Published in final edited form as:

Clin Pharmacol Ther. 2009 January ; 85(1): 78–85. doi:10.1038/clpt.2008.186.

Elucidating Rifampin's Inducing and Inhibiting Effects on Glyburide Pharmacokinetics and Blood Glucose in Healthy Volunteers: Unmasking the Differential Effect of Enzyme Induction and Transporter Inhibition for a Drug and Its Primary Metabolite

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Abstract

The effects of single doses of intravenous ciprofloxacin and rifampin, multiple doses of rifampin, on glyburide exposure and effect on blood glucose levels in 9 healthy volunteers were investigated. The single intravenous dose of rifampin significantly increased the AUCs of glyburide and metabolite. Blood glucose levels dropped significantly in comparison to when glyburide was dosed alone. Multiple doses of rifampin induced liver enzymes leading to a marked decrease in glyburide exposure and in blood glucose measurements. When intravenous rifampin was given after multiple doses of rifampin, the inhibition of hepatic uptake transporters masked the induction effect, however, relative changes in AUC for glyburide and its hydroxyl metabolite were the same as that seen under non-induced conditions. The studies reported here demonstrate how measurements of both the parent drug and its primary metabolite are useful in unmasking simultaneous drug-drug induction and inhibition effects and characterizing enzymatic versus transporter mechanisms.

Keywords

glyburide; rifampin; induction; inhibition; transporter-enzyme interplay

INTRODUCTION

Elimination of physiological substrates and drugs from the portal circulation involves uptake into hepatocytes as the obligatory first step. At the molecular level, the uptake of bulky amphiphilic and hydrophilic compounds into hepatocytes is primarily mediated by members of the organic anion transporting polypeptide (OATP) family located at the basolateral membrane of hepatocytes. Among which, OATP-1B1, also known as liver specific

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Presented at the 2008 American Society for Clinical Pharmacology and Therapeutics Annual Meeting, April 2, Orlando (Abstract PT-03) by Dr. Zheng as an ASCPT Presidential Trainee Award Winner.

Conflict of Interest

The authors declared no conflict of interest.

transporter-1 (LST-1) or OATP2 (1–3), is the most abundant with expression exclusively in the liver. A previous *in vitro* study demonstrated that OATP2B1 (OATP-B), located in liver, intestine, and kidney, mediated uptake of glyburide (4). There are many examples of drug-drug interactions at the level of hepatic cytochrome P450, but changes in metabolism can also occur by either inhibition or induction of relevant transporter protein(s) in the liver (5,6). We have previously demonstrated that inhibition of hepatic uptake transporters can decrease hepatic elimination even when hepatic enzymes are unaffected for digoxin and erythromycin in hepatocytes (7,8), digoxin and atorvastatin in isolated perfused rat livers (9,10) and atorvastatin in humans (11). A particularly confounding interaction occurs when enzymes and transporters are upregulated but the upregulating drug can also inhibit hepatic uptake transporters as recently addressed by Lam et al. (12). In the present work we investigate these confounding interactions for glyburide in humans, and report how the differential effect of enzyme induction and transporter inhibition may be unmasked by measuring the parent drug and its primary metabolite.

Glyburide, also known as glibenclamide, is a second-generation oral hypoglycemic agent used worldwide for more than 3 decades. Glyburide is well absorbed in the intestine and eliminated by extensive metabolism in the liver. CYP2C9 seems to be the principal CYP involved, with CYP3A4 playing a more minor role (13). Previous studies showed that genetic polymorphisms of CYP2C9 markedly affect the pharmacokinetics of glyburide and carriers of the CYP2C9 variant *3 had decreased oral clearances of glyburide (14,15). Glyburide is metabolized to two active metabolites, 4-trans-hydroxyglyburide and 3-cis-hydroxyglyburide, which retain 75% and 50%, respectively, of the parent compound's hypoglycemic activity. Clinical case reports showed that rifampin and ciprofloxacin exhibit interactions with glyburide, leading respectively to decreased and increased glyburide effects. A study in patients and a case report indicate that multiple doses of rifampin decreased the effects of glyburide (16,17). Further investigations showed that rifampin decreased area under the concentration-time curve (AUC) of glyburide by 39% and maximal concentration (C_{max}) by 22%. The blood glucose decremental $AUC_{(0-7)}$ (net area below baseline) and the maximum decrease in the blood glucose concentration were –44% and –36%, respectively, by concomitant rifampin versus control (18). In a clinical case report a diabetic patient taking glyburide, who was prescribed ciprofloxacin, developed prolonged hypoglycemia that persisted for over 24 hours (19). The mechanism of these interactions remains to be determined.

