

In vivo and in vitro studies exploring the pharmacokinetic interaction between bosentan, a dual endothelin receptor antagonist, and glyburide

Background: In a clinical trial with patients with chronic heart failure, a higher incidence of elevated levels of liver transaminases was observed during concomitant treatment with bosentan, a dual endothelin receptor antagonist, and glyburide (INN, glibenclamide), a sulfonylurea-type antidiabetic drug, than with treatment with bosentan alone. This study was conducted to investigate a possible pharmacokinetic interaction between bosentan and glyburide.

Methods: In a randomized, 2-way crossover study, 12 healthy volunteers received treatments A and B. Treatment A consisted of 125 mg bosentan twice a day for 10 days plus concomitant 2.5 mg glyburide twice a day on days 6 to 10. Treatment B consisted of 2.5 mg glyburide twice a day for 10 days plus concomitant 125 mg bosentan twice a day on days 6 to 10. Plasma concentrations of bosentan and its metabolites and of glyburide were measured on days 5 and 10 of treatment A and treatment B, respectively.

Results: Bosentan reduced the area under the concentration-versus-time curve of glyburide approximately 40% ($P < .05$). Glyburide decreased the area under the concentration-versus-time curve of bosentan and its metabolites 20% to 30% ($P < .05$). Results of in vitro experiments showed that glyburide is metabolized by cytochrome P450 (CYP) 2C9, 2C19, and 3A4. No interaction was observed on the level of serum protein binding.

Conclusions: The plasma levels of both bosentan and glyburide were reduced after concomitant administration. This finding is consistent with a CYP3A4-inducing potential of both drugs. The observed pharmacodynamic interaction between bosentan and glyburide in patients with chronic heart failure cannot be explained by a pharmacokinetic interaction. (Clin Pharmacol Ther 2002;71:253-62)

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Endothelin 1 is one of the most potent and longest-lasting endogenous vasoconstrictors known. Since its discovery in 1988,¹ the role of endothelin 1 in pathophysiologic processes has been the topic of numerous investigations.^{2,3} Bosentan is an orally active, nonpeptidic,

competitive dual endothelin receptor antagonist with high affinity for both endothelin A and endothelin B receptors.⁴ It is in development for the management of chronic heart failure⁵ and pulmonary hypertension.⁶ The pharmacokinetics and pharmacodynamics of bosentan have been described previously.⁷⁻⁹ The drug has a low systemic plasma clearance of approximately 12 L/h and a volume of distribution of approximately 40 L. Steady-state plasma concentration is reached after 3 to 5 days, and the elimination half-life is approximately 5 to 6 hours after both single- and multiple-dose administration of 100 to 200 mg. The metabolism of bosentan depends on cytochrome P450 (CYP) 2C9 and 3A4 isozymes, and excretion of the metabolites into the bile constitutes the main pathway of elimination. The following 3 metabolites have been identified:

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the phenol metabolite (Ro 47-8634), the hydroxy metabolite (Ro 48-5033), and the hydroxy-phenol metabolite (Ro 64-1056),⁷ of which only the hydroxy metabolite is pharmacologically active (Actelion Pharmaceuticals Ltd, Allschwil, Switzerland; data on file). The hydroxy-phenol metabolite is a secondary metabolite formed from both the phenol and the hydroxy metabolites, and both CYP2C9 and 3A4 appear to play a role in its formation. The proposed metabolic pathways of bosentan in humans and the chemical structures of the metabolites are shown in Fig 1.

Glyburide (INN, glibenclamide) is widely used in the treatment of patients with type 2 diabetes mellitus. It is an orally active blood glucose-lowering drug of the sulfonylurea class. Diabetes is a frequently occurring comorbid condition among patients with chronic heart failure. In the REACH-1 (Research on Endothelin Antagonism in Chronic Heart Failure) trial, in which the long-term effects of endothelin blockade on patients with chronic heart failure were investigated, the incidence of elevated levels of liver transaminases was higher among bosentan-treated patients (43 of 244, 17.6%) than among placebo-treated patients (5 of 126, 4.0%). This incidence increased to 28.6% (10 of 35) in the subgroup of patients receiving 500 mg bosentan twice a day and glyburide 2.5 to 40 mg per day.¹⁰ In REACH-1, the bosentan-induced elevations in liver transaminase levels were asymptomatic and reversible and did not result in any signs of clinically significant liver disease.^{10,11}

