Clarithromycin substantially increases steady-state bosentan exposure in healthy volunteers

Christoph Markert, Yvonne Schweizer, Regina Hellwig,
Theresia Wirsching, Klaus-Dieter Riedel, Juergen Burhenne,
Johanna Weiss, Gerd Mikus & Walter E. Haefeli

*Department of Clinical Pharmacology and Pharmacoepidemiology, University of Heidelberg,
Heidelberg, Germany*

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Exposure to the endothelin antagonist bosentan depends on the activity of the cytochrome P450 isozymes CYP3A4 and polymorphic CYP2C9 and the activity of organic anion-transporting polypeptides (OATP) 1B1 and 1B3.
- Clarithromycin is a potent inhibitor of CYP3A4 and OATP1B1, suggesting that concomitant therapy will result in a major increase in bosentan exposure.

WHAT THIS STUDY ADDS

- Our study shows that clarithromycin substantially increases the exposure to bosentan and the active hydroxy-metabolite, suggesting that dose reductions may be necessary.
- The CYP2C9 and OATP1B1 polymorphisms had no influence on bosentan pharmacokinetics.

Correspondence

Professor Walter E. Haefeli MD,
Department of Clinical Pharmacology and
Pharmacoepidemiology, University of
Heidelberg, Im Neuenheimer Feld 410,
69120 Heidelberg, Germany.
Tel.: +49 6221 56 8740
Fax: +49 6221 56 4642
E-mail:
walter.emil.haefeli@med.uni-heidelberg.de

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AIMS

The aim of this study was to assess the effect of the cytochrome P450 (CYP) 3A4 and organic anion-transporting polypeptide (OATP) 1B1 inhibitor clarithromycin on the pharmacokinetics of bosentan. We also aimed to evaluate the impact of CYP2C9 and SLCO1B1 (encoding for OATP1B1) genotypes and their combination.

METHODS

We assessed the effect of the OATP and CYP3A inhibitor clarithromycin on bosentan pharmacokinetics at steady state and concurrently quantified changes of CYP3A activity using midazolam as a probe drug. Sixteen healthy volunteers received therapeutic doses of bosentan (125 mg twice daily) for 14 days and clarithromycin (500 mg twice daily) concomitantly for the last 4 days, and bosentan pharmacokinetics was assessed on days 1, 10 and 14.

RESULTS

Clarithromycin significantly increased bosentan area under the plasma concentration–time curve of the dosing interval 3.7-fold and peak concentration 3.8-fold in all participants irrespective of the genotype. Clarithromycin also reduced CYP3A activity (midazolam clearance) in all participants; however, these changes were not correlated to the changes of bosentan clearance.

CONCLUSIONS

Clarithromycin substantially increases the exposure to bosentan, suggesting that dose reductions may be necessary.

Introduction

As a nonselective endothelin-1 receptor antagonist, bosentan has been shown to reduce morbidity in patients with pulmonary arterial hypertension [1], and in a meta-analysis of targeted pulmonary arterial hypertension therapies (including bosentan), mortality also was reduced [2]. Beneficial [1] and (mainly metabolic and hepatic) adverse effects of bosentan [3] are dose dependent, suggesting that the safety margin for bosentan is rather narrow. In addition to dose, exposure to bosentan also depends on the activity of hepatic uptake via organic anion-transporting polypeptide (OATP) transporters OATP1B1 and OATP1B3 [4] and subsequent oxidative metabolism through cytochrome P450 (CYP) 3A4 and 2C9 to the pharmacologically active metabolite Ro 48-5033 (hydroxy-bosentan) and two inactive phenolic metabolites [5]. In addition, *in vitro* data suggest that bosentan is also a substrate of the hepatic efflux transporters P-glycoprotein and bile-salt export pump [6].

In line with these *in vitro* findings, co-medication modifying the activity of these targets has been shown to alter exposure with bosentan critically. As an example, inhibition of OATP1B by ciclosporin may increase plasma concentrations of bosentan by an order of magnitude [7] and therefore this combination is contraindicated. Likewise, inhibition of CYP3A4 by ketoconazole approximately doubles bosentan exposure, thus highlighting the important role of this isozyme in bosentan elimination [8]. Even more impressively, administration of ritonavir, which inhibits CYP3A, P-glycoprotein, OATPs and bile-salt export pump [6], results in a roughly fivefold increase in the exposure of bosentan and its active metabolite [9], stressing that interactions with compounds affecting multiple pathways will probably be more relevant.

