

Bosentan decreases the plasma concentration of sildenafil when coprescribed in pulmonary hypertension

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Aims

To determine whether bosentan decreases the plasma concentration of sildenafil in patients with pulmonary arterial hypertension.

Methods

Ten patients (aged 39–77 years) with pulmonary arterial hypertension in WHO functional class III received bosentan 62.5 mg twice daily for 1 month, then 125 mg twice daily for a second month. Sildenafil 100 mg was given as a single dose before starting bosentan (visit 1) and at the end of each month of bosentan treatment (visits 2 and 3). Sildenafil and its primary metabolite, desmethylsildenafil, were measured in plasma at 0 h and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18 and 24 h using liquid chromatography-tandem mass spectrometry. Statistical analysis was by repeated measures ANOVA, using log transformed data where appropriate.

Results

Treatment with bosentan 62.5 mg twice daily for 4 weeks was associated with a two-fold increase in sildenafil clearance/F and a 50% decrease in the AUC ($P < 0.001$). Increasing the dose of bosentan to 125 mg twice daily led to a further increase in sildenafil oral clearance and decrease in the AUC ($P < 0.001$ vs. 62.5 mg bosentan). The ratio of AUC on bosentan treatment relative to that of visit 1 was 0.47 [95% confidence interval (CI) 0.36, 0.61] for visit 2 and 0.31 (95% CI 0.23, 0.41) for visit 3 ($P < 0.001$). Sildenafil $C_{\max}$ fell from 759 ng mL⁻¹ on visit 1 to 333 ng mL⁻¹ on visit 3 ($P < 0.01$) and there was a significant decrease in the plasma half-life of sildenafil on the higher bosentan dose ($P < 0.05$). The AUC and plasma half-life of desmethylsildenafil was also decreased by bosentan in a dose-dependent manner ($P < 0.01$).

Conclusions

Bosentan significantly decreases the plasma concentration of sildenafil when coadministered to patients with pulmonary hypertension.

Introduction

Pulmonary hypertension encompasses a group of vascular diseases that result in elevated pulmonary artery pressure, leading to progressive hypertrophy and failure of the right ventricle [1]. The treatment options are limited and mortality remains high, particularly in

idiopathic pulmonary arterial hypertension (PAH) and PAH associated with collagen lung disease.

A recent advance in the management of PAH has been the introduction of bosentan, an inhibitor of endothelin A and B receptors. Endothelin is thought to play an important role in the pathogenesis of PAH and, consis-

tent with this, bosentan reduces pulmonary artery pressure and improves exercise capacity and functional class in patients with the condition [2, 3].

Another emerging oral therapy for PAH is sildenafil, a type 5 phosphodiesterase (PDE5) inhibitor. PDE5 is abundant in the pulmonary vasculature where it hydrolyses cyclic guanosine monophosphate (cGMP), a mediator of the vascular actions of nitric oxide and the natriuretic peptides. Sildenafil citrate has been shown to have beneficial effects on pulmonary vascular wall remodelling in animal models of pulmonary hypertension [4, 5] and to improve exercise capacity and pulmonary artery pressure in human disease [6, 7].

Both drugs are well absorbed following oral dosing. Studies of sildenafil 1.25–200 mg given as single doses to healthy subjects show an absolute oral bioavailability of around 40%, a peak plasma concentration at about 1 h and a terminal half-life of between 3 and 6 h [8, 9]. Bosentan has an absolute oral bioavailability of 50%, reaches a peak plasma concentration at 3 h and has a terminal half-life of 5.4 h [10]. Whereas each drug has the potential to modify the progression of PAH and improve patient well-being, neither represents a cure. There is interest in combining the two treatments for additional therapeutic benefit but there is a potential pharmacokinetic interaction. Sildenafil is cleared mostly by hepatic metabolism, predominantly by the P450 enzyme CYP3A4, with N-desmethylsildenafil the main metabolite [11–13]. N-desmethylsildenafil has the same PDE specificity but about half the potency and plasma concentrations are around 40–50% of the parent drug [8, 9]. Bosentan is a known inducer of CYP3A4 as well as a substrate for this enzyme. Thus, steady-state concentrations at 3–5 days are 50% lower, probably because of induction [10]. In this study we have evaluated the effects of chronic treatment with bosentan on the pharmacokinetics of sildenafil citrate in 10 patients with advanced, stable pulmonary hypertension.