Because the bosentan-induced elevations in levels of liver transaminases are dose-dependent,¹⁰ the observed pharmacodynamic interaction between bosentan and glyburide may be explained by increases in bosentan concentration in the presence of glyburide. Interpretation of the pharmacokinetic data obtained in the REACH-1 trial that showed comparable plasma levels of bosentan in patients with or without concomitant use of glyburide (Actelion; data on file) was hampered by the small number of patients and plasma samples obtained, the varying dosages of glyburide used, and the large number of concomitant medications taken by these patients. This study was conducted to investigate the effect of glyburide on the pharmacokinetics of bosentan in healthy subjects. Because abnormalities in liver function, including elevations in transaminase levels, have been reported after treatment with glyburide alone,¹²⁻¹⁴ the effect of bosentan on the pharmacokinetics of glyburide also was investigated. To explain some of the findings obtained in this interaction study, *in vitro* experiments were performed to elucidate the CYP isozyme or isozymes involved in the metabolism

of glyburide and to investigate a possible interaction at the level of protein binding.

METHODS

Subjects. Twelve healthy male ($n = 9$) and female ($n = 3$) white subjects (age range, 18-35 years) were recruited to participate in this study. The study protocol was approved by an ethics committee (Freiburger Ethik-Kommission International, Freiburg, Germany), and all subjects gave written informed consent before any screening procedures were performed. Subjects were assessed to be healthy on the basis of results of physical examination (including electrocardiography), medical history, and clinical laboratory tests (hematologic testing, blood chemical study, and urinalysis) performed within 3 weeks before first drug administration. The subjects were nonsmokers. During the study, no concomitant medication was allowed with the exception of medications to control adverse events. Restrictions were applied regarding the intake of methylxanthine-containing beverages and the consumption of grapefruit juice. The subjects entered the hospital approximately 12 hours before administration of the study drug on day 1 and stayed in the institute until 12 hours after the last drug intake on day 10 of each treatment period. An end-of-study examination was performed within 7 days after the final drug administration.

Study design. This was an open-label, randomized, 2-way crossover study in which subjects received treatments A and B, administered in the morning and evening after a standardized breakfast and dinner. Treatment A was 125 mg bosentan twice a day for 9 days and a single 125-mg dose on day 10 plus concomitant treatment with 2.5 mg glyburide (Semi-Daonil; Hoechst Marion Roussel, Frankfurt, Germany), twice a day on days 6 through 9 and a single 2.5-mg dose on day 10. Treatment B was 2.5 mg glyburide twice a day for 9 days and a single 2.5-mg dose on day 10 plus concomitant treatment with 125 mg bosentan twice a day on days 6 to 9 and a single 125-mg dose on day 10. Concomitant treatment was initiated only after steady-state conditions of monotherapy had been attained on the basis of the pharmacokinetic characteristics of bosentan⁸ and glyburide¹² and confirmed by means of measurement of drug levels in trough samples (data not shown). A dosage of 125 mg bosentan twice a day was chosen in this study because it is the recommended maintenance dosage for the treatment of patients with pulmonary arterial hypertension.⁶ To minimize the risk of hypoglycemia among the volunteers, a dosage of 2.5

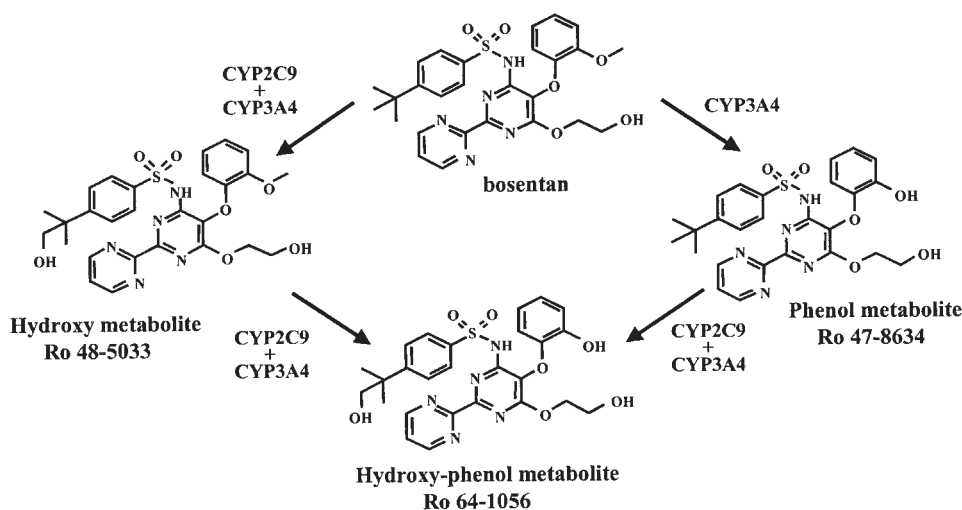


Fig 1. Metabolic pathways and chemical structures of bosentan and its metabolites.

mg glyburide twice a day was chosen. This is the recommended starting dosage in the treatment of patients with diabetes. On all days the meals were standardized, and throughout the two treatment periods the meals were of the same composition. Between the two treatment periods there was a washout period of 19 days. In each period blood samples of 5 ml were collected into ethylenediaminetetra-acetic acid-containing tubes by means of venipuncture or from an indwelling catheter in an antecubital vein just before the morning drug administration on days 1 through 6 and on day 10. In addition, on days 5 and 10, blood samples were withdrawn at 15, 30, 45, 60, and 90 minutes and at 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, and 12 hours after drug administration. Plasma was separated and stored at -20°C pending analysis.