Clarithromycin is another drug known to inhibit OATPs [10], CYP3A4 [11, 12] and P-glycoprotein [13, 14], suggesting that its impact on bosentan pharmacokinetics will also exceed the effects observed with ketoconazole. Moreover, because the contribution of CYP2C9 to bosentan metabolism is estimated to be 40% [5] and because it is a polymorphic enzyme, with ~5% of Caucasians being carriers of less active *2 or *3 alleles (poor metabolizers [15]), it appears possible that the clearance will be lowest during such an interaction in poor metabolizers. Likewise, genetic changes in the activity of OATPs could also modulate the extent of such an interaction.

We evaluated the effect of clarithromycin on bosentan pharmacokinetics in the absence and presence of polymorphisms in the OATP1B1 (*SLCO1B1*) and CYP2C9 genes known to reduce the activity of these targets. Concurrently, we used repetitive midazolam phenotyping [16] to estimate the contribution of CYP3A4 to the overall changes observed.

Methods

Study quality standards

After approval by the responsible Ethics Committee and the Federal Institute of Drugs and Medical Devices (BfArM, Bonn, Germany) this study (EudraCT 2010-021392-93) was conducted at the Clinical Research Center of the Department of Clinical Pharmacology and Pharmacoepidemiology, University of Heidelberg, which is certified according to DIN EN ISO9001:2008. It followed the standards of Good Clinical Practice, the Declaration of Helsinki and the specific legal requirements in Germany. Written informed consent was obtained from each participant before inclusion.

Population

The participants were screened for the presence of the functional *SLCO1B1**15 haplotype and *CYP2C9**2 and *3 alleles. Four groups of healthy volunteers were enrolled, who were classified as follows: (i) carriers of wild-type alleles of *CYP2C9* (extensive metabolizers) and *SLCO1B1* (EM/wt); (ii) carriers of wild-type *CYP2C9* and deficient *SLCO1B1* (EM/def); (iii) poor metabolizers of *CYP2C9* and wild-type *SLCO1B1* (PM/wt); or (iv) deficient for both (PM/def).

None of the participants had been on any regular drug treatment in the past 2 months before the study (except for oral contraceptives in females) and none was taking drugs or substances known to induce or inhibit drug-metabolizing enzymes or transporters within a period of less than 10 times the respective elimination half-life. Grapefruit juice was not allowed for the preceding 3 days before the start of the study. The participants were ascertained to be in a good state of health on the basis of medical history, physical examination and routine laboratory testing (including screening for illicit drugs). Women were required to be nonlactating, to have a negative pregnancy test and to use two independent, effective contraceptive methods. Participants with any of the following conditions were excluded: intolerance to study drugs; regular smoking; excessive alcohol drinking; any condition which could modify the pharmacokinetics of the study drugs under investigation; allergies (except for mild forms of hay fever) or hypersensitivity reactions; participation in another clinical trial within 1 month before the study; moderate or severe liver or renal impairment; blood donation within 6 weeks prior to the study; pulmonary arterial hypertension; and inability to give written informed consent or to communicate well with the investigator.

During the whole study, smoking, alcohol and beverages containing grapefruit juice were not allowed. Twenty-four hours before and on each study day, participants were not allowed to drink caffeinated beverages. Participants were fasted on all study days and received standardized lunch and dinner.

Study design

This was an open, monocentre, fixed sequence, multiple dose study assessing the effect of clarithromycin on bosentan pharmacokinetics dosed to steady state and its relationship with *CYP2C9* and *SLCO1B1* genotypes. Concurrently, intraindividual changes in *CYP3A4* activity were monitored [16].

Study conduct

The study was conducted at bosentan steady state to account for auto-induction of the metabolism [17]. Participants were started on oral bosentan 125 mg daily on day 1 (Tracleer®; Actelion Pharmaceuticals, Freiburg, Germany) and were maintained on 125 mg twice daily from day 2 to day 14. Additionally, from day 11 to day 14 oral clarithromycin ($2 \times 500 \text{ mg day}^{-1}$; Klacid® Pro; Abbott, Wiesbaden, Germany) was co-administered with bosentan. On study days, plasma samples were collected before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 12 h (on day 1 also 24 h) after administration of bosentan to determine pharmacokinetics of bosentan.

