Sildenafil versus Endothelin Receptor Antagonist for Pulmonary Hypertension (SERAPH) Study


Rationale: Phosphodiesterase type 5 (PDE5) inhibition has been proposed for the treatment for pulmonary arterial hypertension (PAH). Objective: This study compared adding sildenafil, a PDE5 inhibitor, to conventional treatment with the current practice of adding bosentan, an endothelin receptor antagonist. Methods: Twenty-six patients with PAH, idiopathic or associated with connective tissue disease, World Health Organization (WHO) functional class III, were randomized in a double-blind fashion to receive sildenafil (50 mg twice daily for 4 weeks, then 50 mg three times daily) or bosentan (62.5 mg twice daily for 4 weeks, then 125 mg twice daily) over 16 weeks. Measurements: Changes in right ventricular (RV) mass (using cardiovascular magnetic resonance), 6-minute walk distance, cardiac function, brain natriuretic peptide, and Borg dyspnea index. Main Results: When analyzed by intention to treat, there were no significant differences between the two treatment groups. One patient on sildenafil died suddenly. Patients on sildenafil who completed the protocol showed significant changes from baseline, namely, reductions in RV mass (−8.8 g; 95% confidence interval [CI], −2.16; n = 13, p = 0.015) and plasma brain natriuretic peptide levels (−19.4 fmol · mL−1; 95% CI, −5.34; p = 0.014) and improvements in 6-minute walk distance (114 m; 95% CI, 67.160; p = 0.0002), cardiac index (0.3 L · min−1 · m−2; 95% CI, 0.10.4; p = 0.008), and systolic left ventricular eccentricity index (−0.2; 95% CI, −0.02; −0.37; p = 0.031). Bosentan improved 6-minute walk distance (59 m; 95% CI, 29.89; n = 12, p = 0.001) and cardiac index (0.3; 95% CI, 0.1, 0.4; p = 0.008). Conclusions: Sildenafil added to conventional treatment reduces RV mass and improves cardiac function and exercise capacity in patients with PAH, WHO functional class III. Safety monitoring is important until more experience is obtained.

Keywords: cardiovascular disease; cardiac magnetic resonance; pharmacology

Pulmonary arterial hypertension (PAH) of unknown etiology or associated with connective tissue disease is a fatal disease with few medical therapies (1). It is characterized by increased pulmonary vascular resistance caused by vasoconstriction, thrombosis, and structural remodeling of pulmonary arterioles, with occlusion of the lumen of some vessels. If untreated, pressure overload of the right ventricle leads to progressive dilatation and hypertrophy and end-stage heart failure within 2 to 3 years (2). Conventional therapy with warfarin, digoxin, and diuretics improves symptoms but has a limited effect on disease progression.

Calcium antagonists, in appropriate patients, and prostacyclin analogs significantly improve exercise capacity and prolong survival but treatment is frequently complicated by side effects (3–6).

The recent approval of bosentan, an orally active, nonselective endothelin receptor antagonist, for PAH has widened the treatment options (7–9). Endothelin-1 is a potent vasoconstrictor and a smooth muscle mitogen. Plasmin and pulmonary vascular tissue levels of this peptide are elevated in PAH (10). Chronic administration of bosentan has been shown to improve cardiopulmonary hemodynamics in patients with PAH in World Health Organization (WHO) functional class III (7) and exercise capacity in patients in WHO functional class III/IV (8), and recent evidence suggests it improves survival (9). It is now an established treatment for these patients, often used first line, with bosentan thought to be the first choice for more severely ill patients.

In the search for alternative therapeutic targets in PAH, cyclic guanosine monophosphate (cGMP)-dependent phosphodiesterase type 5 (PDE5) has emerged as an attractive candidate. PDE5 is abundant in smooth muscle in the lung where it is the main enzyme responsible for cGMP hydrolysis (11). The cGMP mediates the relaxant and antitrophic actions of nitric oxide and the natriuretic peptides in vascular tissues (12, 13). Studies in animal models and open-label studies suggest that chronic inhibition of PDE5 with sildenafil reduces pulmonary arterial pressure (14–16). Some physicians have begun using sildenafil as an alternative to, and in combination with, bosentan (17) in the early management of PAH, but its place is uncertain.

We have conducted a double-blind, randomized, controlled trial to compare the effects of sildenafil and bosentan in patients with PAH, functional class III. We have compared the effects of the two drugs on right ventricular (RV) mass, as measured by cardiovascular magnetic resonance (CMR), together with exercise capacity, assessed by 6-minute walk distance, cardiac function, and circulating cardiac hormones.