Bioanalytic methods. Plasma samples obtained during treatment A were analyzed for bosentan and its 3 metabolites (phenol, hydroxy, and hydroxy-phenol). A slightly modified liquid chromatography assay method with tandem mass spectrometry detection was used, details of which have been described previously.¹⁵ The limits of quantification were 5.0 ng/ml for bosentan, 1.25 ng/ml for the phenol metabolite, and 2.5 ng/ml for the hydroxy and hydroxy-phenol metabolites. The day-to-day coefficients of variation varied between 2.0% and 7.3% for bosentan and its metabolites, and inaccuracy was less than 9%.

Plasma samples obtained during treatment B were analyzed for glyburide with an HPLC method with ultraviolet detection at 226 nm. The analysis of 1-ml plasma samples was performed after acidification and

extraction with an organic solvent (5 ml benzene with 1 μl 0.1-mol/L hydrochloric acid). The organic phase was evaporated to dryness, and the residue was dissolved in mobile phase (47% acetonitrile in 10-mmol/L phosphate buffer, pH 3.7), an aliquot (50 μl) of which was injected into the HPLC system. Quantification of glyburide was performed with an internal standard (glibornuride). The limit of quantification of the assay was 5 ng/ml. The day-to-day coefficients of variation were 6.5% at 15 ng/ml, 4.5% at 50 ng/ml, and 6.7% at 100 ng/ml. Inaccuracy was less than 6%. No glyburide metabolites were quantified in the human plasma samples.

In vitro experiments. Glyburide was incubated with human liver microsomes at a concentration of 10 $\mu\text{mol/L}$ in the presence of a nicotinamide adenine dinucleotide phosphate (NADPH)-regenerating system. Aliquots were obtained at the beginning of the experiment and after 10, 20, and 30 minutes of incubation time. Proteins were precipitated by means of addition of 1 equivalent of methanol [vol/vol] and storage on ice. Samples were centrifuged and supernatants were directly analyzed by means of HPLC. Control experiments were run in the absence of protein or the NADPH-regenerating system to confirm that metabolite formation was CYP-dependent. Chromatographic separations were performed with a combination of a ProC8 column (S-05 μm , 12 nm, 50 $\times$ 2.0 mm internal diameter; YMC, Kyoto, Japan) and an octadecylsilane (ODS) column (S-05 μm , 12 nm, 20 $\times$ 2.0 mm internal diameter; YMC) at a column oven temperature of 30°C and a solvent flow of 400 $\mu\text{l/min}$. Mobile phases were 2.5% hydrochloric acid (pH 3.0,

Table I. Pharmacokinetic parameters of bosentan and its metabolites on days 5 and 10 in 12 healthy volunteers after administration of 125 mg bosentan twice a day for 9.5 days and concomitant administration of 2.5 mg glyburide twice a day for 4.5 days starting on day 6

Variable	Day 5 (no glyburide)	Day 10 (concomitant glyburide)
Bosentan		
C _{max} (ng/ml)	754 (592-960)	571 (434-751)*
t _{max} (h)	2.5 (1.5-5.0)	3.0 (2.0-4.5)
AUC _τ (ng · h/ml)	3495 (2832-4314)	2475 (1897-3230)*
Phenol metabolite		
C _{max} (ng/ml)	26.9 (19.3-37.5)	21.6 (15.1-30.8)*
t _{max} (h)	2.8 (1.5-6.0)	3.3 (2.0-4.5)
AUC _τ (ng · h/ml)	132 (98.4-177)	98.0 (67.2-143)*
Hydroxy metabolite		
C _{max} (ng/ml)	70.8 (55.0-91.2)	53.0 (41.3-68.1)*
t _{max} (h)	4.5 (0.5-12)	4.5 (2.0-6.0)
AUC _τ (ng · h/ml)	428 (332-550)	320 (249-411)*
Hydroxy-phenol metabolite		
C _{max} (ng/ml)	75.0 (59.8-93.9)	61 (47-81)*
t _{max} (h)	4.3 (2.0-8.0)	4.0 (2.0-4.5)
AUC _τ (ng · h/ml)	382 (312-468)	299 (227-394)*

Data are presented as geometric mean value with 95% confidence interval in parentheses for C_{max} and AUC_τ and as median value and range for t_{max}. C_{max}, Peak plasma concentration; AUC_τ, area under the plasma concentration-versus-time curve from time zero to 12 hours; t_{max}, time to reach C_{max}.