Prior to the study and on study days 1, 10 and 14, *CYP3A* activity was monitored after oral doses of 3 mg midazolam using a validated limited sampling strategy (as [16]).

Genotyping of *CYP2C9* and *SLCO1B1*

For genotyping of *CYP2C9**2 (rs1799853), *CYP2C9**3 (rs1057910) and *SLCO1B1**15 (rs2306283, rs4149056), we used the hybridization format on the LightCycler480® (Roche Applied Sciences, Mannheim, Germany), with primers and probes as published previously [18, 19].

Analytical assays

Quantification of bosentan and its major metabolites was performed with a validated liquid chromatography–tandem mass spectrometry method (for details see supplementary material) [20]. The lower limit of quantification was 0.20 ng ml^{-1} for bosentan, 0.125 ng ml^{-1} for desmethyl-bosentan, 0.25 ng ml^{-1} for hydroxy-desmethyl-bosentan and 0.25 ng ml^{-1} for hydroxy-bosentan. Calibration for all drugs was linear, and the coefficients of correlation (r^2) were always >0.99 . The within-day and day-to-day accuracy and precision data of the quality control of bosentan and all metabolites were always within $\pm 15\%$.

The plasma concentrations of midazolam were determined with a validated chromatography–tandem mass spectrometry method as previously described [21]. The calibrated range was $0.525\text{--}150 \text{ ng ml}^{-1}$ for midazolam, with a correlation coefficient >0.99 . The lower limit of quantification was 0.525 ng ml^{-1} . The accuracies were always within $\pm 15\%$, with corresponding precision of $<15\%$ of the coefficient of variation.

Pharmacokinetic analysis and statistical evaluation

Data are presented as point estimates with 95% confidence intervals (CIs) unless indicated otherwise. Standard pharmacokinetic parameters of bosentan and midazolam were determined using Kinetica 5.0 (Thermo Fisher Scientific, Waltham, MA, USA). Peak concentration (C_{max}) was directly obtained from individual data. Area under the plasma concentration–time curve of the dosing interval ($\text{AUC}_{0-\tau}$) and AUC from the time of dosing extrapolated to infinity ($\text{AUC}_{0-\infty}$) were determined using the linear trapezoidal rule. The terminal slope of the concentration–time curve, λ , was calculated by linear regression of the time vs. log concentration data. The metabolic ratio in plasma was calculated as the molar ratio of $\text{AUC}_{0-\infty}$ or $\text{AUC}_{0-\tau}$ of bosentan divided by the sum of $\text{AUC}_{0-\infty}$ or $\text{AUC}_{0-\tau}$ of its metabolites. The apparent oral clearance of bosentan (Cl/F) after the first and repetitive administration was calculated as the dose of bosentan divided by plasma $\text{AUC}_{0-\infty}$ or $\text{AUC}_{0-\tau}$, respectively. Midazolam clearance was estimated using partial AUC values as previously described [16]. The relationship between midazolam and bosentan clearance changes induced by clarithromycin was analysed using Spearman rank correlation. The nonparametric Mann–Whitney *U* test was used to evaluate differences between two samples of independent observations using Prism 5.01 (GraphPad Software, La Jolla, CA, USA). A *P* value <0.05 was considered significant.

Assuming an impact of clarithromycin similar to cyclosporine [9], five participants are enough to detect a mean AUC difference with a power $>80\%$ when applying a *t*-test for paired observations and unequal variances at a two-sided significance level of 5%. Due to multiplicity issues, no confirmatory power calculation was used for the assessment of the influence of the haplotypes on bosentan pharmacokinetics.

Results

After screening >500 healthy volunteers, we enrolled a total of 16 participants (15 Caucasians and one Hispanic) with a mean age ($\pm \text{SD}$) of $31.6 (\pm 7.8)$ years and a body mass index of $23.0 (\pm 2.4) \text{ kg m}^{-2}$ in the four groups. Six participants were EM/wt (three females), four were EM/def (two females), five were PM/wt (one female; four participants with *CYP2C9**2/*3, one with *CYP2C9**2/*2 genotype), and one male was PM/def (*CYP2C9**2/*3).