METHODS

Selection of Patients

Consecutive patients attending the Hammersmith Hospital during February 2002 to September 2003 with PAH that was either idiopathic or associated with connective tissue disease (scleroderma or systemic lupus erythematosus) and who were eligible for bosentan therapy were considered for the study. The PAH diagnosis was based on cardiac catheter data (mean pulmonary artery pressure at rest, > 25 mm Hg) obtained within the preceding 12 months and the results of extensive screening for other causes according to current guidelines (18). All patients were symptomatic despite conventional therapy with diuretics, digoxin, and anticoagulants, and their 6-minute walk distance was required to be between 150 and 450 m at entry. Exclusion criteria included the following: elevated baseline liver enzymes (> three times the upper limit of the normal range), previous bosentan or sildenafil treatment, and the urgent need for prostanoid therapy on clinical grounds.

The study was performed according to the 1975 Declaration of Helsinki (modified in 1983) and in adherence to local guidelines for good clinical practice. The protocol was approved by the local ethics committees.
review committee, and written, informed consent was obtained from all patients.

**Study Design**

The study was double blind, and 26 patients were randomized to receive either sildenafil or bosentan for 16 weeks. The medication was blinded in identical-looking gelatin capsules and randomized using a computer-generated random list by the Hammersmith Hospital pharmacy. Bosentan (62.5 mg twice daily) or sildenafil (50 mg twice daily) was given for the first 4 weeks. Patients were then up-titrated to sildenafil (50 mg three times daily) or bosentan (125 mg twice daily, with midday placebo tablet) for a further 12 weeks. All patients were then transferred to open-label bosentan treatment in the fifth month.

**Outcome Measures**

The following tests were performed at baseline and 16 weeks: ECG, transthoracic echocardiogram, CMR, 6-minute walk distance, Borg dyspnea index (performed immediately after the 6-minute walk), and Kansas City Cardiomyopathy Quality-of-Life questionnaire (19). Blood sampling for hormone levels was performed at baseline, 4 weeks, and for routine biochemistry at baseline and 4-week intervals.

The primary efficacy measure was predefined as a change in RV mass from baseline as measured by CMR; we hypothesized that RV hypertrophy is a response to increased pulmonary artery pressure (PAP) and a decrease in RV mass would be expected to follow a clinically significant and sustained reduction in PAP. The secondary measures of efficacy were change in 6-minute walk distance, cardiac index, Borg dyspnea index, quality of life, and plasma B-type natriuretic peptide (BNP) levels from baseline. For comparison, plasma C-type natriuretic peptide levels were also measured. Safety was assessed by an independent committee who recorded adverse events.

**Imaging**

CMR was performed with a 1.5-T scanner (Sonata; Siemens, Erlangen, Germany), with surface phased-array receiver coil and prospective electrographic triggering. Assessment of left ventricular (LV) and RV volumes/mass was performed as previously described (20) using steady-state free precession techniques. Imaging parameters were as follows: repetition time/echo time of 3.2/1.6 milliseconds, in-plane pixel size of 2.3 × 1.4 mm, section thickness of 7.0 mm, flip angle of 60°, and acquisition time of 12 heartbeats. Breath-hold scout images and subsequent vertical and horizontal long-axis cine images were initially acquired. Short-axis sequential slices were piloted from these, starting from the atrioventricular ring, covering both ventricles to beyond the apex (21).

Analysis of the acquired images was performed using dedicated software (CMR tools; Cardiovascular Imaging Solutions, London, UK). Tracing of epicardial and endocardial borders of short-axis slices in end-diastole (image phase with the largest cavity area) and endocardial border in end-systole (smallest cavity area) allowed calculation of right and left myocardial volume and end-diastolic and end-systolic volumes (Figure 1). From this, stroke volume and ejection fraction could be derived. For mass calculation, the myocardial volume was multiplied by the specific density of myocardium (taken as 1.05 g/cm3). Cardiac output was calculated from the CMR images using the LV stroke volume multiplied by heart rate, and cardiac index was then derived by normalization to body surface area.

