Combination therapy with bosentan and sildenafil in Eisenmenger syndrome: a randomized, placebo-controlled, double-blinded trial†

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Aims
To evaluate the efficacy of combining the dual endothelin receptor antagonist, bosentan, and the phosphodiesterase-5-inhibitor, sildenafil, in patients with Eisenmenger syndrome.

Methods and results
The study was a randomized, placebo-controlled, double-blinded, cross-over design. Patients with Eisenmenger syndrome (n = 21) were treated open label with bosentan for 9 months. After 3 months, sildenafil/placebo was added for 3 months, and a cross-over was performed for the last 3 months. At baseline and after 3, 6, and 9 months, patients were examined with 6 min walk test, oxygen saturations, N-terminal pro-brain natriuretic peptide, New York Heart Association (NYHA) classification, cardiac catheterization, and magnetic resonance imaging. The primary endpoint was changed in 6 min walk distance (MWD). Bosentan improved the 6 MWD (377 vs. 414 m, P = 0.001), pulmonary vascular resistance (PVR) (28 vs. 22 wood, P = 0.01), and pulmonary blood flow (2.6 vs. 3.5 L/min, P = 0.01). Adding sildenafil to bosentan did not improve the 6 MWD significantly (21 vs. 8 m, P = 0.48), but increased saturation at rest (2.9 vs. –1.8%, P < 0.01).

Conclusion
In Eisenmenger syndrome, treatment with bosentan significantly improved walking distance, pulmonary blood flow, and PVR. Adding sildenafil to bosentan did not significantly improve walking distance but did increase saturation at rest.

Keywords
Congenital heart defects • Heart septal defects • Pulmonary hypertension

Introduction
Until recently, the only effective treatment of Eisenmenger syndrome was lung (and heart) transplantation. In 2006, Galie et al.¹ presented the results from the BREATHE 5 study demonstrating a beneficial effect of bosentan on 6 min walk distance (MWD) in patients with Eisenmenger syndrome.

Combining different pulmonary vasodilators in the treatment of patients with pulmonary arterial hypertension has previously shown promise.²–⁴ No randomized clinical trials have examined the effect of combining pulmonary vasodilators in patients with Eisenmenger syndrome.

The purpose of the present study was to examine the effect of bosentan alone and in combination with sildenafil in patients with Eisenmenger syndrome. The primary endpoint was change in 6 MWD, secondary endpoints included changes in oxygen saturation at rest and during exercise, N-terminal pro-brain natriuretic peptide (NT-proBNP), New York Heart Association class, pulmonary blood flow, ratio between pulmonary and systemic blood flow, and pulmonary vascular resistance (PVR).
Methods

Patients

Patients ≥18 years of age were recruited from the Department of Cardiology at Copenhagen University Hospital Rigshospitalet in Copenhagen, Denmark between January 2006 and April 2007. Eisenmenger syndrome should be documented with cardiac catheterization as pulmonary hypertension and a right-to-left shunt through a non-restrictive congenital heart defect. Only patients who were not treated with any kind of pulmonary artery vasodilators were eligible.

Study design

The study was a randomized, placebo-controlled, double-blinded cross-over trial. At baseline, patients underwent a 6 min walk test (6MWT), oxygen saturation measurement at rest and during exercise, NT-proBNP measurement, NYHA classification, cardiac catheterization, and cardiac magnetic resonance imaging (MRI).

All patients were treated open label with bosentan for the entire study period (9 months). During the first 2 weeks, the patients received bosentan 62.5 mg b.i.d. and thereafter bosentan 125 mg b.i.d. for the remaining of the trial. Evaluation identical to baseline examinations was performed after 12 weeks and patients were randomized to sildenafil 25 mg t.i.d. for 2 weeks followed by 50 mg t.i.d. for 10 weeks or matching placebo as add-on therapy to bosentan. After this period, evaluation identical to baseline examinations was performed again and a cross-over was performed so that patients receiving sildenafil were now treated with placebo and vice versa. After further 12 weeks, a final evaluation was performed. During the entire trial, liver enzymes and blood pressure were measured at least once every month.

Randomization was performed by Copenhagen County Hospital Pharmacy, and the trial was fully monitored according to rules for good clinical practice by the GCP unit in Copenhagen.

Study procedures

Six minutes walk test was performed according to standard procedures and carried out by trained study nurses. Oxygen saturation was measured before and at the end of the 6 MWT. N-terminal pro-brain natriuretic peptide was analysed using the ECLIA assay from ROCHE. Trained study nurses performed NYHA classifications according to standard criteria.

