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RESEARCH PAPER

Effects of St John's wort and *CYP2C9* genotype on the pharmacokinetics and pharmacodynamics of gliclazide

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Background and purpose: Patients commonly take complementary medicines in conjunction with conventional drugs without clear evidence of safety or the risk of herb–drug interactions. The aim of this study was to assess potential pharmacokinetic (PK) and pharmacodynamic (PD) interactions between St John's wort and gliclazide in healthy subjects with different cytochrome P450 2C9 (*CYP2C9*) genotypes.
Experimental approach: A crossover controlled study was conducted in 21 healthy subjects. Each received gliclazide (80 mg) either alone or during 15 days treatment with St John's wort. The area under the plasma concentration–time curve ($AUC_{0-\infty}$), apparent clearance (CL/F) and elimination half-life ($t_{1/2}$) of gliclazide and incremental changes in glucose and insulin AUC_{0-4} were compared. *CYP2C9*2* and *CYP2C9*3* alleles were identified using PCR followed by restriction enzyme digestion analysis.
Key results: St John's wort significantly altered gliclazide pharmacokinetics in all except for four healthy subjects. The mean ratio and 90% confidence interval (CI) of gliclazide $AUC_{0-\infty}$ and CL/F were 0.67 (0.55–0.81) and 1.50 (1.24–1.81), respectively, after St John's wort treatment. St John's wort decreased gliclazide $t_{1/2}$, with mean ratio and 90% CI of 0.85 (0.74–0.93). There were no significant changes in glucose or insulin AUC_{0-4} after St John's wort treatment and no significant differences according to *CYP2C9* genotype.
Conclusions and implications: Treatment with St John's wort significantly increases the apparent clearance of gliclazide which is independent of *CYP2C9* genotype. People with diabetes receiving this combination should be closely monitored to evaluate possible signs of reduced efficacy.
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Keywords: pharmacokinetics; pharmacodynamics; gliclazide; St John's wort; CYP2C9; genotype

Abbreviations: AUC, area under the plasma gliclazide concentration–time curve; CI, confidence interval; CL/F, apparent clearance; C_{max}, maximum concentration; CYP2C9, cytochrome P450 2C9; k_{el}, elimination rate constant; t_{max}, time to C_{max}

Introduction

The incidence of type 2 diabetes is increasing in the community. Dietary modification and hypoglycaemic medicines such as gliclazide have important roles in the control of diabetes and the prevention of secondary complications of this disease. Recent survey data indicate that 52% of Australian consumers had used complementary and alternative medicines in 2004 (MacLennan *et al*., 2006). Approximately, half of these consumers also took prescription medicines at the same time (Kristoffersen *et al*., 1996; MacLennan *et al*., 2006). Furthermore, 31% of diabetic patients have been reported to take alternative medicines, most in conjunction with conventional medicines (Ryan *et al*., 2001). St John's wort (*Hypericum perforatum*) is one of the most widely used herbal medicines in Western countries, and it has been used for centuries for a variety of diseases, but most commonly for depression (Ryan *et al*., 2001). A systematic review of clinical trials concluded that St John's wort interacts with many medicines, including many clinically important drugs such as warfarin and cyclosporin, leading to reduced effectiveness (Mills *et al*., 2004).

Gliclazide (1-(3-azabicyclo[3.3.0]oct-3-yl)-3-para-tolylsulphonylurea) is a most widely used sulphonylurea drug for type 2 diabetes (Figure 1). The clinical use of gliclazide is

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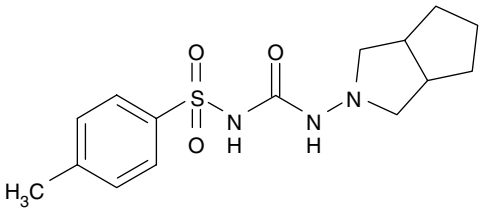

Figure 1 Chemical structure of gliclazide.