Two-dimensional and Doppler echocardiography was performed using standard techniques on commercially available equipment (Philips Ultrasound, ATL 5000 and Agilent 5500; Croydon, London, UK) with a predefined imaging protocol. The maximal tricuspid regurgitant jet velocity was assessed by determining the peak regurgitant velocity (v) in the continuous wave Doppler flow profile obtained from the cardiac apex. The tricuspid regurgitant Doppler signal represents the pressure difference between the RV and right atrium during systole. Systolic pulmonary arterial pressure was calculated using the formula $4v^2$ + right atrial pressure. Right atrial pressure was assessed by clinical examination of the jugular venous pressure, and echocardiographic diameter of the inferior vena cava and its change with inspiration. The right atrial volume was calculated at end-systole from the apical four-chamber view using the diameter and the long-axis length of the atrium according to the equation: volume = $(\pi D^2L)/6$, where $D$ = minor axis (cm), $L$ = major or long axis (cm) (22). The LV eccentricity index, a measure of the degree of septal displacement, was measured at end-diastole and end-systole from parasternal short-axis projections of the LV. It was calculated as the ratio of the major axis of the LV cavity parallel to the septum at the level of the chordae (a), divided by the minor axis perpendicular to and bisecting the septum on the same section (b) (eccentricity index = a/b) (23). The RV Doppler (Tei) index was calculated as described previously (24) using the length of two time intervals in the formula $(a - b)/b$, where a equals the time between the onset of QRS complex and onset of tricuspid inflow and b is the ejection time of RV outflow.

The measurements from images were made by an experienced operator who was unaware of each patient’s clinical information.

**Hormone Assays**

Assays for plasma BNP and C-type natriuretic peptide were performed on plasma extracted using C18 columns with appropriate specific competitive immunoluminometric assays. Standards and plasma extracts were reconstituted in assay buffer (25) and preincubated with specific antibodies for BNP and C-type natriuretic peptide (Bachem UK Ltds, St. Helens Meyerside, UK) in ELISA plates coated with anti-rabbit IgG. After 24 hours at 4°C, biotinylated tracers, which were prepared in-house and purified by HPLC (25), were added. Detection was achieved using methyl-acridinium-labeled streptavidin, with chemiluminescence elicited by sequential injections of H2O2 (in HNO3) and NaOH.

**Statistical Analysis**

On the basis of reproducibility measurements of RV mass in patients with heart failure (mean difference, 0.7 ± 6 g) (20), 12 patients in each group would allow us to detect a change of 3.5 g in RV mass from baseline to 16 weeks with 80% power. Statistical analysis was performed by intention to treat (where missing values were replaced with 0 m for the 6-minute walk and the last measurement carried forward for other

![Figure 1. True fast imaging with steady-state precession (FISP) cine images demonstrating the piloting of short-axis images from the horizontal long-axis image (a). Epicardial and endocardial myocardial borders of right and left ventricle are manually traced in end-diastole (b) and the endocardial border in end-systole (c). These images are from a patient with pulmonary arterial hypertension. Right ventricular hypertrophy and increased trabeculation can be seen.](image-url)
observations) and on data from all patients who completed the protocol. After tests for normality, the baseline characteristics of the two groups and the significance of the differences from baseline to 16 weeks within and between treatment groups were examined using a t test. All p values were two-tailed and 95% confidence intervals (CIs) were calculated for differences within and between treatment groups.

RESULTS

Twenty-six patients were recruited to the study (Table 1 and Figure 2). The baseline characteristics of the two treatment groups did not differ significantly (Table 1). All were in WHO functional class III and the majority of patients had idiopathic PAH. Thirteen patients assigned to sildenafil and 12 receiving bosentan completed the protocol and provided data for group analysis. One patient assigned to sildenafil died suddenly during Week 14 of treatment. A BNP level measured in a blood sample from this patient taken at Week 4 showed a fall from 115 fmol · ml⁻¹ at baseline to 65 fmol · ml⁻¹, but no other follow-up data are available.

Cardiac Mass and Hemodynamic Measurements

Baseline RV mass was markedly elevated in both treatment groups compared with healthy individuals of a similar age (range, 30–80 g [20]; Table 1). There was no significant difference between the two treatment groups, either at baseline or 16 weeks. Further analysis of data from patients on sildenafil who completed the protocol revealed a significant reduction in RV mass (p = 0.015) compared with baseline (Table 2). Imputing a value of no change for the patient who died also supported a significant reduction in RV mass (–8.1 g, p = 0.016, n = 14). No significant reduction was observed with bosentan treatment (p = 0.172).

Individual responses are shown in Figure 3. No change was seen in LV mass.