**P* < .05, versus day 5.

phase A) and acetonitrile (phase B). Chromatographic separations were achieved with a gradient starting at 5% of phase B and increasing to 30% of phase B within 2 minutes. Phase B was further increased to 75% within 10 minutes and then kept constant for 3 minutes. Detection and quantification of glyburide and metabolites were performed with ultraviolet detection at 236 nm. No efforts were made to identify the structures of the glyburide metabolites.

Human CYP isoforms responsible for the metabolism of glyburide were identified with two independent approaches. Glyburide was incubated at a substrate concentration around its Michaelis-Menten constant (K_m) of 20 μmol/L in the presence of CYP isozyme-specific inhibitors; that is, methoxsalen (10 μmol/L) for CYP2A6, sulfaphenazole (5 μmol/L) for CYP2C9, quinidine (3 μmol/L) for CYP2D6, and troleandomycin (100 μmol/L) for CYP3A4. For incubations with methoxsalen and troleandomycin, human liver microsomes were incubated in the presence of the inhibitor and an NADPH-regenerating system for 10 minutes before addition of the substrate. Glyburide was incubated with recombinant human CYP isoforms CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 expressed in baculovirus-infected insect cells (Gentest Corporation, Woburn, Mass). The assays were performed with 10-μmol/L glyburide and 20-nmol/L enzyme in the presence of an NADPH-regenerating system. The reactions were stopped after 0, 10, 20, and

30 minutes by means of addition of 1 equivalent of methanol [vol/vol]. Control experiments, sample measurements, and analysis were performed as described earlier.

The mutual serum protein-binding interactions between bosentan and glyburide were investigated with equilibrium dialysis at 37°C. Bosentan and glyburide were tested in concentrations up to 10 μg/ml and 0.5 μg/ml, respectively; that is, at concentrations approximately 10-fold the plasma concentrations achieved in this study.

Pharmacokinetic and statistical evaluations. The pharmacokinetic evaluation for bosentan, its metabolites, and glyburide was performed with model-independent methods.¹⁶ The peak plasma concentration (C_{max}) and the time to C_{max} (t_{max}) were read directly from the concentration-time data. The area under the plasma concentration-versus-time curve from 0 to 12 hours (AUC_τ) was estimated with use of the linear trapezoidal rule. Pharmacokinetic parameters were analyzed descriptively. Geometric mean value and 95% confidence intervals or, for t_{max}, median value and range were calculated. The plasma concentration in the figures was expressed as the arithmetic mean value ± SEM.

ANOVA with the factors *treatment*, *day*, and *subject(treatment)* was conducted on the logarithmically transformed variables C_{max} and AUC_τ to explore the effect of concomitant treatment with bosentan on the

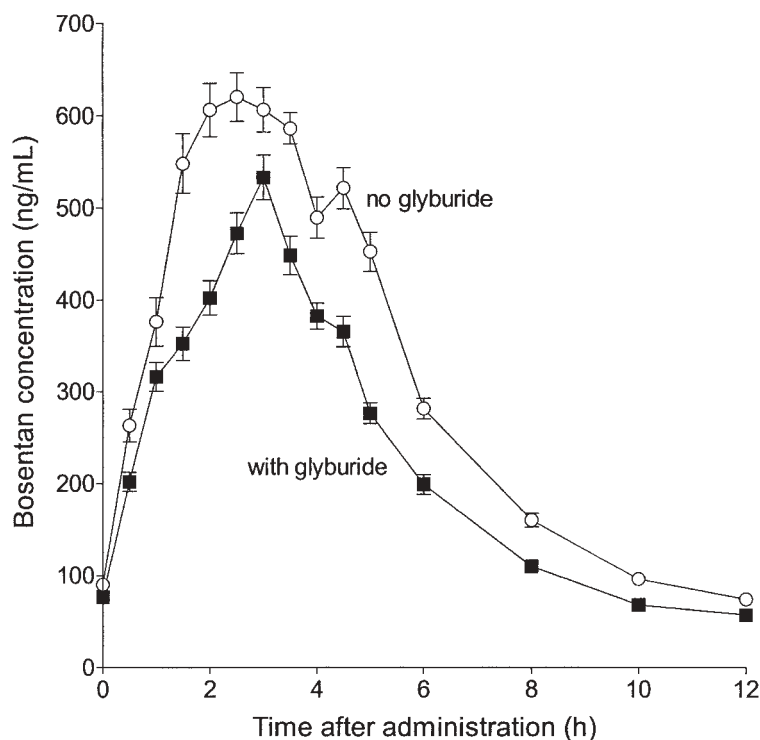


Fig 2. Plasma concentration (mean value $\pm$ SEM) of bosentan on day 5 (circles) and day 10 (squares) in 12 healthy volunteers after administration of 125 mg bosentan twice a day for 9.5 days and concomitant administration of 2.5 mg glyburide twice a day for 4.5 days starting on day 6.