Cardiac catheterization was done using a local analgesic; a 6 Fr sheath was inserted into a femoral vein and a 4 Fr sheath into a femoral artery. Mixed venous oxygen saturation was calculated as the mean of two measurement in the superior vena cava and two measurements in the inferior vena cava. The oxygen saturation in the pulmonary trunc was calculated as a mean of three measurements. Oxygen saturation in the pulmonary veins was measured directly in patients with atrial septal defect, otherwise it was estimated to 95%. Systemic oxygen saturation was measured in the femoral artery. The values of pressure were recorded in right atrium, right ventricle, pulmonary artery, and femoral artery. Left atrial pressure (LAP) was measured directly in case of atrial septal defect, otherwise estimated to 95%.

Brain natriuretic peptide was analysed using the ECLIA assay from Roche Diagnostics, Denmark. NT-proBNP measurement, NYHA classification, and cardiac magnetic resonance imaging (MRI). The study was conducted according to the most recent amendments to the Declaration of Helsinki and in adherence to good clinical practice guidelines. The local ethic committee (journal number 02-293240) and the Danish Medical Agency (journal number 2612-3086) approved the protocol. Written informed consent was obtained from all patients.

Statistics

The sample size was decided with a power calculation for the 6 MWD for the sildenafil vs. placebo group. The level of significance was chosen to be 0.05, the power was 0.90, the estimated standard deviation 40 m, the minimal detectable difference 50 m and four potential dropouts. Only patients completing the trial were included in the analyses. Test for normality of data was done using Kolmogorov–Smirnov test. Variables were normal distributed except for Qs during sildenafil and NT-proBNP but non-parametric testing did not change the results for these variables. Comparisons between continuous data were done by paired t-test and between data in categories by Fishers exact test. There were three repeated measurements for every patient in the crossover part of the trial. Two differences were computed and these were analysed using paired t-tests. Analyses were also performed using a linear mixed effects model including an interaction term to test for carry-over effect. Correlations were done using Pearson’s correlation. For linear regression analyses, we used backward elimination, variables were excluded if probability of F was <0.10. All confidence intervals (CI) were constructed to have coverage of 95% and are reported in brackets. A level of significance of 5% was chosen and all tests were two-sided. Statistical calculations were performed using SPSS software (version 13.0).

Results

Twenty-one patients with known Eisenmenger syndrome were screened for the study. All the screened patients fulfilled the inclusion criteria and all were included in the study. No code breaks were done during the study. Two patients were excluded during the study (one due to side-effects of the medication and one due to death). The flowchart of the study is presented in Figure 1. Demographic data from all patients are presented in Table 1.

Bosentan treatment

During the initial bosentan treatment, the primary endpoint, i.e. the 6 MWD increased in 16 patients, was unchanged in two patients and decreased in three patients. The mean increase in walk
distance was 37 m (95% CI 17–58 m, \(P < 0.01\)) from 377 to 414 m. Of the secondary endpoints, pulmonary blood flow measured with cardiac catheterization and PVR improved significantly, other effect parameters only showed insignificant changes. Data are presented in Table 2.

**Combination treatment**

Adding sildenafil to bosentan increased six primary endpoint i.e. the MWD by 21 m (95% CI –3 m to 45 m) from 422 to 443 m, which borders on significance (\(P = 0.08\)). However, the 6 MWD also increased when adding placebo to bosentan (8 m, 95% CI –6 m to 32 m, \(P = 0.50\)), and there was no statistical significant difference between the effect of sildenafil and placebo as add-on therapy to bosentan (\(P = 0.48\)). Among secondary endpoints, only saturation at rest changed significantly. The effect of combination therapy on effect parameters is presented in Table 3 and figures of changes in selected endpoints are presented in Figures 2 and 3.

Using a linear mixed effects model gave similar results. Testing for interaction in the linear mixed effects model yielded no evidence of carry-over effect.

**Patients with atrial septal defects**

Only two patients had atrial septal defects, one of them dropped out of the study due to headache when receiving sildenafil. Excluding patients with atrial septal defects from the analysis did not change any of the results except for the ratio between pulmonary and systemic blood flow in the bosentan part of the trial that lost significance (\(P = 0.07\)).

**Correlation between effect parameters**

The 6 MWD was significantly correlated with saturation at rest \((r = 0.46, P < 0.01)\), NT-proBNP \((r = -0.50, P < 0.01)\), systemic blood flow measured with cardiac catheterization \((r = 0.31, P < 0.01)\), pulmonary/systemic blood flow measured with cardiac catheterization \((r = 0.36, P < 0.01)\), and PVR \((r = -0.42, <0.01)\). Correlations between differences detected with effect parameters showed that only secondary effect parameter that correlated significantly with the primary endpoint was saturation at rest \((r = 0.34, P < 0.01)\). In order to reveal significant association between the primary endpoint and secondary endpoints, multiple linear regression analyses were performed. All secondary endpoints were included as covariates and the only endpoint that remained in the model was saturation at rest (B 3.7, 95% CI –0.2 to 7.5; \(P = 0.06\)).