There was no significant difference between the two treatments in measurements of cardiac function. For patients completing the protocol, cardiac index increased significantly from baseline on both treatments (p < 0.01), and this was associated with a significant reduction in eccentricity index during systole in the sildenafil group (p = 0.031; Table 2). The Tei index did not change, and neither treatment affected systemic blood pressure or heart rate.

Exercise Capacity

The mean 6-minute walk distance increased on both treatments, with no difference between the treatment groups when analyzed by intention to treat. The mean 6-minute walk in the sildenafil group increased by 75 m (with 0 m imputed for Week 16 for the patient who died) compared with 59 m in the bosentan group. Individual data for those patients who completed the protocol are shown in Figure 3 and Table 2. Borg dyspnea index scores for these patients showed no significant change on either treatment: sildenafil (–1.5 [95% CI, –3, 0], p = 0.058) and bosentan (0.2 [95% CI, –1.4, 1.7]).

Hormone Measurements

Baseline plasma BNP levels were elevated in both treatment groups (sildenafil, 67 fmol · ml⁻¹ [95% CI, 25, 109]; bosentan, 50 fmol · ml⁻¹ [95% CI, 21, 80]) compared with those seen in health (< 20 fmol · ml⁻¹). The change in BNP levels with treatment did not differ significantly between the two treatment groups. However, BNP levels fell significantly from baseline with sildenafil (p = 0.014). For all patients assigned to sildenafil, BNP levels fell by 18 fmol · ml⁻¹ (p = 0.014), if the baseline BNP value for the patient who died is carried forward to Week 16, and 21 fmol · ml⁻¹ (p = 0.006), if the Week 4 value is used at 16 weeks. Data for all patients who completed the protocol are

![Figure 2. SERAPH study profile.](Image)
given in Table 2. Plasma C-natriuretic peptide levels were also elevated (sildenafil, 2.7 fmol·ml⁻¹ [95% CI, 1.6, 3.7]; bosentan, 2.7 fmol·ml⁻¹ [95% CI, 0.8, 4.7]; normal, < 1) but did not change significantly with treatment (Table 2).

Quality of Life and Safety

There was no significant difference in quality of life between the two treatments, analyzed by intention to treat (imputing the lowest possible score for the patient who died). For those completing the protocol, quality-of-life scores improved significantly on sildenafil treatment (27 points; 95% CI, 19, 35) but not on bosentan (6 points; 95% CI, –6, 17; Table 2). In addition to the death, one patient in the sildenafil group with a longstanding history of paroxysmal atrial flutter was admitted with an episode of palpitations, which settled spontaneously. Three patients on bosentan had unscheduled visits to the hospital because they felt less well; two had clinical evidence of fluid retention requiring an increase in dose of their diuretic treatment; one patient developed hemoptysis, which settled spontaneously. No clinically significant change in hematologic or routine biochemical measurements (including liver function) was observed. No patient was withdrawn from treatment or required a change in dose of study medication.

DISCUSSION

This study compared the effect of adding sildenafil to conventional therapy for PAH with the response to adding bosentan. It is unusual among studies in PAH in that it used an active treatment as a comparator and the change in RV mass as a primary endpoint. There were no significant differences between the two treatment groups in any endpoint when analyzed by intention to treat. An interesting observation, however, is that treatment with sildenafil was associated with a reduction in RV mass from baseline, which was not seen in the bosentan-treated patients from the same center over the 4-month period of study. Change in RV mass has been discussed but is not currently recognized as an endpoint in clinical trials in PAH (26). The accurate measurement of RV mass by echocardiography is difficult. In contrast, CMR measurements show good reproducibility and are well validated (20) and inclusion of CMR in PAH clinical trials has been encouraged (26). Unfortunately, at present, there are few published data in this area and so the clinical importance of a reduction in RV mass of 8 to 9 g over 4 months is uncertain.

Physiologically, a reduction in RV mass is an appropriate response to a reduction in pulmonary vascular resistance. PDE5 is abundant in pulmonary vascular (and bronchial) smooth muscle, where it is the major pathway for cGMP degradation.
PDE5 inhibition augments the relaxant and antimito-
genic actions of local and circulating cGMP-dependent factors, nitric oxide, and natriuretic peptides on the pulmonary vascula-
ture, and studies have shown that sildenafil reduces PAP in
PAH, acutely (16) and chronically (29). More recently, data have
emerged indicating that PDE5 is present in cardiac myocytes (30)
and suggesting that sildenafil may have a direct antihypertrophic
action on cardiac muscle (31). It is unknown whether a direct
antihypertrophic action on the RV is helpful in PAH.