pharmacokinetics of glyburide and vice versa. The Wilcoxon signed-rank test was used for analysis of $t_{\max}$. The analyses were performed with SAS version 6.12 (SAS Institute, Cary, NC). A 2-tailed P value was used, the required level of significance being $P < .05$.

Safety and tolerability. Vital signs (systolic and diastolic blood pressure and heart rate) were measured before the morning drug administration on each treatment day and at several time points after the morning drug administration on days 5 and 10 of each treatment period with subjects in the sitting position. Clinical laboratory tests, in addition to those at screening and at the end-of-study examination, were performed on a blood sample taken before dosing in the morning on days 5 and 10 of each treatment. All adverse events reported during the study were recorded and rated as mild, moderate, or severe. The tolerability data were evaluated descriptively. Clinical laboratory values were compared with the normal range supplied by the analyzing laboratory.

RESULTS

All subjects completed the entire study in accordance with the protocol, and pharmacokinetics could be eval-

uated for each subject. During treatment A, concomitant use of glyburide was associated with a lower plasma concentration of bosentan and of the phenol, hydroxy, and hydroxy-phenol metabolites on day 10 than on day 5 (Table I, Fig 2). Glyburide reduced the mean AUC_t of bosentan and the phenol, hydroxy, and hydroxy-phenol metabolites 29%, 26%, 25%, and 22% (all $P < .05$), respectively (Table I). Similar decreases were observed for $C_{\max}$ (Table I). Glyburide had no significant effect on the $t_{\max}$ of bosentan and its metabolites (Table I). During treatment B, bosentan decreased the plasma concentration of glyburide (Table II, Fig 3). The mean $C_{\max}$ and AUC_t of glyburide were reduced 22% and 40% (both $P < .05$), respectively, in the presence of bosentan (Table II). Bosentan had no significant effect on the $t_{\max}$ of glyburide (Table II). Individual decreases in AUC_t of bosentan and glyburide are shown in Fig 4.

The results of the incubations of glyburide with human liver microsomes and recombinant human CYP isoforms are presented in Tables III and IV, respectively. In the in vitro incubations with glyburide, formation of two major (M1 and M4) and two minor (M2, M3) metabolites was

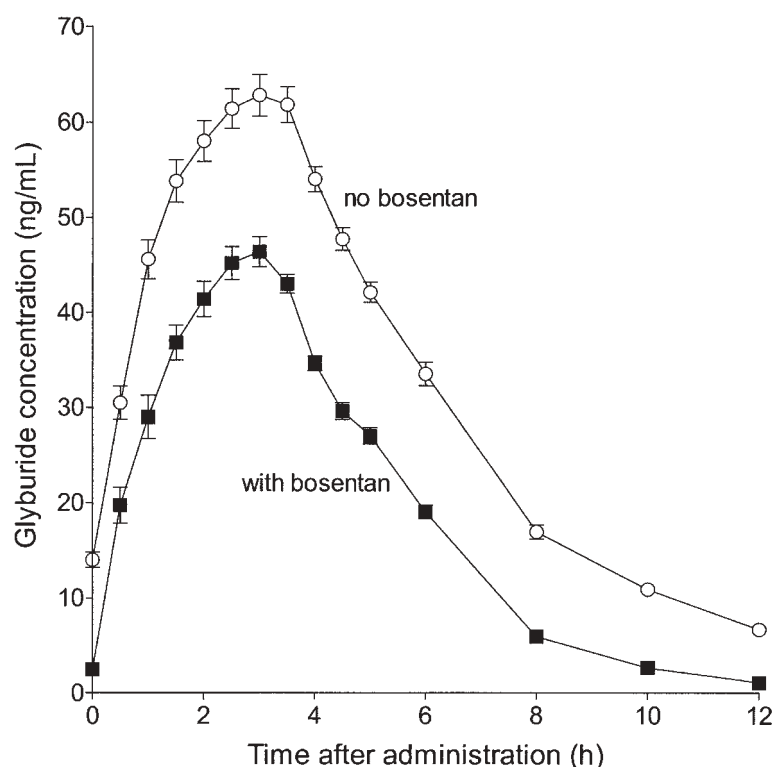


Fig 3. Plasma concentration (mean value $\pm$ SEM) of glyburide on day 5 (circles) and day 10 (squares) in 12 healthy volunteers after administration of 2.5 mg glyburide twice a day for 9.5 days and concomitant administration of 125 mg bosentan twice a day for 4.5 days starting on day 6.