Methods

Subjects

Patients attending the Hammersmith Hospital between June 2003 and February 2004 with PAH or pulmonary hypertension related to chronic thromboembolic disease were recruited to the study. All were in WHO functional class III and eligible for bosentan therapy according to standard clinical criteria. The diagnosis of pulmonary hypertension was based on a mean pulmonary artery pressure greater than 25 mmHg at rest obtained from cardiac catheter data within the preceding 12 months. Exclusion criteria were: previous treatment with bosentan or sildenafil, elevated baseline liver enzymes (greater

than three times the upper limit of the normal range) and the urgent need for prostanoid therapy on clinical grounds.

Approval for the study was obtained from a local ethics review committee (Hammersmith, Queen Charlotte's & Chelsea and Acton Hospitals Research Ethics Committee) and written informed consent was obtained from all patients.

Protocol

Patients were admitted to hospital on three occasions 1 month apart with repetition of the following protocol. After admission, patients were fasted from 23.00 h until lunchtime the next day. After a baseline blood sample (09.00 h) patients were given sildenafil 100 mg. Blood samples were collected 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18 and 24 h post dose. Subjects received a standard lunch and dinner after the 3- and 10-h blood samples, respectively. Subjects were discharged on the third day following standard assessment. Patients received bosentan 62.5 mg twice daily between visits 1 and 2 and bosentan 125 mg twice daily between visits 2 and 3. The morning dose of bosentan was given with sildenafil 100 mg on visits 2 and 3.

Patients were taking the following medications: warfarin ($n = 9$), frusemide ($n = 5$), spironolactone ($n = 4$), amlodipine ($n = 3$), diltiazem ($n = 2$), amiodarone ($n = 2$), digoxin ($n = 2$), iron sulphate ($n = 2$), lansoprazole ($n = 2$), ranitidine ($n = 1$), pravastatin ($n = 1$), simvastatin ($n = 1$), gliclazide ($n = 1$), carbimazole ($n = 1$), prednisolone ($n = 1$), azathioprine ($n = 1$), didronel ($n = 1$), diazepam ($n = 1$). No changes to these medications were made during the course of the study.

Plasma concentrations of sildenafil and its primary metabolite desmethylsildenafil were measured using liquid chromatography-tandem mass spectrometry on silica column with an aqueous-organic mobile phase [14]. The lower limit of quantification for both analytes was 1.00 ng mL⁻¹. The assay was validated with a linear calibration range of 1.00–500 ng mL⁻¹. During sample analysis analytical quality control (QC) samples were prepared at 3, 30 and 350 ng mL⁻¹ of sildenafil and desmethylsildenafil. A further QC sample (sildenafil and desmethylsildenafil concentration 700 ng mL⁻¹, diluted 1 : 10) was prepared where dilution of samples into range occurred. The overall imprecision values (CVs) for the analysis of plasma QC samples at concentrations of 3, 30 and 350 ng mL⁻¹ were 9.2, 3.6 and 3.5%, respectively, for sildenafil and 8.6, 7.9 and 5.2%, respectively, for desmethylsildenafil. The mean inaccuracy (bias) of the assay ranged from 0.9 to +4.7% for sildenafil and 3.0 to +1.9% for desmethylsildenafil over the QC con-

centration range (including dilution QCs). The specificity of the assay was demonstrated in plasma containing bosentan. Both sildenafil and desmethylsildenafil are stable in human plasma for up to 12 months when stored at -20°C or at -70°C . Additionally, both components are stable for at least three freeze-thaw cycles.

Clinical assessment

On each hospital admission patients underwent a standard full physical examination. Routine blood tests included full blood count, renal and liver function tests. Systemic blood pressure was measured using an automated recorder (Dinamap Pro300) before and after sildenafil administration.

Data analysis

Individual concentration-time curves for both sildenafil and desmethylsildenafil were evaluated using SigmaPlot version 8, Systat Software UK Limited, 24 Vista Centre, 50 Salisbury Road, Hounslow, London TW4 6JQ. Data were analysed by nonlinear least squares regression analysis, fitting a single exponential decline from $T_{\max}$ to the last measured concentration. The following pharmacokinetic parameters were determined for both sildenafil and desmethylsildenafil: the apparent terminal elimination phase rate constant (k_{el}) from the nonlinear curve fit, terminal half life ($t_{1/2}$) from $k_{\text{el}}/0.693$, the area under the plasma concentration-time curve from zero time extrapolated to infinity (AUC) directly from the experimental data with extrapolation to infinity using C_t/k_{el} , the maximum observed plasma concentration ($C_{\max}$) determined by visual inspection of the data and clearance/F (for sildenafil) from Dose/AUC. Statistical analysis was by repeated measures ANOVA, using log-transformed data where appropriate (i.e. all parameters other than k_{el} and clearance/F). Data for the different treatment arms (visits) were compared with each other using a *post hoc* test for repeated measures with Bonferroni correction (Systat 11] Systat Software, London, UK). Results have been expressed as mean $\pm 95\%$ confidence interval (CI) with back transformation of logarithmic data as necessary.