initiated at 40 mg daily and may increase up to 320 mg daily in divided doses. It has high bioavailability (approximately 100%) from tablets and gliclazide's absorption is not affected by food (Shima *et al*., 1982; Hong *et al*., 1998). Compared to other sulphonylureas, gliclazide has an intermediate half-life of approximately 11 h (Campbell *et al*., 1991; Park *et al*., 2003). Gliclazide is extensively metabolized in the liver (Davis *et al*., 2000) to at least eight identified metabolites (Oida *et al*., 1985; Campbell *et al*., 1991; Taylor *et al*., 1996). Tolylmethyl hydroxylation (to form methyl-hydroxy-gliclazide) followed by oxidation to the corresponding carboxylic acid (to form carboxygliclazide) is the major metabolic pathway of gliclazide. These two metabolites account for 59% of the dose recovered in urine 96 h later (Oida *et al*., 1985). Hydroxylation of the azabicyclo-octyl moiety has the potential to form seven monohydroxylated metabolites. Four of these metabolites (6α, 6β, 7α and 7β positions) have been identified in urine, which represented 1, 20, 6 and 14% of urinary recovered dose 96 h after dose, respectively (Oida *et al*., 1985). Gliclazide metabolites have no hypoglycaemic activity and plasma concentrations are very low, representing only a small fraction of gliclazide concentrations *in vivo* (Campbell *et al*., 1980; Lupo and Bataille, 1987). There is no evidence that transporters are involved in gliclazide disposition. Elliot *et al*. (2007) recently identified the human cytochrome P450 catalysing the rate-limiting pathways of gliclazide metabolism and found that cytochrome P450 2C9 (CYP2C9) is the major contributor to gliclazide metabolic clearance, although CYP2C19 may also be involved in tolymethyl hydroxylation of gliclazide.

CYP2C9 is a polymorphic enzyme with 29 variant alleles described (www.cypalleles.ki.se/). The three most common alleles are *CYP2C9*1* (wild-type), *CYP2C9*2* and *CYP2C9*3*. *CYP2C9*2* and *CYP2C9*3* alleles are associated with lower enzyme activity both *in vitro* and *in vivo* (Lee *et al*., 2002; Soga *et al*., 2004; Topic *et al*., 2004). CYP2C9 polymorphism also plays an important role in individual differences in hypoglycaemic effects of sulphonylurea agents (Kirchheiner *et al*., 2005). The *CYP2C9*2/*2* genotype carriers have a lower apparent clearance (CL/F) of glyburide compared to the individuals with *CYP2C9*1/*1*, but this contrast is not seen with the other sulphonylurea drugs. The carriers of one or two *CYP2C9*3* alleles tend to have lower CL/F for most of the sulphonylurea drugs. However, there are only limited clinical data available on gliclazide pharmacokinetics and/or pharmacodynamics in relation to *CYP2C9* genotype groups despite the suggestion that individual gene differences are potentially important factors that determine a patient's response to therapy.

The aims of this study were to investigate the effects of St John's wort on the pharmacokinetics and pharmacodynamics of gliclazide in healthy subjects and to identify sources of individual variation by examining gliclazide pharmacokinetics and pharmacodynamics in relation to *CYP2C9* polymorphisms.

Methods

Subjects

This study was approved by the St Vincent's Hospital and University of Sydney Human Research Ethics Committees (Sydney, Australia). A total of 21 healthy volunteers (15 male, 6 female; age range, 19–37 years; weight range, 53–91 kg) were recruited and screened for *CYP2C9* genotype after giving written informed consent. Health status was assessed by medical history, physical examination and routine laboratory tests (including fasting blood glucose concentration).