Although the enzyme induction effect of rifampin on glyburide has been reported, the hepatic uptake inhibition effect of rifampin on glyburide has not been studied previously. We propose that an uptake transporter such as OATP may control the access of a dual OATP/CYP substrate such as glyburide to the enzymes in the liver. In the presence of an acute dose of an OATP inhibitor such as rifampin (20), we expected a reduction in glyburide hepatic metabolism since the bulk of drug entering the hepatocytes is reduced and the availability of drug to CYP is decreased. We hypothesize that the presence of rifampin in the systemic circulation will markedly modulate the glyburide pharmacokinetic and the pharmacodynamic effect observed after multiple dose rifampin. In the present study we test this hypothesis utilizing single and multiple dosing clinical drug interactions.

RESULTS

Safety assessment

No serious adverse events occurred during or after the study. No clinical relevant abnormalities were found in the vital signs and the safety laboratory measurements during or post-treatments. One subject experienced hypoglycemia, with a blood glucose level of 40mg/dl that was corrected by giving oral glucose. One subject withdrew from the study

after visit 3 because of allergy to rifampin. Data obtained from this subject were not included in the analysis.

Effect of IV infusion of ciprofloxacin on the pharmacokinetics of glyburide and 4-hydroxyglyburide (Treatment B)

No significant changes in any parameter were observed when ciprofloxacin was co-administered with glyburide. No further comparisons of Treatment B will be discussed as the results did not differ from Treatment A.

Effect of IV infusion of rifampin on the pharmacokinetics of glyburide and 4-hydroxyglyburide (Treatment C)

A single IV infusion of rifampin significantly increased the $AUC_{0-\infty}$ of glyburide by $125 \pm 51.5\%$ ($p < 0.001$) and C_{max} by $81 \pm 28\%$ ($p < 0.01$) compared with the control phase, Treatment A (Table III). The $AUC_{0-\infty}$ of 4-hydroxyglyburide was also increased $45 \pm 37\%$ by rifampin ($p < 0.01$) and C_{max} of the 4-hydroxyglyburide was increased by $34 \pm 47\%$ ($p = 0.06$) in comparison to the control (Table IV). T_{max} values remained statistically unaltered by rifampin, for both glyburide and its metabolite.

The oral steady-state volume of distribution (V_{ss}/F) of glyburide was extensively decreased in the presence of rifampin by $60 \pm 29\%$ ($p = 0.01$), whereas the oral clearance (CL/F) was also markedly decreased by $53 \pm 13\%$ ($p < 0.001$) (Table III). The single IV infusion of rifampin did not change the apparent terminal half-life ($t_{1/2}$) of glyburide (Table III), nor the half-life of its metabolite (Table IV).

Effect of multiple oral doses of rifampin on the pharmacokinetics of glyburide and metabolite 4-hydroxyglyburide (Treatment E)

When glyburide was administered three days after the multiple oral doses of rifampin (and two days following the second IV dose of rifampin), but when no unchanged rifampin was present in the plasma, the $AUC_{0-\infty}$ of glyburide was significantly decreased by $63 \pm 11\%$ ($p < 0.001$) and C_{max} by $48 \pm 21\%$ ($p < 0.01$) compared with the control phase (Table III). The $AUC_{0-\infty}$ of 4-hydroxyglyburide was decreased $67 \pm 6\%$ by rifampin ($p < 0.0001$) and C_{max} of the 4-hydroxyglyburide was decreased by $44 \pm 11\%$ ($p < 0.001$) (Table IV). T_{max} values remained statistically unaltered by rifampin, for both glyburide and its metabolite.