Table II. Pharmacokinetic parameters of glyburide on days 5 and 10 in 12 healthy volunteers after administration of 2.5 mg glyburide twice a day for 9.5 days and concomitant administration of 125 mg bosentan twice a day for 4.5 days starting on day 6

Variable	Day 5 (no bosentan)	Day 10 (concomitant bosentan)
Glyburide		
C_{max} (ng/ml)	68.3 (55.5-84.0)	53.3 (41.3-68.8)*
t_{max} (h)	3.0 (1.5-6.0)	3.0 (1.0-5.0)
AUC_t (ng · h/ml)	369 (300-453)	223 (186-267)*

Data are presented as geometric mean value with 95% confidence interval in parentheses for C_{max} and AUC_t and as median value with range in parentheses for t_{max} .

* $P < .05$, versus day 5.

observed. Formation of metabolites M1 and M4 was entirely dependent on CYP3A4, as evidenced by the nearly complete absence of these substances in the presence of troleandomycin and the results of incubation with recombinant CYP3A4. Metabolite M2 was formed mainly by CYP2C19 with a minor contribution by CYP2C9. Inhibition of CYP2C9 by sulfaphenazole slightly decreased the formation rate of M2 in human liver microsomes. Metabolite M3 was formed in incubations with recombinant isoforms CYP2C9 and CYP3A4. In

human liver microsomes, inhibition of formation of M3 was observed in the presence of sulfaphenazole and troleandomycin. Glyburide was not metabolized by recombinant isoforms CYP1A2 or CYP2D6, nor was formation of any of its metabolites significantly decreased in the presence of quinidine or methoxsalen. At the concentrations tested, bosentan and glyburide had no effect on serum protein binding.

Six adverse events were reported in this study, as follows: 1 case of mild headache, 1 case of mild fatigue, and

Table III. Normalized residual metabolite formation rates in human liver microsomes in the presence of CYP isozyme-specific inhibitors

<i>Inhibitor (enzyme)</i>	<i>M1</i>	<i>M2</i>	<i>M3</i>	<i>M4</i>
None	100	100	100	100
Methoxsalen (CYP2A6)	106	NE	97	100
Sulfaphenazole (CYP2C9)	100	88	87	102
Quinidine (CYP2D6)	100	94	95	105
Troleandomycin (CYP3A4)	0	64	66	6

Values are formation rates expressed as the percent of control, that is, no inhibitor present. NE, Not evaluated because of chromatographic interference.

Table IV. Normalized metabolite patterns of glyburide in incubations with recombinant human CYP isozymes

<i>Metabolite</i>	<i>CYP1A2</i>	<i>CYP2C9</i>	<i>CYP2C19</i>	<i>CYP2D6</i>	<i>CYP3A4</i>
M1	ND	ND	ND	ND	2
M2	ND	1	6	ND	ND
M3	ND	1	ND	ND	1
M4	ND	ND	ND	ND	13
Glyburide	100	98	94	100	84

Determined at a substrate concentration of 10 μ mol/L with 20 nmol/L recombinant enzyme for 30 minutes. Values represent normalized peak areas. Identical ultraviolet absorbance of glyburide and its metabolites at the wavelength used is assumed. ND, Not detected.

1 case of elevated ALT level during treatment A and 1 case of moderate headache, 1 case of mild abdominal discomfort, and 1 case of elevated AST and ALT level during treatment B. All events resolved without sequelae. The 2 cases of asymptomatic elevated liver enzyme levels occurred in 2 male subjects during the first treatment period on day 10; that is, during concomitant treatment with bosentan and glyburide. In the subject receiving treatment A, ALT level was increased to approximately 4-fold the upper limit of normal. In the subject receiving treatment B, AST and ALT levels were elevated to 9- and 2-fold the upper limit of normal, respectively. Both cases resolved during the washout period, and during the second treatment period, no increases in liver enzyme levels were found. No pattern of abnormal laboratory values or vital signs was detected during the study to suggest a treatment effect. The few abnormalities not mentioned were judged not clinically relevant.

DISCUSSION

This study was an investigation of a possible pharmacokinetic interaction between bosentan and glyburide. It was found that concomitant administration of bosentan and glyburide resulted in decreased exposure to both drugs. These results refute the hypothesis that increased bosentan concentrations in the presence of glyburide are the basis for the observed increased incidence of elevation in liver transaminase levels in patients with chronic heart failure taking both drugs.