Pharmacogenetic study (PCR-RFLP analysis)

Saliva (2 ml) or blood samples (4 ml) were obtained from each subject. DNA was extracted and used to detect the different alleles of *CYP2C9*. Genomic DNA was isolated from saliva using Oragene DNA Self-Collection Kit (DNA Genotek, Ottawa, ON, Canada) or blood using Qiagen blood kit (Qiagen, Doncaster, VIC, Australia). Detection of the *CYP2C9*2* and *CYP2C9*3* alleles were carried out using a polymerase chain reaction based restriction fragment length polymorphism (polymerase chain reaction-RFLP) analysis. The primers and restriction sites (*Ava*II restriction site for the *CYP2C9*2* allele, *Nsi*I and *Kpn*I for the *CYP2C9*3* allele) used in the present study were as described by Sullivan-Klose *et al*. (1996). Subjects in whom neither *CYP2C9*2* nor *CYP2C9*3* was detected were characterized as *CYP2C9* wild type (**1/*1*).

Briefly, PCR was performed in a final reaction volume of 50 μl, which included 20 μl 2.5 × HotMasterMix (Eppendorf, Westbury, NY, USA), approximately 250 ng of human genomic DNA and the primer pairs (0.25 μM). PCR amplification was performed with an initial melting step at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for *CYP2C9*2* (59 °C for *CYP2C9*3*) for 30 s and extension at 72 °C for 30 s. Upon completion of 35 cycles, the reaction was continued at 72 °C for 10 min for final extension. Aliquots of each PCR reaction (16 μl for *Ava*II and *Nsi*I, 10 μl for *Kpn*I) were subjected to restriction enzyme analysis with 20 U *Ava*II (*CYP2C9*2*) and *Nsi*I (*CYP2C9*3*) or 10 U *Kpn*I (*CYP2C9*3*). After overnight incubation at 37 °C, digested products were separated by electrophoresis using 2% agarose gels (Progen, Darra, QLD, Australia) run at 110 V for 60 min for *CYP2C9*2* or 2.5% agarose gels run at 110 V for 50 min for *CYP2C9*3*; the gels were stained with ethidium bromide and the restriction fragments were visualized (Gel Doc, Bio-Rad, Segrate, Milan, Italy).

Clinical study

A sequential crossover two-treatment study was performed, with at least a 4-day washout period between the two

treatments. After an overnight fast, a single dose of 80 mg gliclazide (Diamicron, Servier Laboratories, Hawthorn, VIC, Australia) was administered either alone or, on the last day (Day 15) treatment, with 300 mg St John's wort (Kira, LI 160 extract, Lichtwer Pharma, Berlin, Germany) given three times a day. An oral 75 g dose of glucose (Glucaid Clear, Fronine, Riverstone, NSW, Australia) was given 0.5 h after gliclazide intake. Food and drink (with the exception of water) were not allowed for the first 4 h after gliclazide intake. All participants were asked not to drink caffeine-containing beverages and to refrain from grapefruit from the day before until 2 days after taking gliclazide. Venous blood (EDTA tube) was taken for gliclazide and insulin quantification at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 24, 36 and 48 h after gliclazide administration. Plasma was obtained by centrifugation at 1500 *g* for 15 min and stored at −20 °C before the assay. Capillary blood samples were used to measure glucose levels at 0, 0.5, 1, 1.5, 2, 2.5, 3 and 4 h after gliclazide administration.

Analytical methods

Plasma gliclazide concentrations were measured by reversed-phase HPLC using a C18 column (150 × 4.6 mm) by the method of Park *et al*. (2004) with minor modifications. The separation was achieved using a mobile phase of 40 mM KH_2PO_4 (pH 4.6, 56%) and acetonitrile (44%) at a flow rate of 1.2 ml min^{-1} with UV detection at a wavelength of 227 nm. In brief, 0.5 ml of plasma, 20 µl of glibenclamide (internal standard, 50 µg ml^{-1}) and 0.5 ml of acetonitrile was transferred to a 10 ml plastic tube. After vortexing for 10 s, chloroform (4 ml) was added followed by vigorous shaking (1 min) and then centrifugation at 1500 *g* for 15 min. The lower organic layer (3 ml) was transferred into a 5 ml glass tube and dried under a stream of nitrogen at 40 °C. The residue was reconstituted with mobile phase (150 µl), vortex mixed for 20 s, then transferred to a microcentrifuge tube and centrifuged at 1500 *g* for 2 min, and 50 µl of the upper layer was taken for HPLC analysis. The limit of detection for gliclazide was 50 ng ml^{-1}. The intraday and interday coefficients of variation for gliclazide were all less than 11% at concentrations of 0.5, 3 and 8 µg ml^{-1} ($n = 5$).