Combination effect of single IV infusion of rifampin and multiple oral doses of rifampin on the pharmacokinetics of glyburide and metabolite 4-hydroxyglyburide (Treatment D)

When a rifampin IV infusion was administered one day after the oral rifampin induction regimen, the glyburide AUC (Treatment D) was more than double that observed in Treatment E when no rifampin was present ($120 \pm 56\%$, $p < 0.0001$). C_{max} for Treatment D was significantly higher than for Treatment E ($92 \pm 76\%$, $p < 0.01$). Yet when Treatment D is compared with the control Treatment A, $AUC_{0-\infty}$ of glyburide is significantly decreased, but only by $22 \pm 21\%$ ($p = 0.02$), versus the 120% change noted for Treatment D, while the $9 \pm 0.3\%$ decrease in C_{max} is not significant (Table III). The directional changes in AUC and C_{max} for 4-hydroxyglyburide were the same as for the parent drug. The decrease in metabolite AUC for Treatment D compared to control is $46 \pm 10\%$ ($p < 0.0001$), while the decrease in C_{max} was not significant (Table IV). The AUC and C_{max} in Treatment D for metabolite were significantly greater than for Treatment E ($67 \pm 44\%$, $p < 0.001$, $49 \pm 44\%$, $p < 0.01$, respectively) (Table IV). T_{max} values remained statistically unaltered by rifampin, for both glyburide and its metabolite. The mean residence time of the parent drug was significantly decreased following multiple rifampin doses versus control (Treatment D $39 \pm 70\%$, $p = 0.01$; Treatment E $47\% \pm 25\%$, $p < 0.01$) but was unaffected by the presence of IV

rifampin (Table III). In contrast for the metabolite, its MRT was significantly decreased following multiple rifampin doses (Treatment E vs A, 59 + 53% decrease, $p < 0.05$), but when IV rifampin was present the metabolite MRT decrease was only 23% and not significant vs control (Table IV).

Comparison of effect on single IV infusion of rifampin under noninduced vs induced conditions

It is apparent in Table III that all changes in pharmacokinetic parameters ($C_{\max}$, $AUC_{0-\infty}$, CL/F, MRT and V_{SS}/F) caused by the single IV infusion of rifampin were of the same relative magnitude under both noninduced (Treatment C vs Treatment A) and induced (Treatment D vs Treatment E) conditions. No significant differences were found for any of the ratio comparisons in the last two rows of Table III. This was also observed for the metabolite (last two rows of Table IV).

Effect of rifampin on the AUC ratios of 4-hydroxyglyburide to glyburide

The AUC ratios of the 4-hydroxy metabolite to glyburide were significantly decreased when rifampin was present in the plasma in both Treatments C and D versus the control (Treatment A), as well as versus Treatment E (ANOVA, $p < 0.0001$), and no significant differences were found between Treatments A, B and E, or between Treatments C and D (Fig. 1 and Table V).

Effect of rifampin on the urine excretion of glyburide and 4-hydroxyglyburide and renal clearance of glyburide

Urine accumulated excretion of glyburide and its metabolite within 12h post glyburide dosing were significantly increased in Treatment C in comparison to the control Treatment A ($p < 0.001$, $p < 0.05$ respectively), and in comparison to Treatment D and Treatment E ($p < 0.001$ for both) (Table VI). Urine accumulated excretions of glyburide metabolite in Treatments D and E were decreased significantly compared to the control ($p < 0.001$) (Table VI), while that in Treatment D was significantly greater (60+40%, $p < 0.01$) in comparison to Treatment E. Renal clearance of glyburide and the metabolite did not significantly change between treatments (Table VI).

Pharmacodynamic effect of rifampin on the AUC of decremental blood glucose level

A statistically significant decrease in the decremental AUC_{0-12} of blood glucose was observed during the rifampin IV (Treatment C) phase in comparison to other treatment groups (repeated ANOVA, $p < 0.0001$) (Fig. 2). One subject experienced hypoglycemia during this phase of the study.

DISCUSSION

The objective of this clinical study was to investigate if a single intravenously administered dose of ciprofloxacin or rifampin would influence the pharmacokinetics and pharmacodynamics of a single oral dose of glyburide. We also investigated whether the presence of rifampin in the blood can counter balance the enzyme induction effect of multiple dose rifampin. In the present clinical study, rifampin, a potent OATP inhibitor (11,20,21), and ciprofloxacin were chosen as potential blockers of the *in vivo* OATP1B-mediated hepatic uptake of glyburide. We hoped that the potential confounding interactions in the intestine either at the enzyme or transporter levels could be minimized when ciprofloxacin and rifampin were given intravenously. Our present results demonstrate that the OATP inhibitor, rifampin, given as a single intravenous infusion, significantly increased the plasma AUC and $C_{\max}$ values of glyburide and its hydroxy metabolites, leading to an

