interstitial lung diseases [8]. In accordance with previous studies, the herein investigated IPF subjects showed evidence of a profound V_A/Q mismatch, with a mean shunt flow of 8.5% of total perfusion in

absence of a significant perfusion of low V_A/Q areas. Such shunt flow may largely determine the alveolar-arterial oxygen gradient, as has been suggested recently [13]. It is almost self-evident that introduction of any systemic vasodilatory principle may be of harm in these patients due to interference with the hypoxic pulmonary vasoconstriction. In fact, such disadvantageous effect had already been described for patients with interstitial lung diseases and secondary pulmonary hypertension upon use of systemic prostacyclin [8]. In the current study, oral application of 125mg bosentan did not result in any change in the V_A/Q distribution with the exception of one patient, who showed a marked increase in the perfusion of low V_A/Q areas after bosentan intake. This patient, however, did neither show any clinical signs of hypoxemia (increased dyspnea or breathing effort, increased cyanosis) nor did he show significant alterations in the other measurable parameters (desaturation, drop of arterial pO_2, change in CO), suggesting that the disturbances in V_A/Q distribution were not profound enough to forward clinical changes at rest. They might, however, have reached clinical importance under physical exercise, which was not tested in this study, because the patient was withdrawn in accordance the original study protocol. In view of the MIGET and hemodynamic data from all bosentan treated patients, we conclude that – similar to observations made with sildenafil intake in patients with lung fibrosis [14] - application of 125mg bosentan in IPF subjects in general seems to be well tolerated in patients with mild to moderate IPF. We feel it may nevertheless be necessary to assess exercise capacity shortly after first bosentan administration in subsequent studies in order to get some more insight into the relevance of the V_A/Q mismatch observed in one patient with IPF in this study.

Another important objective of this study was to evaluate the tolerability and safety of a chronic application of bosentan over 12 weeks in IPF subjects. In general, the reported AEs (or safety profile) were comparable to those previously been published in other studies

employing bosentan [15]. They included symptoms such as flushing, abnormal hepatic function, leg oedema, headache and anaemia, and all were reversible upon down titration of bosentan or upon cessation of the study drug application. However, respiratory infections and pneumonia were recorded in 7 cases (see table 5) and, in fact, two patients receiving bosentan during the 3-month study period died due to severe pneumonia (see narratives in online supplement) and two during the extension phase. In general, respiratory infections are not uncommon in IPF subjects and both, the underlying disease as well as the frequently used steroid/immunosuppressant therapy may favour these events. In detail, it has been shown that patients with end-stage pulmonary diseases undergoing transplantation are frequently colonized with haemophilus influenzae [16]. Next to the architectural changes that may account for such colonization, loss of the lung-specific host defence properties (Collectins SP-A, SP-D, α-defensins [17, 18, 19]) or suppression of effector T-cell and activated macrophage function by TGF-β [20] may account for such increased susceptibility towards bacterial strains. As an alternative explanation, acute exacerbation of IPF may have occurred in our patients, as suggested previously [21, 22]. However, in view of the clinical course, with dramatic deterioration of the patients within 1-2 days, the proof of systemic infection by highly elevated CRP values and the also of infectious agents, we do believe that infection and not acute exacerbation was the underlying cause of deterioration in our patients. In the recently published interferon γ-1β trial, the frequency of respiratory tract infections, severe pneumonias and death due to severe pneumonia was reported to be 56%, 8% and 1.8% respectively in the placebo group (n=168) [23]. Thus, respiratory infections are not uncommon in IPF. Although we think that the overall number of patients studied herein is much too small to give any robust information, we can, of course, not definitely rule out the possibility that bosentan treatment may increase the susceptibility to lung infection. In the recently finished and larger BUILD 1 study, no lung infection excess was attributed to

bosentan [I. Leconte, S. Roux, personal communication]. In addition, a literature search did not forward any specific concern in this regard. We therefore do believe that the rate of infection observed in this study in bosentan-treated patients is not much different from that in untreated IPF subjects, but respiratory infections should be carefully monitored in future studies employing bosentan in IPF.

Finally, this study was not designed for evaluation of efficacy and the available data clearly do not allow any final conclusion regarding this aspect. Short-term observation over a 3-months study period was, however, possible in 9 out of 12 patients and did not indicate a significant change within this treatment period. SP-D values in serum were significantly down-regulated under bosentan treatment in a subset of enrolled patients in whom serum samples were available from baseline and end of study and this may indicate some beneficial action of bosentan on the underlying disease process and thus a better prognosis [19]. In light of the overall limited influence of other treatment modalities in IPF [23-25], further assessment of the therapeutic efficacy of bosentan in IPF seems to be warranted.