The number of subjects recruited to the study was based on the assumption that a decrease in area-under-plasma concentration-curve (AUC) of $400 \text{ ng ml}^{-1} \text{ h}^{-1}$ would mean a one-third decrease in availability of circulating sildenafil to tissues. Assuming an SD of 400 for the average intrasubject variation, 13 subjects would permit the detection of a decrease in area-under-plasma concentration-curve (AUC) of $400 \text{ ng ml}^{-1} \text{ h}^{-1}$ with 90% power at $P < 0.05$, whereas such a change could be detected in 10 subjects with 80% power.

Results

Ten patients (39–77 years of age) with WHO functional class III PHT were recruited into the study (Table 1).

The mean pharmacokinetic values for sildenafil given alone and after coadministration with bosentan are shown in Table 2. The corresponding plasma concentration profiles are shown in Figure 1. Coadministration of bosentan 62.5 mg twice daily for 4 weeks was associated with a two-fold increase in sildenafil clearance/F and a 50% decrease in AUC ($P < 0.001$). Increasing the dose of bosentan to 125 mg twice daily led to a further increase in sildenafil clearance and decrease in AUC ($P < 0.001$ vs. 62.5 mg bosentan). The ratio of AUC on bosentan treatment relative to that on the control day showed a dose-dependent decrease [visit 2/visit 1, 0.47 (95% CI 0.36, 0.61) compared with visit 3/visit 1, 0.31 (0.23, 0.41), $P < 0.001$]. Sildenafil $C_{\max}$ fell from 759 ng ml^{-1} at baseline to 333 ng ml^{-1} on coadministration with bosentan 125 mg ($P < 0.01$) and there was a significant decrease in the plasma half-life ($t_{1/2}$) of sildenafil on the higher bosentan dose ($P < 0.05$). There was no significant difference in the ratios for $C_{\max}$ between visit 3 and visit 2 vs. visit 1. The AUC and plasma half-life of desmethylsildenafil was also decreased by bosentan in a dose-dependent manner ($P < 0.01$). The ratio $\text{AUC}_{\text{desmethylsildenafil}}/\text{AUC}_{\text{sildenafil}}$ showed a significant, dose-dependent increase following treatment with bosentan by approximately two-fold.

Two patients reported mild headache and ankle swelling after initiating bosentan therapy. There was a trend towards a decrease in baseline systemic blood pressure with increasing dose of bosentan ($P = 0.59$, Table 3). There was a small fall in systemic blood pressure during the first hour after receiving sildenafil on all three visits. This was asymptomatic, did not reach statistical significance at any individual visit, and there was no significant difference between the three visits. There was no significant change in plasma liver enzyme concentrations for the group but a two-fold rise from baseline was seen in two patients on the higher dose of bosentan.

Table 1

Patient demographics (mean $\pm$ SD)

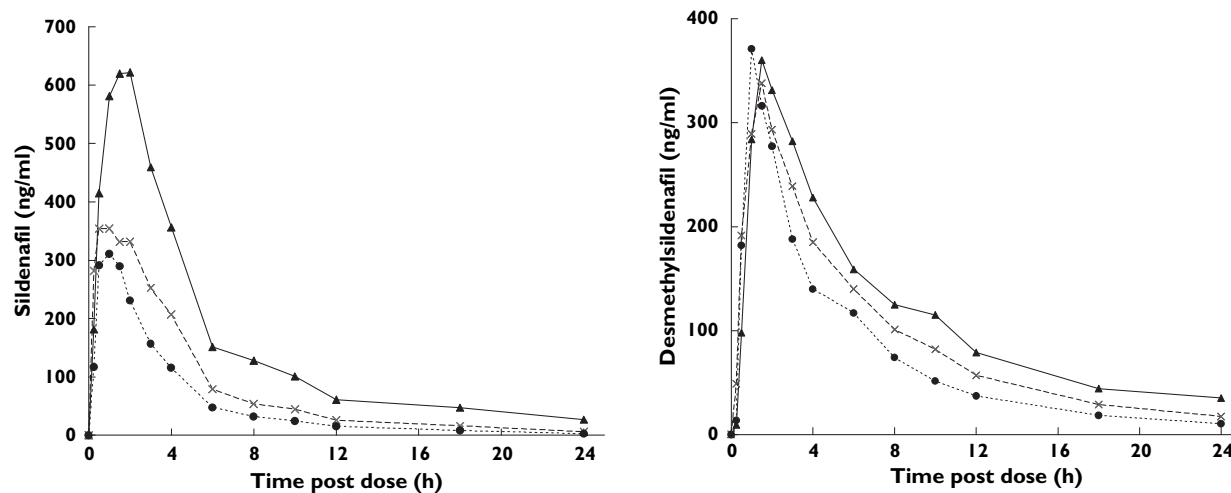
Age (years)	59.9 $\pm$ 11.8
Male/female	5/5
Body mass index	26.7 $\pm$ 3.29
Ethnic group, Caucasian (C)/black Caribbean (B-C)	C (8)/B-C (2)
Aetiology, primary (P)/chronic thromboembolic (CTE)	P (4)/CTE (6)