enhancement of glyburide's therapeutic effect. Glyburide and its active metabolite, 4-hydroxyglyburide, formed after intestinal and hepatic metabolism, are mainly eliminated by metabolism. Comparison of MRT values in Table III for glyburide and in Table IV for the metabolite show that the oral rifampin regimen markedly induces both drug and the metabolite elimination. The significantly higher glyburide AUC and $C_{\max}$ with concomitant IV rifampin in Table III (Treatment C vs. control Treatment A and Treatment D vs. Treatment E under induction conditions) indicates that rifampin reduced the amount of parent drug getting into the liver by blocking the hepatic uptake transporter. This effect was the same under both induced and non induced conditions as can be seen by the ratio comparisons in Table III for $C_{\max}$ and AUC.

Note also in Table III that the ratio for CL/F and V_{SS}/F change the same under both induced and non-induced conditions and that these two parameters change in parallel. Often when CL/F and V_{SS}/F change in parallel this is an indication that the interaction is affecting bioavailability, F, rather than clearance and volume (22), or alternatively that the interacting drug is affecting protein binding. The latter can not be true because clearance and volume are decreasing, not increasing as would be expected if protein binding of the parent drug was decreased by the interacting drug. Here because we measured both parent drug and primary metabolite we know that the interacting effect is not due to a change in F, since the ratios of metabolite to parent drug in Table V only change when iv rifampin is present, not under conditions of induction vs non induction. Apparently rifampin inhibition of uptake in the liver is complimented by uptake in other unidentified tissues resulting in decreases in both CL and V_{SS} . It is likely that other tissues also have expression of transporters that are capable of mediating the uptake of glyburide and can be inhibited by rifampin.

As we observed previously for our atorvastatin-rifampin interaction study (11), AUC and $C_{\max}$ of the primary metabolite in Table IV were also increased for the same comparisons, and the effect was similar under induced and non induced conditions as can be seen in the ratio comparisons in Table IV. However, the effect on the parent drug was greater than that observed for the metabolite (comparison of ratio values in Tables III and IV) suggesting that glyburide is a better substrate for the uptake transporter than its primary metabolite.

Urinary excretion of glyburide and its metabolite were significantly increased (Table VI) while renal clearance of both compounds were unchanged suggesting that rifampin did not affect kidney elimination, and that changes in both glyburide and metabolite urinary excretion are a reflection of rifampin changes in AUC. Glyburide is also a substrate of P-glycoprotein (P-gp) (23), but we have shown that rifampin does not inhibit Pgp (10). In our study, AUC and $C_{\max}$ were increased 125% and 81%, respectively, following IV rifampin, reflecting the importance of hepatic uptake transporter in the disposition of glyburide.

Despite administering only half of the therapeutic dose of glyburide to the subjects, a single dose of rifampin significantly increased the glyburide therapeutic effect, leading to hypoglycemia in one subject. The 4-hydroxy metabolite of glyburide exhibits 75% potency of the parent compound, but since the exposure of the metabolite is only 2–4% of the parent compound (Tables III and IV), it appears that the parent compound caused the observed increased pharmacologic effect.

Conventionally, rifampin is a potent inducer of CYP3A in both the liver and the intestine (24,25). Rifampin also induces P-gp and MRP2 via the pregnane X receptor-dependent mechanism (26–28). Many drugs are co-substrates for CYP enzymes and P-gp, exhibiting clinically relevant drug–drug interactions owing to the co-induction of *CYP3A4* and *MDR1* genes by rifampin. In our study, we found that multiple doses of rifampin induce liver enzymes and possibly efflux transporters, leading to a significant decrease in glyburide

exposure following oral dosing, which is consistent with previous studies and clinical observations (16–18, 29). However, when rifampin was present in the blood after enzyme induction, the AUC and C_{max} were only decreased 22% and 8% in comparison to 63% and 81%, respectively, during the enzyme induction phase. Our findings are consistent with the observations of Bidstrup et al. (30) where after the rifampin induction regimen, the AUC of repaglinide, another antidiabetic drug, was decreased less in the presence of rifampin than when rifampin was not measurable.