Pharmacokinetics of bosentan and its metabolites is shown in Tables 1 and 2 and Figure 1. Due to auto-induction of its metabolism, bosentan exposure at steady state (day 10) was reduced to 57% and apparent oral clearance increased by 73% of the corresponding value on day 1. The AUC of hydroxy-bosentan and the molar metabolic ratio (bosentan/hydroxy-bosentan) decreased to 75 and 77%, respectively. Furthermore, AUCs of

Table 1

Point estimates (95% confidence intervals) of bosentan and its metabolites compared with steady state values in 16 healthy participants before and during clarithromycin administration

Bosentan	First dose	All (n = 16) With clarithromycin
C_{max}	1.29 (0.97, 1.73)	3.82 (2.84, 5.13)
AUC	1.74 (1.34, 2.24)	3.73 (2.85, 4.89)
Cl/F	0.58 (0.45, 0.74)	0.27 (0.20, 0.35)
Metabolic ratio	1.33 (1.16, 1.52)	1.63 (1.47, 1.82)
AUC of hydroxy-bosentan (Ro 48-5033)	1.33 (1.07, 1.66)	3.05 (2.32, 3.99)
Molar metabolic ratio (bosentan/hydroxy-bosentan)	1.31 (1.15, 1.48)	1.23 (1.10, 1.37)
AUC of desmethyl-bosentan (Ro 47-8634)	1.41 (1.23, 1.62)	1.43 (1.14, 1.80)
AUC of hydroxy-desmethyl-bosentan (Ro 64-1056)	1.25 (1.06, 1.47)	1.52 (1.27, 1.82)

Point estimates of bosentan and its metabolites are shown. These parameters have no units – in contrast to geometric means (Table 2). Abbreviations are as follows: AUC, area under plasma concentration–time profile; Cl/F , apparent oral clearance; C_{max} , peak plasma concentration; and metabolic ratio, molar plasma ratio of AUC of bosentan divided by the sum of the AUCs of its three metabolites (Ro 48-5033, Ro 47-8634 and Ro 64-1056).

Figure 1

Concentration–time profiles of bosentan after 10 days of treatment in 16 healthy volunteers stratified according to *CYP2C9* genotype and *SLCO1B1* genotype. Extensive metabolizers of *CYP2C9* are shown as filled symbols, poor metabolizers as open symbols, wild-type *OATP1B1* carriers are shown as squares, and the defective variant as circles. Values are shown as means $\pm$ SD. ■, *CYP2C9* EM/ *OATP1B1* wt; ●, *CYP2C9* EM/*OATP1B1* def; □, *CYP2C9* PM/*OATP1B1* wt; ○, *CYP2C9* PM/*OATP1B1* def

desmethyl-bosentan and hydroxy-desmethyl-bosentan were also decreased to 71 and 80%, respectively. The molar metabolic ratio of the AUC of bosentan divided by the sum of the AUCs of all metabolites was reduced to 75%.

The pharmacokinetics of bosentan and its metabolites was similar in carriers of individual haplotypes both on day 1 and on day 10 and when extensive ($n = 10$) and poor metabolizers of *CYP2C9* ($n = 6$) were pooled irrespective of the *OATP1B1* status (Supplementary Tables S1–3).

Figure 2

Effect of 4 days of clarithromycin co-administration (▲) on bosentan pharmacokinetics (steady state; ○). Pooled data of 16 healthy volunteers with different *CYP2C9* and *SLCO1B1* genotypes

Co-administration of clarithromycin substantially increased peak concentrations of bosentan (by 282%) and $AUC_{0-\tau}$ (by 273%; Figure 2 and Tables 1 and 2). The extent of accumulation was similar in all genotype groups (Figure 3). The AUCs of hydroxy-bosentan, desmethyl-bosentan and hydroxy-desmethyl-bosentan were increased by 204, 44 and 52%, respectively. The molar metabolic ratio of bosentan/hydroxy-bosentan and the molar metabolic ratio of the AUC of bosentan divided by the sum of AUCs of all metabolites increased by 23 and 63%, respectively (Tables 1 and 2).