Two mechanisms may explain the observed decrease in exposure to bosentan and glyburide when the drugs are coadministered. First, the chosen fixed-sequence study design in which the control part always preceded the drug combination part cannot exclude a time of treatment effect. Second, the absorption, distribution, metabolism, or elimination may be affected through, for example, induction of metabolizing enzymes or transporter proteins. The fixed-sequence design was chosen because it resembles clinical practice more closely than does a crossover design with 3 periods (bosentan alone, glyburide alone, and the combination). Because concomitant treatment was started only when steady-state conditions of the first drug had been achieved, it is unlikely that a time effect can explain the observed reductions in drug concentration.

The pharmacokinetics of glyburide in the absence of bosentan is in good agreement with that previously published.¹⁷ Bosentan has been shown to be a moderate inducer of CYP3A4, CYP2C9, and CYP2C19 but not of CYP1A2. Evidence of this finding has been obtained in in vitro experiments with human hepatocytes (Actelion; data on file), from drug-drug interaction studies with healthy volunteers treated with warfarin sodium¹⁸ and cyclosporine (INN, ciclosporin)¹⁹ and from multiple-dose studies with bosentan that showed autoinduction.⁸ Because little is known about the enzymes involved in the metabolism of glyburide, which is extensively metabolized in the liver,²⁰ we performed in

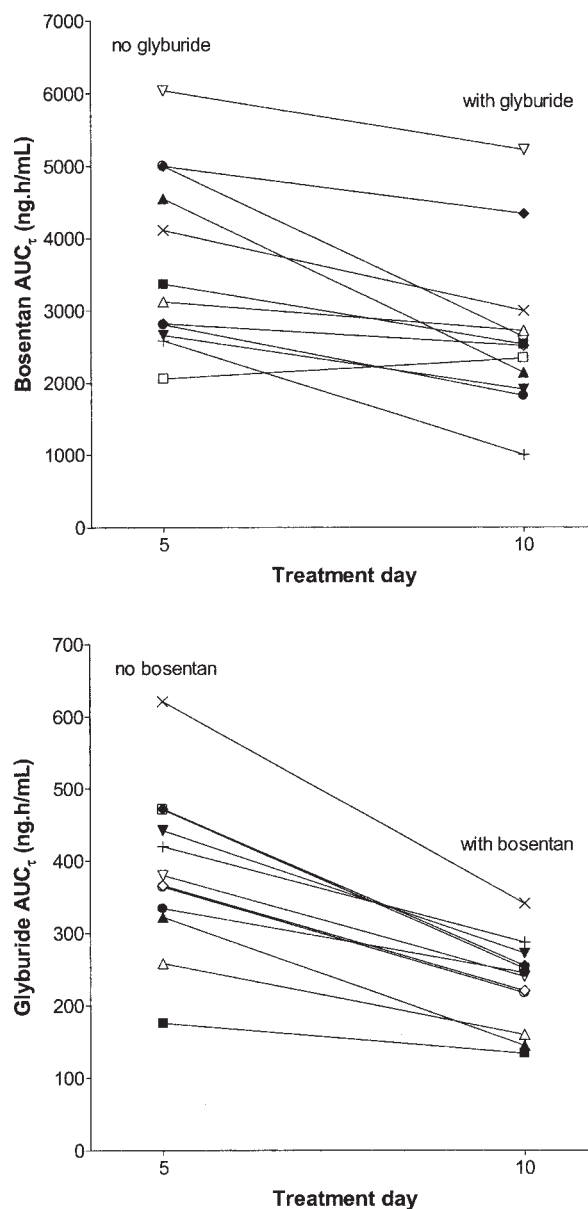


Fig 4. Individual AUC_τ of bosentan (*top*) and glyburide (*bottom*) in 12 healthy volunteers on treatment day 5 (no concomitant drug) and day 10 (with concomitant drug).

vitro experiments to identify the CYP isozymes that metabolize glyburide. The results showed that glyburide is metabolized in vitro by CYP2C9, CYP2C19, and CYP3A4. The relative contribution of each CYP isozyme to the in vivo metabolism of glyburide cannot be predicted from these results. CYP1A2, CYP2A6, and CYP2D6 did not play a significant role in the metabolism of glyburide.

The reduced plasma concentrations of glyburide in the presence of bosentan thus are consistent with the CYP2C9-, CYP2C19-, and CYP3A4-inducing potential of bosentan. The magnitude of the effect of bosentan (on average, a 40% decrease in the AUC of glyburide) is such that reduced efficacy of glyburide should be considered when this drug is given concomitantly with bosentan. A drug-drug interaction between rifampin (INN, rifampicin), a strong inducer of CYP2C9 and CYP3A4,²¹ and glyburide has been reported previously.²² Although plasma concentrations were not measured, elevated blood glucose levels in the presence of rifampin indicated reduced plasma levels of glyburide.²² In vitro screening for inducers of CYP3A4 revealed that glyburide could induce this enzyme.²³ This finding would be consistent with the observed decrease in plasma levels of bosentan.