Ciprofloxacin had no effect on any glyburide pharmacokinetic parameter, indicating that the reported interaction of ciprofloxacin and glyburide is probably not caused by blocking hepatic uptake of glyburide. At this point we are unable to explain this interaction.

Most previous studies have investigated the important role played by transporters in the renal and hepatobiliary excretion of many poorly metabolized drugs. This paper and a relatively small number of other publications (8–12, 23, 31–33) have shown the importance of transporter interaction in terms of changes in drug metabolism. Organic anion-transporting polypeptides (OATPs) represent a family of important proteins involved in the membrane uptake of physiological substrates and drugs in humans. They play an important role in controlling the extent of uptake into hepatocytes and thus control the availability of substrates for metabolism by the liver cytochrome P450 enzymes and the subsequent excretion of drugs into bile. There are numerous examples of drug-drug interactions at the level of hepatic cytochrome P450. Inhibition or induction of these enzymes by different interacting drugs is a common mechanism that can potentially result in drug toxicity or lack of efficacy. However, drug-drug interactions may also occur by inhibition of relevant uptake transporter protein(s) in the liver that can counteract the enzyme induction effect.

In conclusion, by inhibiting a hepatic uptake transporter, a single iv rifampin dose was shown to inhibit the liver uptake of glyburide and its primary metabolite, increase exposure to active drug and significantly lower blood glucose. Multiple doses of rifampin induced liver enzymes, and possibly transporters, leading to a marked decrease in glyburide exposure. Inhibition of the uptake transporter by iv rifampin after rifampin enzyme induction markedly masked the total induction effect of rifampin. The relevant message from this study in terms of drug development is that if rifampin is present in plasma when rifampin induction effects are being clinically evaluated for an OATP substrate, the effects of induction may be markedly underestimated. We also demonstrated in this study how measurements of both the parent drug and its primary metabolite are useful in unmasking simultaneous drug-drug induction and inhibition effects and characterizing enzymatic versus transporter mechanisms.

METHODS

Subjects

We recruited 10 healthy volunteers for this study, one of whom withdrew during the study (Table I). All subjects were screened via a medical history, physical examination and blood and urine chemistries to check hepatic and renal function, and to ensure that no subjects were anemic. Only subjects exhibiting the CYP2C9 *1/*1 genotype were included in the study. Exclusion criteria included allergy to study drugs, major medical or psychiatric conditions, obesity, pregnancy, lactation, drug or alcohol abuse, and use of prescription medications. The study protocol was approved by the Committee on Human Research and the General Clinical Research Center at UCSF. Written informed consent was obtained from each subject. The subjects were financially compensated for participation.

Experimental protocol

A prospective randomized, open-label, partial crossover clinical pharmacokinetic study was done on the General Clinical Research Center at UCSF consisting of five visits (Table II). Visits 1, 2, 3 and 4 were separated by a one week washout period and visit 5 was carried out 2 days after visit 4. On visits 1 and 2, each subject received an oral 1.25 mg dose of glyburide. This low dose was chosen due to our concern that inhibition of hepatic uptake could lead to significant hypoglycemia, even following a single dose. On one of two visit days, each subject also received a 200mg intravenous dose of ciprofloxacin infused over 20 minutes just before the oral dose of glyburide (Treatment B). Treatments A and B were randomized by odd-even random number draw. On visit 3, all subjects received 600mg iv doses of rifampin followed immediately by a 1.25 mg oral dose of glyburide (Treatment C). After a 7-day washout period, all subjects received 600mg oral doses of rifampin once daily for 6 days to induce liver enzymes (and transporters) (Table II). On visit 4, the day after the rifampin induction regimen, all subjects received a 600mg iv dose of rifampin infused over 30 minutes followed immediately by a 1.25mg oral dose of glyburide (Treatment D). On visit 5, two days after visit 4, all subjects received only a 1.25mg oral dose of glyburide (Treatment E).

On visits 1, 2 and 3, venous blood samples (8 mL) were drawn before dosing and ½, 1, 2, 3, 4, 6, 8, 12 and 24 hours after glyburide dosing. Subjects also provided a baseline urine sample and collected urine (at intervals of 4 hours) up to 24 hours after glyburide dosing. On visits 4 and 5, the 24hr blood and 12–24hr urine collections were omitted. Blood glucose level was measured immediately after each blood sampling by glucometer. Additional blood glucose measurements were obtained when deemed necessary by the study physician. Plasma was separated after the determination of blood glucose and stored at –80°C until analysis.