Plasma insulin concentration was determined using the Coat-A-Count ^{125}I-labelled radioimmunoassay (Bio-Mediq DPC, Los Angeles, CA, USA). Capillary blood glucose concentration was measured immediately after each sample was taken using a commercial glucose meter (Roche ACCU-CHEK Go, Castle Hill, NSW, Australia).

Data analysis

The area under the plasma gliclazide concentration–time curve (AUC) up to the last concentration measured (AUC_{0-t}) was determined using the linear trapezoidal rule. The AUC was extrapolated to infinity ($AUC_{t-\infty}$) using C_t/k_{el}, where C_t is the last measured gliclazide concentration and k_{el} is the elimination rate constant determined from the terminal slope of the log concentration–time plot. Elimination half-life ($t_{1/2}$) was calculated as $\ln 2/k_{el}$. The maximum concentration (C_{max}) and the time to C_{max} (t_{max}) were obtained by the inspection of the concentration–time data. The CL/F was calculated as dose/$AUC_{0-\infty}$.

The glucose and insulin concentrations were measured to determine the pharmacodynamic effects. The incremental area under the blood glucose and insulin concentration–time curves (AUC_{0-4}) were calculated to adjust for the individual variation of the baseline glucose concentrations using the approach described by Park *et al*. (2003). The maximum glucose and insulin concentration after glucose loading was determined by observation of the concentration–time data.

Statistical analysis

All data are presented as mean ± s.d. and 95% confidence interval (CI) except t_{max}, which is presented as median and range. The pharmacokinetic parameters with and without St John's wort treatment were compared using the paired Student's *t*-test. The mean residual error was calculated using ANOVA (Stata 5.0, Stata Corp, College Station, TX, USA) of log-transformed parameters considering treatment and used to calculate the 90% CI of the ratio of log-transformed pharmacokinetic parameters comparing control (gliclazide alone) and treatment phase (gliclazide and St John's wort). A 90% CI that did not include 1.0 was considered significantly different.

Results

Effects of St John's wort on pharmacokinetics of gliclazide

St John's wort significantly reduced gliclazide $AUC_{0-\infty}$ and C_{max} in 17 of 21 healthy subjects, and the results of all 21 subjects are presented here (Figures 2 and 3). The mean ratio ($n = 21$) and 90% CI of gliclazide $AUC_{0-\infty}$ and CL/F are shown in Table 1. After St John's wort treatment, gliclazide C_{max} decreased by 22%, whereas the CL/F increased by nearly 50%. Furthermore, the mean $t_{1/2}$ of gliclazide after St John's wort treatment decreased from 10.3 to 8.7 h.

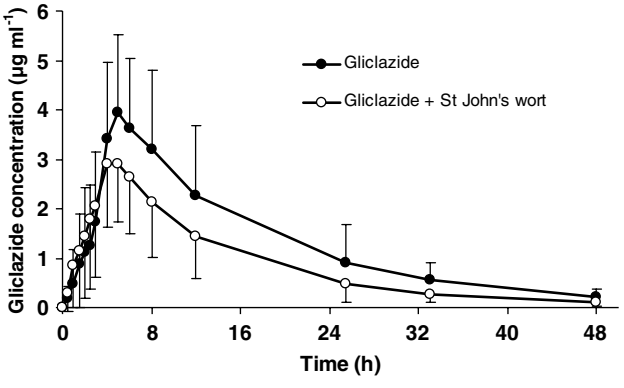

Figure 2 Mean plasma gliclazide concentration–time profile in control and St John's wort treatment phases. Error bars represent s.d. ($n = 21$).