Given that bosentan undergoes little first-pass metabolism, it is puzzling why in the presence of glyburide the C_{max} of bosentan decreased. An alternative explanation for the observed reduction in both C_{max} and AUC of bosentan and its metabolites may be that glyburide reduces the absorption of bosentan, for example, by inhibiting a transporter protein involved in the uptake of bosentan in intestinal cells or by inducing P-glycoprotein. P-Glycoprotein has been shown effective at pumping drugs out of the intestinal cell into the intestinal lumen, thereby limiting absorption.²⁴ At present, it is unknown whether and, if so, for which drug transporter proteins bosentan is a substrate. It should be noted that glyburide in vitro has been shown to be an inhibitor rather than an inducer of P-glycoprotein.²⁵ By means of induction of P-glycoprotein, however, bosentan may decrease the concentration of glyburide. An interaction study with digoxin, the pharmacokinetics of which are influenced by inhibition²⁶ and induction²⁷ of P-glycoprotein, showed that concomitant administration of 500 mg bosentan twice a day reduced the exposure to digoxin by only approximately 12%.²⁸ This result shows that bosentan does not inhibit P-glycoprotein but may be a weak inducer of this transporter protein. Although digoxin is mainly excreted unchanged, 10% to 20% is metabolized at least in part by CYP3A,²⁹ and thus induction of CYP3A4 by bosentan may play a role in the small reduction in digoxin exposure. Overall changes in P-glycoprotein activity appear to play a negligible role in the pharmacokinetic interaction between bosentan and glyburide. However, involvement of other drug transporter proteins cannot be excluded. Glyburide does not affect serum protein binding of bosentan and vice versa. Therefore the possibility that the observed

pharmacokinetic interaction is caused by changes in protein binding can be ruled out.

The aforescribed pharmacokinetic interaction between bosentan and glyburide that leads to reduced plasma concentrations of both drugs does not explain the increased incidence of elevated liver transaminase levels among patients with chronic heart failure.¹⁰ Several possible mechanisms are known by which drugs can induce hepatotoxicity.³⁰ Drug-induced cholestasis and enzyme induction leading to accumulation of a hepatotoxic metabolite are two possible mechanisms.

Inhibition of the hepatocellular bile salt export pump (BSEP) has been proposed as a molecular mechanism for the induction of cholestasis.^{31,32} The BSEP is involved in the elimination of bile salts from hepatocytes.³³ As a result of inhibition of the BSEP, increased hepatocyte concentrations of toxic bile salts can induce cell injury.³⁴ This may lead to increased release of liver transaminases into the blood. Bosentan and its minor phenol metabolite,¹⁰ as well as glyburide,³² have been shown to be inhibitors of BSEP *in vitro* with about equal affinity, and increased serum levels of bile acids have been measured in bosentan-treated patients with elevations in liver transaminase levels.¹⁰ In rats, bosentan increased serum bile acids, and coadministration of glyburide resulted in potentiation of this effect.¹⁰ It is thus hypothesized that the increases in liver transaminase levels in patients treated with bosentan may result, at least in part, from inhibition of BSEP¹⁰ and that concomitant administration of glyburide may enhance this effect despite a decrease in the plasma concentration of bosentan.

Because of the chemical structures of bosentan and its 3 identified metabolites, it is unlikely that these compounds covalently bind to cell macromolecules and cause liver damage. Formation of a minor reactive metabolite, however, which could be enhanced by concomitant administration of glyburide as a result of further CYP induction, cannot be excluded.

The treatments were well tolerated. This finding is in agreement with previous observations among healthy volunteers treated with single and multiple doses of up to 2400 mg and 1000 mg bosentan per day, respectively.^{8,9} To our knowledge, elevated levels of liver enzymes as occurred in this study have not been reported in healthy volunteers. It should be noted that both cases were asymptomatic, occurred in the first treatment period, and resolved before start of the second treatment period. Reintroduction was considered justified because the subjects stayed in the institute for the duration of the study and because bosentan had been reintroduced into patients with asymptomatic increases in liver transaminase levels during the clinical devel-

opment phase without negative consequences. In both subjects plasma concentrations of the drug did not differ from those measured in the other subjects.

In conclusion, a pharmacokinetic interaction between bosentan and glyburide led to decreases in the plasma concentration of both drugs. This interaction, however, did not explain the observed increases in liver transaminase levels when bosentan and glyburide were administered concomitantly to patients with chronic heart failure.

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