Encouragingly, the reduction in RV mass with sildenafil was
accompanied by a significant reduction in BNP levels, which
correlate with severity of the condition (32), and systolic eccen-
tricity index, an indication of the pressure in the RV compared
with the LV during contraction. In addition, cardiac output
and 6-minute walk distance improved during treatment. The
6-minute walk distance is widely used for evaluating patients
with moderate to severe pulmonary hypertension in multicenter
clinical studies (8, 33–35). It is an index of a patient’s ability
to perform activities of daily living and it is an independent pre-
dictor of mortality (33). Patients in functional class III on conven-
tional therapy alone show a gradual deterioration in 6-minute
walk distance of around 10 m over 3 to 4 months (7, 8). The
increase in 6-minute walk distance of 114 m from baseline in
patients on sildenafil who completed the protocol is clinically
significant and consistent with that seen in open-label studies
(113–128 m) (29, 36). It should be acknowledged that these
studies, like ours, were based on small patient numbers. A
smaller (~45 m), but clinically important, increase in 6-minute
walk distance has been reported in a large multicenter, placebo-
controlled study of sildenafil, 20 to 80 mg 8 hourly (H. Ghofrani,
presented at the American College Chest Physicians’ meeting,
October 2004). Improvements in 6-minute walk distance seen
with bosentan at 4 months have been reported to be sustained
for many patients over 12 months (37). On the other hand,
studies with beraprost indicate caution in interpreting data from
short-term studies, because, in this case, the early benefit was
lost with long-term treatment (35). Further studies will be needed
to determine the duration of improvement in 6-minute walk
distance with sildenafil beyond 4 months.

The response to bosentan in our study requires further com-
ment. Although the improvements in cardiac index and 6-minute
walk distance from baseline compare favorably with those re-
ported by other studies (8, 38), there was no improvement in
systolic LV eccentricity index or Tei index, seen in larger studies
(38). The relatively small number of patients in our study pro-
vides limited power to detect these changes. Furthermore, one
possible confounding variable is that more patients assigned
to bosentan were taking calcium antagonists, which may have
dominated to the need to adjust diuretic therapy in this group
and delayed the therapeutic effect of the drug. Fluid retention
is a recognized side effect of bosentan, most likely related to
inhibition of the natriuretic effect of endothelin at the ET₄ receptor,
and the presence of a calcium antagonist may not have
helped. Given these considerations, it would be incorrect to
infer from our data that sildenafil is superior to bosentan in the
treatment of PAH.

The introduction of all new treatments should be accompa-
nied by careful safety monitoring, and this applies to sildenafil
use in PAH. One patient assigned to treatment with sildenafil
died suddenly in Week 14 of the study. The postmortem exami-
nation confirmed pulmonary hypertension as the cause of death,
and there was no evidence that it was related to sildenafil or
electrolyte disturbance. Sudden death is a well-recognized com-
plication of pulmonary hypertension and the patient’s death is
likely to have been a chance occurrence. On the other hand,
sildenafil treatment was well tolerated in those patients who
completed the protocol. One patient with a history of paroxysmal
atrial flutter was admitted with a recurrence of arrhythmia, which
settled spontaneously. Collectively, the patients on sildenafil com-
pleting the study reported a significant improvement in quality-
of-life score. The measure used (Kansas City Cardiomyopathy
Quality-of-Life questionnaire) has not been validated for pulmo-

nary hypertension and should be interpreted with that knowl-
edge, although it has been validated in congestive cardiac heart
failure (19).

On balance, this study adds to the increasing body of data
supporting therapeutic benefit from sildenafil in PAH and ex-
tends the management options for this condition. Its place in
the management of PAH still needs to be defined. Sildenafil is not
yet licensed for use in this condition, but data on the response
to treatment with sildenafil 20 to 80 mg 8 hourly have been
submitted to the Food and Drug Administration. There is an-
creasing interest in using sildenafil in combination with other
therapies, including bosentan (17), targeting different signaling
pathways. A pharmacokinetic interaction between these two
drugs, resulting in lower plasma sildenafil levels, indicates that
such combinations need careful pharmacodynamic evaluation (39).

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for attending a meeting; N.T. does not have a financial relationship with a commer-
cial entity that has an interest in the subject of this manuscript; W.G.S. has received
travel expenses from Actelion for attending a meeting; W.A.B. does not have a
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