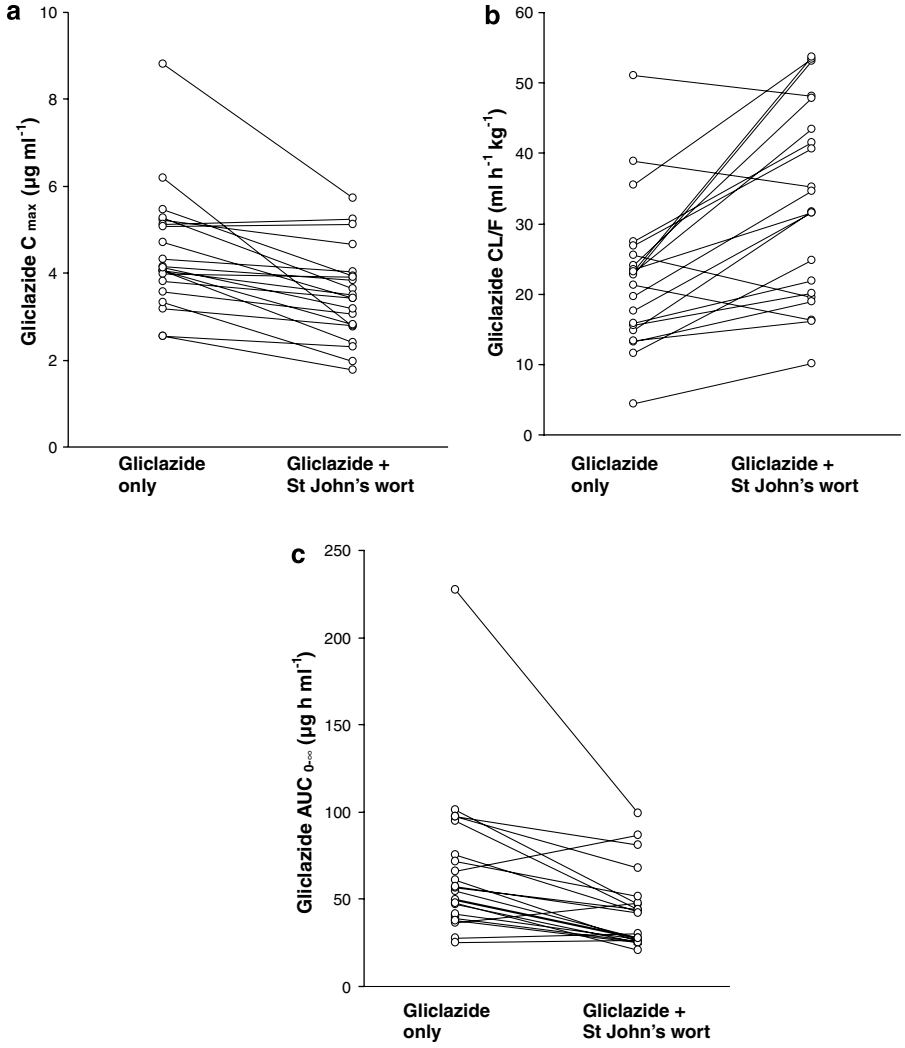

Figure 3 Individual gliclazide (**a**) maximum plasma concentration (C_{max}), (**b**) apparent clearance (CL/F) and (**c**) area under the plasma gliclazide concentration–time curve (AUC) in 21 healthy subjects after single oral dose of 80 mg gliclazide before and during co-administration with St John's wort (300 mg three times daily for 15 days).

Table 1 Pharmacokinetic parameters of gliclazide in 21 healthy subjects after a single oral dose of 80 mg gliclazide or co-administered with St John's wort (300 mg three times daily)

Pharmacokinetic parameter	*Gliclazide alone*	*Gliclazide and St John's wort*	*Ratio (90% CI)*
t_{max} (h)	5 (3–8)	4 (1.5–6)	NA
C_{max} (μg ml^{-1})	4.5 (3.9–5.0)	3.5 (3.0–4.0)	0.78 (0.72–0.85)
Half-life (h)	10.3 (9.1–11.4)	8.7 (7.8–9.6)	0.85 (0.74–0.93)
CL/F (l h^{-1})	1.5 (1.2–1.8)	2.2 (1.9–2.6)	1.50 (1.24–1.81)
$AUC_{0-\infty}$ (μg h ml^{-1})	67.2 (48.6–85.7)	43.6 (33.9–53.2)	0.67 (0.55–0.81)