The *CYP3A* activity as measured by midazolam clearance (Cl_{met}) at baseline was 684 ml min^{-1} (95% CI 519, 902 ml min^{-1}). Midazolam clearance changed substantially during this study, whereas acute bosentan administration had no effect [compared with baseline, point estimate (95% CI) 1.02 (0.89, 1.17)], the clearance of midazolam increased 3.2-fold after 10 days of bosentan [3.17 (2.39, 4.20)]. Co-administration of clarithromycin decreased midazolam clearance to 14% of its value at bosentan steady state (Figure 4) corresponding to a decrease to 44% of midazolam clearance at baseline [0.44 (0.34, 0.58)]. The changes in bosentan clearance were not correlated with those of midazolam (Figure 5).

Safety

Overall, 53 adverse events occurred in nine participants; all were transient, none was serious, and none resulted in dropouts. Before co-administration of clarithromycin, 26 adverse events occurred in nine participants (nasopharyngitis, nasal congestion, headache, loss of appetite, nausea, flatulence, night sweats, dizziness, fatigue, epigastric pain, diarrhoea and eczema). During clarithromycin co-administration, 27 adverse events in nine participants were noted (increase of creatine kinase, increase of

Table 2

Geometric means (95% confidence intervals) of bosentan and its metabolites in 16 healthy participants after a single dose, at steady state and during clarithromycin administration

Bosentan	First dose	All (n = 16) Steady state	With clarithromycin
C_{max} (ng ml ⁻¹)	1890 (1540, 2310)	1460 (1120, 1900)	5570 (4600, 6740)
AUC (h ng ml ⁻¹)	11 900 (9730, 14 500)	6830 (5330, 8750)	25 500 (21 700, 29 900)
Cl/F (ml min ⁻¹)	176 (144, 214)	305 (238, 391)	81.7 (69.7, 95.8)
Metabolic ratio	8.66 (7.37, 10.2)	6.52 (5.69, 7.46)	10.6 (8.99, 12.6)
AUC of hydroxy-bosentan (Ro 48-5033; h ng ml ⁻¹)	662 (507, 863)	497 (357, 693)	1510 (1130, 2030)
Molar metabolic ratio (bosentan/hydroxy-bosentan)	18.4 (14.8, 23.0)	14.1 (11.7, 17.1)	17.3 (13.5, 22.3)
AUC of desmethyl-bosentan (Ro 47-8634; h ng ml ⁻¹)	165 (131, 208)	117 (92.9, 148)	168 (122, 231)
AUC of hydroxy-desmethyl-bosentan (Ro 64-1056; h ng ml ⁻¹)	529 (431, 649)	423 (327, 546)	641 (558, 738)

Abbreviations are as for Table 1.

Figure 3

Changes in bosentan clearance (Cl/F) after co-administration of clarithromycin for 4 days. Extensive metabolizers of CYP2C9 are shown as filled symbols, poor metabolizers as open symbols, wild-type OATP1B1 carriers are shown as squares, and the defective variant as circles

aspartate transaminase, taste disorder, diarrhoea, nasopharyngitis, headache, nasal congestion, dizziness, concentration difficulty, tachycardia, abdominal pain, fatigue, epigastric pain, eczema, tinnitus and xerostomia).

Discussion

When clarithromycin was added to an established bosentan regimen, bosentan exposure increased by 273%. This is more than twice the extent observed with the potent CYP3A inhibitor ketoconazole [8], suggesting that

Figure 4

Percentage changes in midazolam clearance induced by bosentan and co-administration of clarithromycin in 16 healthy volunteers

alternative clearance pathways were concurrently affected. Two further arguments suggest that not only CYP3A was involved. First, changes in midazolam clearance did not correlate with the respective changes in bosentan clearance and second, the percentage change in bosentan clearance was less than the respective change in midazolam clearance, suggesting that this interaction is only partly explained by CYP3A inhibition and other pathways must be involved that are not shared by midazolam.