Analytic methods

The concentrations of glyburide and its main metabolite were measured using a liquid chromatography/tandem mass spectrometry (LC/MS/MS) system, consisting of a 717 plus autosampler (Waters Corporation, Milford, MA), and a Quattro LC Ultima (Micromass, Manchester, UK) detector with electrospray positive ionization mode. The multiple reaction monitor (MRM) was set at 559.6 – 440.8 *m/z* for glyburide, 575.2 – 440.5 *m/z* for 4-hydroxy-glyburide, 564.2 – 445.8 *m/z* for glipizide, the internal standard. Chromatography was performed on an Agilent, XDB C18 column (4.6 × 50 mm, 5 µm particle size, Agilent Technologies, Palo Alto, CA). Mobile phase A was 20% acetonitrile containing 0.05% acetic acid and 5 mM ammonium acetate and mobile phase B was 80% acetonitrile containing 0.05% acetic acid and 5 mM ammonium acetate. The gradient elution time program was set as follows: 0–1.5 min, B, 20–100%; 1.5–4.0 min, B, 100%; 4.0–4.5 min, B, 100–20%; The flow rate was 1.0 ml/min from 0–4.5 min; increased to 1.5 ml/min at 4.6 min and maintained at 1.5 ml/min from 4.6–6.5 min. The run time for each sample was 6.5 min. Twenty-five percent of the flow liquid was split into the mass system. The sample cone voltage and collision energy for all analytes and internal standard were set at 30 V and 20 eV, respectively.

The method for glyburide and metabolite was validated from 0.2 to 100 ng/ml in plasma. The lower limit of quantitation for glyburide and its metabolite was 0.2 ng/ml. The intra- and inter-day coefficients of variation were below 15% at relevant concentrations (n = 10). Ciprofloxacin and rifampin did not interfere with the assay. Calculations were performed with MassLynx 3.5 software (Micromass, Manchester, UK).

Data Analysis

Pharmacokinetic Analysis—Pharmacokinetic parameters were estimated from plasma concentration data via noncompartmental analysis using WinNonlin Professional software (Version 3.1; Pharsight Corporation, Mountain View, CA). The total area under the plasma concentration-time curve (AUC) was estimated using the linear/logarithmic trapezoidal method (for the up/down portions of the curve, respectively) up to the last measured concentration that was above the lower detection limit, which occurred in all cases at 24-hour for the first three visits and 12-hour for the last two visits when the subjects were induced. This area was extrapolated to infinity ($AUC_{0-\infty}$) by the addition of the last measured concentration divided by the apparent terminal disposition rate constant λ_z , determined by regression analysis of the terminal portion of the log plasma concentration-time curve. The apparent terminal half-life ($t_{1/2}$) was estimated from the terminal rate constant as $t_{1/2} = \ln 2 / \lambda_z$. Oral clearance (CL/F) was calculated as $Dose / AUC_{0-\infty}$. The steady-state volume of distribution (V_{ss}/F) was calculated as $V_{ss}/F = MRT \times CL/F$. The values of CL/F and V_{ss}/F were normalized by body weight. The mean residence time (MRT) for glyburide was calculated as the ratio of the area under the first moment curve $AUMC_{0-\infty}$ divided by $AUC_{0-\infty}$, minus the estimated mean absorption time (MAT) [i.e. $MRT = (AUMC_{0-\infty} / AUC_{0-\infty}) - MAT$]. MAT was the reciprocal of first-order absorption rate constant, determined by fitting the oral data to a 2-compartmental model with first-order absorption. The MRT of the 4-hydroxy metabolite was calculated for each treatment as $AUMC_{0-\infty} / AUC_{0-\infty}$ for the metabolite minus the same ratio for the parent glyburide.

Statistical Analysis

The number of subjects ($N = 10$) was based on a power analysis to detect an effect size of 30% in glyburide AUC assuming a standard deviation of 50% of the effect size with $\alpha = .05$ and $\beta = 0.2$. Results are expressed as mean $\pm$ SD. The pharmacokinetic parameters of glyburide and its metabolite were analyzed by use of the paired t test for any comparisons between two treatment groups. The other pharmacokinetic parameters were compared across 5 treatments with repeated-measures ANOVA with the Tukey post hoc test, comparing each treatment group with the glyburide group as the control. Logarithmic transformation of C_{max} , $t_{1/2}$, V_{ss}/F , CL/F and AUC values was performed before statistical analysis, and 95% confidence intervals were calculated for the geometric mean ratios (treatment phase values as a percentage of the control phase values) of these variables. The data were analyzed with GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). Differences were considered statistically significant at $p < 0.05$.