Table 2

Pharmacokinetic values for sildenafil and desmethylsildenafil

	Sildenafil 100 mg	Bosentan 62.5 mg bd	Bosentan 125 mg bd
<i>Sildenafil</i>			
AUC (ng ml ⁻¹ h)	3420 (2660, 4390)	1600 (1060, 2420)***	1060 (715, 1560)***†††
C _{max} (ng ml ⁻¹)	759 (550, 1050)	421 (264, 670)**	333 (217, 513)**
Clearance (ml min ⁻¹)/F	512 ± 153 (403, 621)	1170 ± 501 (814, 1530)**	1740 ± 655 (1280, 2210)***††
k _{el} (h ⁻¹)	0.32 ± 0.10 (0.25, 0.40)	0.38 ± 0.16 (0.26, 0.49)	0.52 ± 0.21 (0.37, 0.67)*
t _{1/2} (h)	2.24 (1.77, 2.83)	2.01 (1.48, 2.73)	1.42 (1.09, 1.85)*
<i>Desmethylsildenafil</i>			
AUC (ng ml ⁻¹ h)	2529 (1892, 3373)	1936 (1282, 2931)*	1585 (1069, 2350)***†††
C _{max} (ng ml ⁻¹)	378 (297, 482)	382 (272, 535)	379 (277, 518)
k _{el} (h ⁻¹)	0.19 ± 0.08 (0.13, 0.24)	0.30 ± 0.21 (0.15, 0.45)	0.39 ± 0.22* (0.23, 0.55)
t _{1/2} (h)	4.04 (2.96, 5.48)	2.78 (1.77, 4.38)*	2.05 (1.35, 3.11)**
AUC _{desmethylsildenafil}	0.740	1.21	1.50
AUC _{sildenafil}	(0.580, 0.944)	(0.94, 1.55)***	(1.14, 1.98)***†

Data are expressed as the mean ± SD (lower and upper 95% confidence intervals), calculated on the log transformed data with back transformation as appropriate (see text for details). *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001 vs. baseline; †, ††, ††† vs. bosentan 62.5 mg.

**Figure 1**

Mean plasma concentration-time curves for (A) sildenafil and (B) desmethylsildenafil following administration of sildenafil 100 mg alone (▲) after 4 weeks' bosentan 62.5 mg b.d. (×) and after 4 weeks' bosentan 125 mg b.d. (●). *n* = 10

Table 3

Haemoglobin, serum creatinine and liver enzyme (with normal ranges) data and blood pressure for each visit

	Visit 1	Visit 2	Visit 3
Haemoglobin (11.4–14.2 g dl ⁻¹)	15.5 (13.7, 17.3)	14.9 (13.3, 16.5)	14.9 (13.4, 16.5)
Creatinine (60–110 µmol l ⁻¹)	104 (95, 118)	103 (92, 115)	108 (96, 119)
Alanine transaminase (0–31 IU l ⁻¹)	27 (21, 42)	29 (18, 40)	33 (16, 50)
Aspartate transaminase (0–31 IU l ⁻¹)	31 (24, 36)	32 (25, 38)	33 (22, 45)
Systolic BP (mmHg)	–	–	–
Baseline	117 (104, 130)	111 (100, 122)	108 (94, 122)
1 h post dose	108 (94, 121)	104 (95, 112)	98 (89, 107)
3 h post dose	110 (97, 124)	101 (95, 108)	106 (97, 115)
24 h post dose	112 (102, 122)	111 (104, 119)	103 (97, 109)
Diastolic BP (mmHg)	–	–	–
Baseline	73 (63, 84)	70 (63, 76)	69 (62, 76)
1 h post dose	69 (59, 79)	66 (60, 73)	63 (58, 68)
3 h post dose	69 (59, 79)	68 (60, 75)	67 (60, 74)
24 h post dose	73 (66, 81)	73 (68, 79)	63 (57, 69)

Data are mean with lower and upper 95% confidence intervals.