The design of our study does not allow us to identify the alternative pathways affected by clarithromycin unequivocally. However, a likely explanation is the involvement of hepatic uptake by OATP1B1 and OATP1B3, both of which are inhibited by clarithromycin *in vitro* [10], whereas OATP1B3 is not affected by ketoconazole [22]. *In vivo* evidence for this mode of interaction is also provided by the interaction of clarithromycin with the OATP1B substrate pravastatin, which is not a CYP3A substrate, leading to a twofold increase when combined [23]. Finally, inhibition of

Figure 5

Relationship between absolute changes in metabolic clearance of midazolam (Cl_{met}) and apparent oral clearance of bosentan (Cl/F) induced by clarithromycin compared with corresponding steady state values of healthy volunteers. Extensive metabolizers of CYP2C9 are shown as filled symbols, poor metabolizers as open symbols, wild-type OATP1B1 carriers are shown as squares, and the defective variant as circles. r_s = Spearman's rank correlation coefficient

P-glycoprotein by clarithromycin could also have contributed to this interaction, because bosentan appears also to be a substrate of this efflux transporter [6]. In contrast, CYP2C9 is probably not involved, because clarithromycin does not inhibit this isozyme [24] and because formation of the hydroxylated metabolites did not differ from the formation of the phenol metabolite, which is produced only by CYP3A4 [5].

Auto-induction of bosentan is a well-known, dose-dependent phenomenon, which decreases exposure in the first week of treatment [8]. The extent of AUC change was similar to earlier trials, and induction certainly affected CYP3A, leading to reduced exposure also to midazolam on day 10. However, the changes in bosentan clearance again exceeded the changes in midazolam clearance, thus suggesting that other pathways were also induced. In addition to CYP3A substrates, such as sildenafil [25] or simvastatin [26], bosentan has also been shown to induce CYP2C9 *in vitro* [27] and to induce the metabolism of the CYP2C9 substrate S-warfarin *in vivo* [28]. Moreover, as an inducer of pregnane X receptor [17], bosentan affects a multitude of targets, including P-glycoprotein [27]. However, *in vivo* this translates into only mild reductions of P-glycoprotein substrates, such as digoxin [29], suggesting that this is not a major area of auto-induction.

Recruitment of volunteers with deficient OATP1B1 uptake was difficult, and only one CYP2C9 poor metabolizer with the deficient *SLCO1B1**15 haplotype was found. Therefore, a statistical analysis according to genotypes was not possible for all subgroups and is only exploratory in nature. However, in the pooled analysis of the five participants with deficient OATP1B1-mediated

uptake, bosentan pharmacokinetics was similar to those in participants with functional uptake, suggesting that a potential difference was not large and certainly not clinically relevant. This may have been expected, because OATP1B3 also contributes to hepatic bosentan uptake [4]. Likewise, another study in Japanese patients evaluated the *SLCO1B1**5 polymorphism (rs4149056), which is part of the *SLCO1B1**15 haplotype, and did not find a difference in bosentan pharmacokinetics. However, this earlier study enrolled only heterozygous carriers of the mutation, who may not express a fully deficient phenotype [30].

Somewhat more surprising and unexplained was the fact that the absence of functional CYP2C9 alleles appeared to have no influence on bosentan single-dose and steady-state pharmacokinetics, although bosentan metabolism is reported to depend entirely on CYP3A and CYP2C9, and the contribution to the metabolism of the latter is considered to be 40% [5]. This finding could be due to a real lack of difference or to low power to detect genotype-dependent differences.

In conclusion, clarithromycin substantially increases the exposure to bosentan and its active hydroxy metabolite, suggesting that vascular effects may be increased. Hence, in such a combination, close monitoring and lower maintenance doses may be required.

Competing Interests

WEH has received speakers' fees for educational meetings and honoraria for attending advisory boards or data safety monitoring boards from various pharmaceutical companies, including Actelion, Bayer, Berlin Chemie, GlaxoSmithKline, Grünenthal, Novartis, Ono and Roche. The other authors have no competing interests to declare.

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REFERENCES

- Rubin LJ, Badesch DB, Barst RJ, Galie N, Black CM, Keogh A, Pulido T, Frost A, Roux S, Leconte I, Landzberg M, Simonneau G. Bosentan therapy for pulmonary arterial hypertension. *N Engl J Med* 2002; 346: 896–903.
- Galiè N, Manes A, Negro L, Palazzini M, Bacchi-Reggiani ML, Branzi A. A meta-analysis of randomized controlled trials in pulmonary arterial hypertension. *Eur Heart J* 2009; 30: 394–403.
- Spangler ML, Saxena S. Warfarin and bosentan interaction in a patient with pulmonary hypertension secondary to bilateral pulmonary emboli. *Clin Ther* 2010; 32: 53–6.