Acknowledgments

These studies were carried out in part in the General Clinical Research Center, Moffitt Hospital, University of California, San Francisco, with funds provided by the National Center for Research Resources, 5 M01 RR-00079, U.S. Public Health Service. During the course of this work, Dr. HongXia Zheng was supported by National Research Service Award T32 GM07546 from the National Institute of Health.

The authors thank the nurses at General Clinical Research Center, Moffitt Hospital, University of California at San Francisco for their help with this study.

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Figure 1.

AUC ratios of 4-hydroxyglyburide to glyburide in nine healthy volunteers on each treatment group. * Significantly different than control ($p < 0.05$).

Figure 2.
Change of blood glucose level in nine healthy volunteers on each treatment group

Figure 3.
AUC ratios of 4-hydroxyglyburide to glyburide in nine healthy volunteers on each treatment group. * Significantly different than Control ($p<0.05$).

Figure 4.
Change of blood glucose level in nine healthy volunteers on each treatment group

Table I

Demographic characteristics of the nine healthy volunteers completing the study

Characteristic	
BMI (Kg/m ²)	
Mean (SD)	25 (4.5)
Age (years)	
Mean (SD)	41.6 (11.2)
Race, n(%)	
Caucasian	3 (33%)
African American	2 (22%)
Asian/Pacific Islander	3 (33%)
Hispanic	1 (12%)
Sex, n(%)	
Male	6 (67%)
Female	3 (33%)

Table II

Clinical protocol

Visit	Treatment	Day	Protocol	
1	A/B	1	1.25 mg oral glyburide	OR 1.25 mg oral glyburide + 200 mg iv ciprofloxacin (20 min prior)
2	B/A	8	1.25 mg oral glyburide + 200 mg iv ciprofloxacin (20 min prior)	OR 1.25 mg oral glyburide
3	C	15	600 mg iv rifampin over 30 min immediately followed by 1.25 mg oral glyburide	
	D	22	600 mg oral rifampin	
	D	23	600 mg oral rifampin	
	D	24	600 mg oral rifampin	
	D	25	600 mg oral rifampin	
	D	26	600 mg oral rifampin	
	D	27	600 mg oral rifampin	
4	D	28	600 mg iv rifampin over 30 min immediately followed by 1.25 mg oral glyburide	
5	E	30	1.25 mg oral glyburide	

Blood samples on days 1, 8, 15, 28 and 30 at 0, ½, 1, 2, 3, 4, 6, 8, 12 and 24 hrs (24 hr sample omitted days 28 and 30). Urine collections on the same days at baseline, 0–4, 4–8, 4–12, and 12–24hr (12–24hr omitted on days 28 and 30).

Table III
Pharmacokinetic variables for glyburide in nine healthy volunteers for the five treatments

Treatment	C _{max} (ng/ml)	AUC _{0-∞} (ng/ml h)	CL/F (ml/h/kg)	T _{max} (h)	t _{1/2} (h)	MRT (h)	V _{ss} /F (ml/kg)
A. Glyburide control phase							
Mean±SD	384±135	1893±682	10.1±3.1	3(2-4)	4.7±1.3	4.5±2.0	49±33
B. Ciprofloxacin IV phase							
Mean±SD	364±145	1997±698	9.54±2.80	3(2-6)	5.1±1.6	4.9±1.8	50±24
Mean % of control	95	107	96		108	119	123
95% CI (% of control)	74-117	92-123	82-110		74-140	91-147	82-164
C. Rifampin IV phase							
Mean±SD	693±274 *	4123±1424 *	4.59±1.20 *	3(2-6)	3.7±0.7	3.3±1.4	16±7 *
Mean % of control	181	225	47		84	84	40
95% CI (% of control)	159-202	185-264	37-57		55-83	49-119	18-63
D. Rifampin IV/PO phase							
Mean±SD	327±91.7	1370±335 *	13.3±3.8	3(2-4)	1.9±0.3	2.0±1.1 *	28±18
Mean % of control	91	78	137			61	81
95% CI (% of control)	69-114	61-95	108-167			8.9-114	23-140
E. Rifampin PO phase							
Mean±SD	182±56.7 *	664±262 *	29.5±12.0	3(2-4)	2.0±0.9	2.0±0.5 *	64±32
Mean % of control	52	37	297			53	179
95% CI (% of control)	36-68	28-46	224-371			34-73	95-262
Ratio C to A	1.8±0.28	2.2±0.51	0.47±0.13			0.84±0.46	0.40±0.29
Ratio D to E	1.9±0.76	2.2±0.56	0.48±0.12			1.1±0.7	0.53±0.35