We conclude that bosentan treatment appears to be well tolerated in IPF patients. Gas exchange properties under exercise and susceptibility towards respiratory infections should, however, be followed up very closely in the forthcoming phase III trials.

Acknowledgments

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Table 1 Baseline characteristics of study patients and outcome

Characteristic	Value
Total number of patients	12
Age	60.6 ±9.5 years
Male/female	9 / 3
Smoking habits	
Never smoker	4
Ex smoker	7
Current smoker	1
FVC at screening	56 ±15 (% of predicted)
Preceding deterioration of FVC	-11.2 ±15.7% in 220 ±178days
DLco at screening	37 ±10 (% of predicted)
Completed study (12 week)	9
Death (due to severe pneumonia)	2
Severe V_A/Q mismatch d1	1

Data provided as means ± SD, unless otherwise specified.

d, day; DLco, diffusion capacity for carbon monoxide; FVC, forced vital capacity; SAE, Serious Adverse Event; Q, perfusion; Va, mean ventilation.

Table 2 Gas Exchange upon NO and Bosentan application

Parameter	Baseline n=12	NO n=12	Bosentan n=12
Shunt flow [% of CO]	8.48 (6,32;10,65)	11.05 (5.28;16.82)	6.12 ** (4.64;7.59)
Low V_A/Q perfusion [% of CO]	0.33 (-0.22;0.87)	1.83 (-0.37;4.02)	2.79 (-1.53;7.11)
Normal V_A/Q perfusion [% of CO]	91.13 (88.96;93.29)	87.12 (79.56;94.67)	91.08 (86.97;95.19)
Mean V_A	1.06 (0.76;1.36)	1.03 (0.80;1.26)	1.01 (0.80;1.23)
Mean Q	0.69 (0.55;0.83)	0.62 (0.49;0.74)	0.56 (0.44;0.69)
Log SDV_A	0.62 (0.49;0.74)	0.64 (0.51;0.77)	0.70 (0.59;0.81)
Log SDQ	0.62 (0.50;0.73)	0.70 (0.49;0.91)	0.76 (0.56;0.95)
Minute ventilation [l/min]	6.83 (5.53;8.12)	6.59 (5.53;7.65)	6.75 (5.50;8.00)
O_2 saturation [%]	97.22 (95.99;98.44)	95.96 (94.49;97.42)	96.29 (94.88;97.71)

Values are means (95 % confidence limits), under baseline conditions and subsequent to nitric oxide (NO) inhalation, or oral bosentan, respectively, using multiple inert gas elimination technique (MIGET). CO, cardiac output.

Mean perfusion (Q) and mean ventilation (Va) represent the mean ventilation/perfusion ratio for perfusion and ventilation, respectively. Log SDQ and Log SDV_a represent the dispersion of these data estimated by the log SD of perfusion and ventilation, respectively. Shunt represents portion of perfusion to non-ventilated areas (V/Q 0-0.001).

Low Va/Q areas represent portion of perfusion to areas with reduced ventilation (V/Q 0.001 – 0.01). p-value determined using paired signed rank test * $p < 0.05$, **$p < 0.01$

Table 3 Hemodynamics upon NO and bosentan application

Paramter	Baseline n=12	NO n=12	Bosentan n=12
PVR [dyn*sec/cm^5]	209.43	152.16 **	186.36
	(150.79;268.07)	(102.84;201.49)	(132.65;240.07)
Mean PAP [mmHg]	22.42	18.58 ***	19.75 **
	(17.82;27.02)	(14.37;22.79)	(15.39;24.11)
Cardiac index [l/min/m^2]	3.11	3.22	2.99
	(2.90;3.31)	(3.01;3.44)	(2.73;3.24)
Cardiac output [l/min]	6.03	6.29	5.74
	(5.43;6.62)	(5.54;7.03)	(5.19;6.29)
SVR [dyn*sec/cm^5]	1258.40	1233.02	1328.35
	(1097.94;1418.86)	(1077.89;1388.14)	(1161.97;1494.74)
Mean SAP [mmHg]	93.58	94.58	94.33
	(87.35;99.82)	(89.37;99.80)	(88.01;100.65)
Pulse rate [bpm]	83.83	82.00 *	79.25 **
	(76.72;90.95)	(74.78;89.22)	(71.26;87.24)
PCWP [mmHg]	5.91	6.09	5.55
	(3.91;7.91)	(4.20;7.98)	(3.50;7.59)