- 4 Treiber A, Schneider R, Häusler S, Stieger B. Bosentan is a substrate of human OATP1B1 and OATP1B3: inhibition of hepatic uptake as the common mechanism of its interactions with cyclosporin A, rifampicin, and sildenafil. *Drug Metab Dispos* 2007; 35: 1400–7.
- 5 Dingemanse J, van Giersbergen PLM. Clinical pharmacology of bosentan, a dual endothelin receptor antagonist. *Clin Pharmacokinet* 2004; 43: 1089–115.
- 6 Hartman JC, Brouwer K, Mandagere A, Melvin L, Gorczynski R. Evaluation of the endothelin receptor antagonists ambrisentan, darusentan, bosentan, and sitaxsentan as substrates and inhibitors of hepatobiliary transporters in sandwich-cultured human hepatocytes. *Can J Physiol Pharmacol* 2010; 88: 682–91.
- 7 Binet I, Wallnöfer A, Weber C, Jones R, Thiel G. Renal hemodynamics and pharmacokinetics of bosentan with and without cyclosporine A. *Kidney Int* 2000; 57: 224–31.
- 8 van Giersbergen PL, Halabi A, Dingemanse J. Single- and multiple-dose pharmacokinetics of bosentan and its interaction with ketoconazole. *Br J Clin Pharmacol* 2002; 53: 589–95.
- 9 Dingemanse J, van Giersbergen PLM, Patat A, Nilsson PN. Mutual pharmacokinetic interactions between bosentan and lopinavir/ritonavir in healthy participants. *Antiviral Ther* 2010; 15: 157–63.
- 10 Seithel A, Eberl S, Singer K, Auge D, Heinkele G, Wolf NB, Dörje F, Fromm MF, König J. The influence of macrolide antibiotics on the uptake of organic anions and drugs mediated by OATP1B1 and OATP1B3. *Drug Metab Dispos* 2007; 35: 779–86.
- 11 Rodrigues AD, Roberts EM, Mulford DJ, Yao Y, Ouellet D. Oxidative metabolism of clarithromycin in the presence of human liver microsomes. Major role for the cytochrome P4503A (CYP3A) subfamily. *Drug Metab Dispos* 1997; 25: 623–30.
- 12 Gorski JC, Jones DR, Haehner-Daniels BD, Hamman MA, O'Mara EM Jr, Hall SD. The contribution of intestinal and hepatic CYP3A to the interaction between midazolam and clarithromycin. *Clin Pharmacol Ther* 1998; 64: 133–43.
- 13 Hughes J, Crowe A. Inhibition of P-glycoprotein-mediated efflux of digoxin and its metabolites by macrolide antibiotics. *J Pharmacol Sci* 2010; 113: 315–24.
- 14 Rengelshausen J, Göggelmann C, Burhenne J, Riedel K-D, Ludwig J, Weiss J, Mikus G, Walter-Sack I, Haefeli WE. Contribution of increased oral bioavailability and reduced non-glomerular renal clearance of digoxin to the digoxin-clarithromycin interaction. *Br J Clin Pharmacol* 2003; 56: 32–8.
- 15 Van Booven D, Marsh S, McLeod H, Carrillo MW, Sangkuhl K, Klein TE, Altman RB. Cytochrome P450 2C9-CYP2C9. *Pharmacogenet Genomics* 2010; 20: 277–81.
- 16 Katzenmaier S, Markert C, Riedel KD, Burhenne J, Haefeli WE, Mikus G. Determining the time course of CYP3A inhibition by potent reversible and irreversible CYP3A inhibitors using a limited sampling strategy. *Clin Pharmacol Ther* 2011; 90: 666–73.
- 17 van Giersbergen PL, Gnerre C, Treiber A, Dingemanse J, Meyer UA. Bosentan, a dual endothelin receptor antagonist, activates the pregnane X nuclear receptor. *Eur J Pharmacol* 2002; 450: 115–21.
- 18 Burian M, Grösch S, Tegeder I, Geisslinger G. Validation of a new fluorogenic real-time PCR assay for detection of CYP2C9 allelic variants and CYP2C9 allelic distribution in a German population. *Br J Clin Pharmacol* 2002; 54: 518–21.