**Adverse events**

Two patients experienced headache during sildenafil treatment but only one was unable to complete the study. Another patient with known thrombocytopenia had a fatal cerebral haemorrhage during treatment with bosentan as monotherapy, but this was not regarded as a side-effect to the treatment. No patients experienced a rise in liver enzymes above normal levels, and no patients experienced systemic hypotension during the trial.
In patients with Eisenmenger syndrome, combination therapy with the pulmonary vasodilators bosentan and sildenafil was not superior to bosentan as monotherapy with regard to the 6 MWD (primary endpoint). The only secondary endpoint affected by combination therapy was systemic oxygen saturation at rest. No differences in pulmonary blood flow, PVR, or natriuretic peptides were detected. Combination therapy was safe and the only adverse effect observed was headache.

### Discussion

#### Principal findings

In patients with Eisenmenger syndrome, combination therapy with the pulmonary vasodilators bosentan and sildenafil was not superior to bosentan as monotherapy with regard to the 6 MWD (primary endpoint). The only secondary endpoint affected by combination therapy was systemic oxygen saturation at rest. No differences in pulmonary blood flow, PVR, or natriuretic peptides were detected. Combination therapy was safe and the only adverse effect observed was headache.
Study rationale
Three types of pharmacological agents are at present used to treat pulmonary hypertension (calcium channel antagonists are not a drug of choice in patients with Eisenmenger syndrome). Prostacyclin analogs relax vascular smooth muscle cells and inhibit vascular smooth muscle cell proliferation via a second messenger.\textsuperscript{15} Phosphodiesterase type 5 inhibitors potentiate the antiplatelet, antiproliferative, and vasodilator effect of nitrate oxide by inhibiting phosphodiesterase type 5 mediated degradation of the second messenger for nitrate oxide.\textsuperscript{16} Endothelin receptor antagonists reduce vasoconstriction, smooth muscle cell proliferation, and fibrosis in the pulmonary vessels by blocking the endothelin 1 receptor.\textsuperscript{17} The rationale for combination therapy is that a strategy where multiple disease mechanism is addressed simultaneously could potentially augment treatment efficacy as seen in several other diseases.

Strengths and weaknesses of the study
This single-centre study is the first study examining the combination of two oral pulmonary vasodilators in patients with pulmonary hypertension. In comparison with previous studies with pulmonary vasodilators, this study had a very extensive examination protocol with both invasive (cardiac catheterization) and non-invasive (MRI and NT-proBNP) assessment of haemodynamics. Furthermore, the single-centre design ensure that study examinations were
performed uniformly. A weakness in the present study is that bosentan could take more than 3 months to reach its full effect. This hypothesis is supported by the findings in the BREATHE-5 extension study, but need to be confirmed in future trials. An alternative explanation of the continuing improvement could be that after the initial clinical effect of bosentan, several patients started exercising more which could have influenced the 6 MWD, which is supported by the unchanged PVR and pulmonary blood flow found in the present study. However, a longer period of single treatment with bosentan could have made it easier to interpret the effect of add-on therapy. On the other hand, even though Eisenmenger is a slowly evolving disease, a long study period could introduce a problem with physical deterioration due to the natural history of the disease. Future studies of clinical stable patients could investigate whether longer treatment periods would have an impact on the efficacy of combination therapy.

A cross-over design was chosen in the present trial in order to optimize the power of the study. Eisenmenger syndrome is a rare condition, and if a single-centre study is wanted, in order to ensure high data validity, a cross-over design is almost mandatory to ensure enough power in the study. On the other hand, there are some inherent problems in the cross-over design, mainly concerning wash-out time and prolonged treatment effect. However, even though it is possible that there are some sustained effects from the medication even after discontinuation, any clinically significant add-on effect from sildenafil should have been detectable.

In this study, both patients with pre- and post-tricuspid shunts were included. It has been speculated that the development of pulmonary hypertension in the setting of pre-tricuspid shunts has a different pathophysiology than in the setting of post-tricuspid shunts. Including both types of patients in study could potentially weaken the results. However, the post hoc analysis showed that excluding the patients with atrial septal defect from the study did not alter the main results.

Saturation in the pulmonary veins was measured in patients with atrial septal defect or persistent foramen ovale. For other patients, the lung vein saturation was estimated to 95%, which in the presence of lung disease could introduce an error. However, none of the patients had any known lung disease and the cross-over design makes changes more important than absolute values; therefore, any overestimation of lung vein saturation would probably not influence the overall study results.

Most of the measured endpoints improved during both treatment with bosentan and sildenafil, but due to high variability the changes were not significant. This reflects a general problem in evaluating patients with a shunt physiology as seen in Eisenmenger syndrome.