Values are means (95 % confidence limits), under baseline conditions and subsequent to nitric oxide (NO) inhalation or oral bosentan, respectively,

bpm = beats per minute, PAP = pulmonary arterial pressure, PVR = pulmonary vascular resistance, SAP = systemic arterial pressure, SVR = systemic vascular resistance. PCWP = pulmonary arterial wedge pressure.

p-values determined using the paired signed rank test. *$p < 0.05$, ** $p < 0.01$

Table 4 Lung function and 6min walking distance at baseline and at 12 weeks

% of predicted values	Baseline (n = 11)	Week 12 (n = 11)
TLC [% pred]	53.64 (45.20;62.08)	54.73 (44.87;64.58)
VC [% pred]	54.82 (45.28;64.36)	51.36 (38.35;64.38)
FVC [% pred]	55.73 (45.42;66.04)	52.18 (38.32;66.05)
DLco [% pred]	36.73 (30.07;43.39)	33.27 (24.65;41.89)
DLco [% pred for alveolar ventilation]	53.09 (40.57;65.61)	51.73 (38.33;65.12)
6min walking distance [m]	320.9 (232.96;408.84)	302.9* (205.01;400.79)

Values are means (95 % confidence limits). One patient had no post-baseline assessment and was not included in the analyses; the two patients who died were given the worst value at week 12.

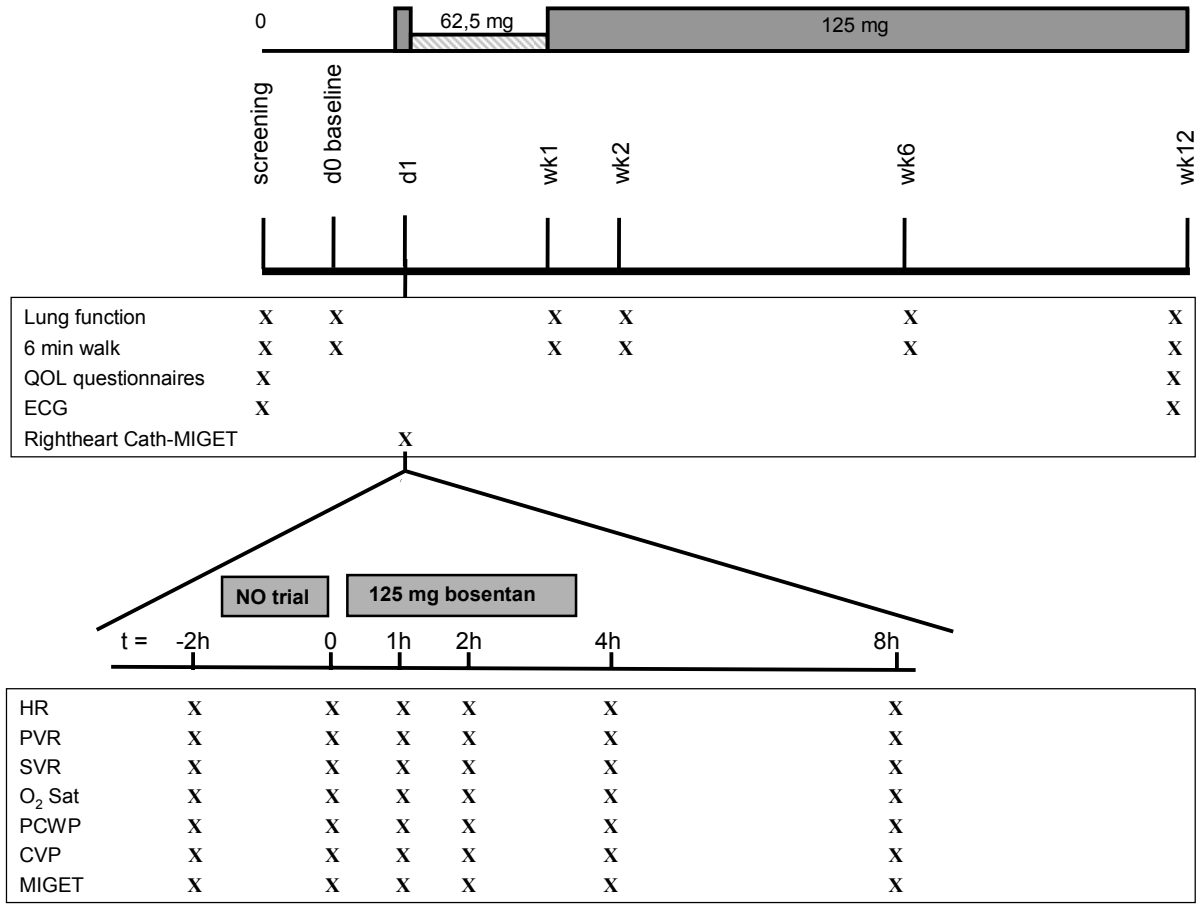
DLco, diffusion capacity for carbon monoxide, FVC, forced vital capacity; TLC, total lung capacity; VC, vital capacity. DLco was normalized for alveolar ventilation.