Discussion

These data demonstrate that bosentan decreases markedly the plasma AUC of both sildenafil and desmethylsildenafil and also the maximum plasma concentration of sildenafil. Bosentan is known to induce CYP3A4 expression in the gut wall and liver and this is the most likely mechanism underlying the interaction with sildenafil. Consistent with this, there was a marked increase in clearance/F for sildenafil. AUC and clearance/F could change as a consequence of an effect on either absorption or metabolism. However, it is known that the modest bioavailability of sildenafil (~40%) is a consequence of presystemic metabolism, with absorption after an oral dose in excess of 90% [8]. In the present study, treatment with bosentan increased the ratio of AUC_{desmethylsildenafil} to AUC_{sildenafil} by two-fold at the higher dose. Hence, the likeliest explanation for the changes in pharmacokinetics of sildenafil following coadministration of bosentan is induction of presystemic metabolism by liver and/or intestine. CYP3A4 is the major enzyme involved in the clearance of sildenafil [12] and there is evidence for its involvement in the elimination of desmethylsildenafil [15]. Increased elimination of desmethylsildenafil by induction of CYP3A4 would explain why the increased rate of metabolism of sildenafil does not lead to increased concentrations of its metabolite.

Further support for a major role for CYP3A4 in sildenafil metabolism comes from the observation that

baseline (prebosentan) AUC values in our study were highest in the two patients who were also taking amiodarone, a potent CYP3A4 inhibitor [16]. Chronic bosentan therapy significantly decreased the AUC from 5784 to 1123 ng ml⁻¹ h⁻¹ in one of these patients. A marginal fall in AUC after 8 weeks' treatment was observed in the other patient. This patient was also taking diltiazem, a weak CYP3A4 inhibitor, for which there is some evidence for an inhibitory effect on sildenafil metabolism [17]. It would appear that the combination was sufficient to offset the induction of sildenafil metabolism by bosentan.

The IC₅₀ for inhibition of PDE5 by sildenafil *in vitro* is 2.7 ng ml⁻¹ [18]. Taking into account its high protein binding (95%), it would appear that the plasma concentration of sildenafil is maintained above the IC₅₀ for between 12 and 18 h following a 100-mg dose. However, following 8 weeks of bosentan, plasma concentrations were above the IC₅₀ for less than 6 h. It is recognized that single-dose administration of sildenafil does not reflect the clinical setting where the drug would be given regularly. Neither the minimum nor the optimum dose of sildenafil for treating pulmonary hypertension have been defined. Doses of 20–100 mg eight-hourly added to conventional therapy (diuretics and warfarin) have been evaluated and found to be effective in improving exercise capacity, with the lower dose as effective as the higher dose in this range (unpublished observations). Given this broad dosing range, the bosentan-induced

decrease in plasma concentration observed in this study might not impair the effectiveness of sildenafil 100 mg given regularly. Nonetheless, it may decrease the effect of lower doses of sildenafil, even if taken eight-hourly.

Both sildenafil and bosentan have the potential to influence systemic vascular tone and therefore systemic blood pressure, but the study was not powered to detect an effect. There was a trend towards a decrease in baseline systemic blood pressure with increasing dose of bosentan. The addition of sildenafil was associated with an asymptomatic fall in systolic blood pressure in the first hour on all three visits, but this did not reach statistical significance.

In summary, bosentan significantly decreases the plasma concentration of sildenafil when coprescribed to patients with pulmonary hypertension. This interaction would be expected to undermine the potential benefits of combining the two drugs.

Contribution of authors

The study was conceived by M. Wilkins. All authors contributed to the study design. A. Boobis assisted with the statistical analysis and interpretation of the data. G. Paul wrote the initial version of the manuscript, which was revised and approved by all contributors.

Competing interests

Actelion Pharmaceuticals UK, who make bosentan, has contributed funds to support the pulmonary hypertension service at Hammersmith Hospital. S. Gibbs has received consultancy fees and M. Wilkins lecture fees from Actelion Pharmaceuticals UK and Pfizer Ltd. G. Paul has received support from Actelion Pharmaceuticals for attending scientific meetings.

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