- 19 Aklillu E, Mugusi S, Ngaimisi E, Hoffmann MM, König S, Ziesenitz V, Mikus G, Haefeli WE, Weiss J. Frequency of the SLCO1B1 388A>G and the 521T>C polymorphism in Tanzania genotyped by a new LightCycler®-based method. *Eur J Clin Pharmacol* 2011; 67: 1139–45.
- 20 FDA, US Department of Health and Human Services. Guidance for industry, bioanalytical method validation. 2001. Available at <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf> (last accessed 20 June 2013).
- 21 Fuchs I, Hafner-Blumenstiel V, Markert C, Burhenne J, Weiss J, Haefeli WE, Mikus G. Effect of the CYP3A inhibitor ketoconazole on the PXR-mediated induction of CYP3A activity. *Eur J Clin Pharmacol* 2013; 69: 507–13.
- 22 Karlgren M, Vildhede A, Norinder U, Wisniewski JR, Kimoto E, Lai Y, Haglund U, Artursson P. Classification of inhibitors of hepatic organic anion transporting polypeptides (OATPs): influence of protein expression on drug-drug interactions. *J Med Chem* 2012; 55: 4740–63.
- 23 Jacobson TA. Comparative pharmacokinetic interaction profiles of pravastatin, simvastatin, and atorvastatin when coadministered with cytochrome P450 inhibitors. *Am J Cardiol* 2004; 94: 1140–6.
- 24 Bruce MA, Hall SD, Haehner-Daniels BD, Gorski JC. In vivo effect of clarithromycin on multiple cytochrome P450s. *Drug Metab Dispos* 2001; 29: 1023–8.
- 25 Burgess G, Hoogkamer H, Collings L, Dingemanse J. Mutual pharmacokinetic interactions between steady-state bosentan and sildenafil. *Eur J Clin Pharmacol* 2008; 64: 43–50.
- 26 Dingemanse J, Schaarschmidt D, van Giersbergen PL. Investigation of the mutual pharmacokinetic interactions between bosentan, a dual endothelin receptor antagonist, and simvastatin. *Clin Pharmacokinet* 2003; 42: 293–301.
- 27 Weiss J, Herzog M, Haefeli WE. Differential modulation of the expression of important drug metabolising enzymes and transporters by endothelin-1 receptor antagonists ambrisentan and bosentan in vitro. *Eur J Pharmacol* 2011; 660: 298–304.
- 28 Weber C, Banken L, Birnboeck H, Schulz R. Effect of the endothelin-receptor antagonist bosentan on the pharmacokinetics and pharmacodynamics of warfarin. *J Clin Pharmacol* 1999; 39: 847–54.

- 29 Weber C, Banken L, Birnboeck H, Nave S, Schulz R. The effect of bosentan on the pharmacokinetics of digoxin in healthy male subjects. *Br J Clin Pharmacol* 1999; 47: 701–6.
- 30 Taguchi M, Ichida F, Hirono K, Miyawaki T, Yoshimura N, Nakamura T, Akita C, Nakayama T, Saji T, Kato Y, Horiuchi I, Hashimoto Y. Pharmacokinetics of bosentan in routinely treated Japanese pediatric patients with pulmonary arterial hypertension. *Drug Metab Pharmacokinet* 2011; 26: 280–7.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Appendix S1

Quantification of bosentan and its major metabolites in plasma

Table S1

Point estimates (95% CI) of bosentan compared to steady-state values in healthy participants with differing CYP2C9 and OATP1B1 polymorphisms before and during clarithromycin

Table S2

Point estimates (95% CI) of bosentan metabolites compared to steady-state values in healthy participants with differing CYP2C9 and OATP1B1 polymorphisms before and during clarithromycin

Table S3

Geometric means (95% CI) of bosentan and its metabolites in healthy participants with differing CYP2C9 and OATP1B1 polymorphisms after single dose, at steady-state and during clarithromycin