Table 5 Summary of AEs (related and unrelated to study drug)

Adverse events	n	%
Respiratory tract infection	5	41.7
Cough	2	16.7
Oedema lower limb	2	16.7
Arthralgia	1	8.3
Bronchospasm	1	8.3
Fungal infection	1	8.3
Hepatic function abnormal	1	8.3
Hypotension	1	8.3
Multi-organ failure	1	8.3
Pneumonia cytomegaloviral	1	8.3
Pneumonia	1	8.3
Respiratory failure	1	8.3
Sepsis	1	8.3
Ventilation/perfusion scan abnormal	1	8.3
Vertigo positional	1	8.3

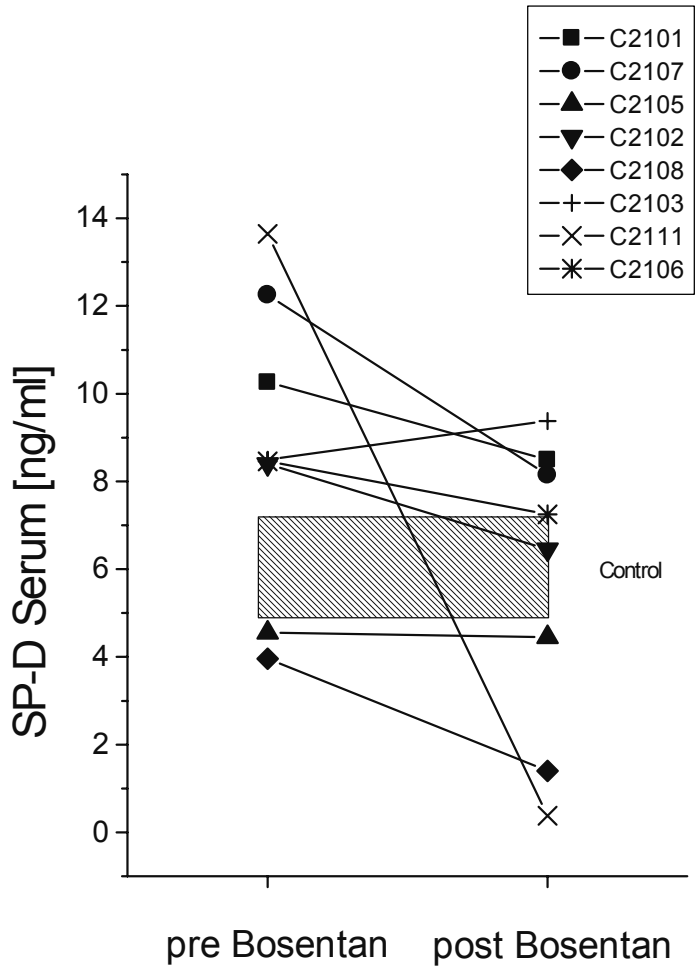
N = 12 patients

Figure 1 Study course and examinations



CVP, central venous pressure; d, day; ECG, electrocardiogram; pressure; h: hour; HR, heart rate; NO, nitric oxide; MIGET, multiple inert gas elimination technique; O_2 Sat oxygen saturation; PCWP, pulmonary capillary wedge; PVR, pulmonary vascular resistance; QoL, quality of life; SVR, systemic vascular resistance; wk, week.

Figure 2 Serum surfactant protein D levels at baseline and week 12



Values are mean ± SEM of serum surfactant protein D (SP-D) levels, as measured by means of ELISA technique. Individual course of values is depicted.