No patient was in atrial fibrillation or flutter, but ventricular and supraventricular ectopic beats were common, making the MRI examination difficult. Even though MRI showed an increase in pulmonary blood flow during bosentan treatment, the high standard deviation of the results at least partly explains the lack of significance. Correlations between MRI and cardiac catheterization were similar for flow measured in the aorta and in the pulmonary artery. This suggests that measuring flow in the dilated pulmonary artery with MRI not a problem per se.

A larger sample size would reduce the risk of a type II error but on the other hand one should critically evaluate the importance of revealing relative small changes in endpoints not shown to influence on the clinical course of the disease.

Both bosentan and sildenafil are cleared mostly by hepatic metabolism, predominantly by the P450 enzyme CYP3A4. It is shown that treatment with bosentan 125 mg b.i.d. decreases area-under-plasma concentration curve for sildenafil to 44% and that treatment with sildenafil increases plasma concentrations of bosentan with about 50%. The clinical impact of these kinetic findings is, however, unknown and in this study the relative high dose of sildenafil (50 mg t.i.d.) ensures that even if the concentration under the curve of sildenafil was reduced to 44% (corresponds to 22 mg t.i.d.), the dosage would still be above the recommended dose by the food and drug administration (20 mg b.i.d.). The logistic regression analyses performed included 7 covariates and only had 38 observations. Therefore, the results from these analyses should be interpreted with caution.

Other studies

That pulmonary vasodilators increase physical capacity and decrease PVR in patients with pulmonary hypertension has been shown in several randomized trials. Even though some studies have included patients with congenital heart disease, only one study (BREATHE-5) has examined the effect of pulmonary vasodilatation on patients with Eisenmenger syndrome. In BREATHE-5, bosentan improved both the PVR (5.9 woods) and the 6 MWT (33.6 m), which is similar to the results in our study. The non-randomized extension of BREATHE-5 showed a further, although non-significant, improvement in exercise capacity.

The combination of vasodilators has previously been examined in two randomized double-blinded clinical trial. The BREATHE II trial failed to show a significant effect of the combination of bosentan and epoprostenol compared with epoprostenol and placebo.
but the recent PACES did show some beneficial effect of adding sildenafil to epoprostenol. Furthermore, several open label and observational studies have suggested that combination therapy is a promising concept.2,3,5–9,11–13,36

Implications
This study confirms earlier findings of bosentan’s beneficial effects on PVR and 6 MWWD when used as first-line therapy in patients with Eisenmenger syndrome. However, the study was not designed to make head to head comparisons between bosentan and sildenafil as monotherapy and future studies could address whether there is a role for sildenafil as first line therapy in patients with Eisenmenger syndrome. The present study shows no benefit in combining bosentan and sildenafil in patients with Eisenmenger syndrome. However, this study does not exclude that some patients could have a beneficial clinical effect of sildenafil when added to bosentan and since this study did not observe any serious side-effects, it could be relevant to try adding sildenafil to bosentan in patients with insufficient response to bosentan or cases of clinical deterioration, fall in exercise capacity or increase in PVR. Due to the intricate nature of the haemodynamics in patients with Eisenmenger syndrome, finding the right effect parameters is still a challenge. In patients with pulmonary hypertension without shunts or with pre-tricuspid shunts, the size, mass, and function of the right ventricle measured by MRI could be an interesting endpoint. However, in the present study, the majority of patients had unrestricted ventricular septal defect, and thus single ventricle physiology, which makes such endpoints less useful. Correlations between our different endpoints showed at best a moderate consistency and although most of the endpoints showed improvement, only few reached statistical significance. Even though clinical endpoints such as death or clinical deterioration would be ideal, the long clinical course and good prognosis of Eisenmenger syndrome make such endpoints difficult to use in this group of patients. Another subject worth considering is whether evaluations should be done at rest (like cardiac catheterization and MRI) where few patients have symptoms or during exercise such as 6 MWTD. Till now, no endpoints have shown superiority to the 6 MWTD and this may therefore still be the test of choice when evaluating patients with Eisenmenger syndrome. Future trials should examine which endpoints are best when evaluating the effect of a treatment in this patient population. It is possible that invasive testing with inhaled nitrite oxide could help identify patients that would benefit from sildenafil as add-on therapy. This was difficult in the present study due to the sample size that did not allow for subgroup analyses and was furthermore not within the scope of this trial but should be investigated in future studies.

Conclusion
In patients with Eisenmenger syndrome, treatment with bosentan significantly improved 6 MWTD, increased pulmonary blood flow, and reduced PVR. Adding sildenafil to bosentan did increase saturation at rest, but did not significantly improve neither walking distance, pulmonary blood flow nor PVR. Adding sildenafil to bosentan was safe and not associated with any serious side-effects.

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