AUC, area under the plasma concentration-time curve; CI, confidence interval; CL/F, oral clearance; t_{1/2}, half-life; V_{ss}/F, oral steady-state volume of distribution; MRT, mean resident time.

T_{max} data are given as median and range

* significantly different from glyburide alone control phase.

Table IV

Pharmacokinetic variables of 4-hydroxyglyburide in nine healthy volunteers for the five treatments

Treatment	C _{max} (ng/ml)	AUC _{0-∞} (ng/ml h)	T _{max} (h)	t _{1/2} (h)	MRT (h)
A. Glyburide control phase					
Mean±SD	9.67±2.51	66.0±23.2	4(3–6)	4.2±1.7	1.6±0.9
B. Ciprofloxacin IV phase					
Mean±SD	9.34±3.17	65.4±20.5	4(3–8)	4.3±1.2	1.1±0.8
Mean % of control	99	101		115	89
95% CI (% of control)	73–125	84–118		80–149	23–154
C. Rifampin IV phase					
Mean±SD	12.5±4.01*	92.6±28.0*	4(3–6)	3.8±0.7	1.5±0.8
Mean % of control	134	145		102	121
95% CI (% of control)	98–170	117–174		72–128	50–192
D. Rifampin IV/PO phase					
Mean±SD	7.46±1.51	34.0±7.42*	4(3–6)	1.8±0.2	0.92±0.22
Mean % of control	81	54			77
95% CI (% of control)	60–103	46–62			41–113
E. Rifampin PO phase					
Mean±SD	5.33±1.69*	22.3±9.59*	4(3–6)	1.4±0.5	0.64±0.60*
Mean % of control	56	33			41
95% CI (% of control)	47–64	29–38			0.1–82
Ratio C to A	1.3±0.5	1.5±0.4			1.2±0.9
Ratio D to E	1.5±0.4	1.7±0.4			1.3±1.3

Table V

AUC ratios of 4-hydroxyglyburide to glyburide in nine healthy volunteers for the five treatments

Treatment	AUCm/AUCp Mean±SD (%)	95% CI
A. Glyburide control	3.67±1.03	2.88–4.47
B. Ciprofloxacin IV	3.34±0.37	3.06–3.62
C. Rifampin IV	2.36±0.61 *	1.89–2.83
D. Rifampin IV/PO	2.52±0.35 *	2.26–2.79
E. Rifampin PO	3.36±0.60	2.90–3.82

AUCm, metabolite area under the curve

AUCp, parent area under the curve

* significantly different from glyburide alone control phase.

Table VI
Accumulated urinary excretion and renal clearance of glyburide and 4-hydroxy metabolite in 9 healthy volunteers

	Glyburide control A	Ciprofloxacin IV B	Rifampin IV C	Rifampin IV/PO D	Rifampin PO E
<u>0–12h accumulated Urine excretion (ug)</u>					
Glyburide					
Mean±SD	1.8±1.1	1.7±0.6	3.9±1.3*	1.4±0.5	1.3±0.7
4-hydroxy-glyburide					
Mean±SD	358±107	325±77	457±72*	208±56*,**	135±40*
<u>CL_r (ml/h/kg)</u>					
Glyburide					
Mean±SD	0.017±0.015	0.015±0.007	0.016±0.007	0.016±0.009	0.031±0.017
4-hydroxy-glyburide					
Mean±SD	91.7±25.8	92.4±23.5	88.2±26.2	87.5±16.7	91.7±27.7

CL_r, renal clearance.

* significantly different from glyburide alone control phase.

** significantly different from Treatment C and E.