Abbreviations: $AUC_{0-\infty}$, area under the plasma gliclazide concentration–time curve extrapolated to infinity; CI, confidence interval; C_{max}, maximum concentration; CL/F, apparent clearance; NA, not applicable; t_{max}, time to C_{max}.
Ratio is presented as mean and 90% CI, and all others are presented as mean and 95% CI.

Effects of St John's wort on pharmacodynamics of gliclazide

Glucose concentration increased after the intake of 75 g glucose in each phase of this study. Gliclazide initially lowered glucose concentrations, which returned to baseline 2.5 h after glucose intake. The mean incremental glucose concentration showed a trend towards a decrease after St John's wort treatment, but this difference did not reach statistical significance (Table 2 and Figure 4a). Similarly, the mean insulin incremental concentration increased slightly in the St John's wort phase of the study compared to the

Table 2 Pharmacodynamic effects of gliclazide in 21 healthy subjects after a single oral dose of 80 mg gliclazide or co-administered with St John's wort (300 mg three times daily)

Pharmacodynamic parameter	*Gliclazide alone*	*Gliclazide and St John's wort*
Blood glucose		
Glucose incremental AUC_{0-4} (mmol h l^{-1})	8.5 ± 4.8	6.2 ± 6.2
Glucose AUC_{0-4} (mmol h l^{-1})	23 ± 2.7	22.2 ± 3.0
Glucose C_{max} (mmol l^{-1})	8.7 ± 1.5	8.6 ± 1.4
Glucose t_{max} (h)	1.2 (1–2.5)	1.1 (1–2)
Plasma insulin		
Insulin incremental AUC_{0-4} (mIU h l^{-1})	180.1 ± 122.2	211.9 ± 159.1
Insulin AUC_{0-4} (mIU h l^{-1})	198.9 ± 141.0	228.1 ± 167.6
Insulin C_{max} (mIU l^{-1})	97.2 ± 53.7	118.0 ± 71.4
Insulin t_{max} (h)	1.6 (1–2.5)	1.3 (1–2)

Abbreviations: AUC_{0-4}, incremental area under the blood glucose and insulin concentration–time curves; C_{max}, maximum concentration; t_{max}, time to C_{max}. Data are presented as mean ± s.d., except for t_{max} data, which are given as median and range.

control phase (Table 2 and Figure 4b). There was considerable interindividual variation observed for both glucose and insulin concentration–time profiles.

Pharmacogenetic results

All subjects were genotyped for *CYP2C9* genes (Table 3). The subjects were grouped into three categories ***1/*1*** (wild type), ***1/*2*** or ***2/*2***, and ***1/*3*** to compare the pharmacokinetic parameters. There was slightly lower CL/F of gliclazide in subjects carrying the *CYP2C9*2* allele, but the difference did not reach statistical significance (Figure 5).

Discussion and conclusions

The mean CL/F of gliclazide in subjects possessing the *CYP2C9*2* allele (homozygous and heterozygous combined) was approximately 25% lower than that observed in wild-type subjects. In similar studies, the CL/F ratios in subjects possessing at least one copy of the *CYP2C9*2* allele (compared with wild-type subjects) were 0.88 for tolbutamide (Kirchheiner *et al*., 2002a) and 0.9 for glyburide (Kirchheiner *et al*., 2002b). Furthermore, the CL/F of gliclazide in subjects who were heterozygous for the *CYP2C9*3* allele was not significantly different from wild-type subjects in the present study, which is consistent with the reported findings for glyburide (Kirchheiner *et al*., 2002b). By contrast, for carriers of the *CYP2C9*3* allele, the CL/Fs of glipizide (Kidd *et al*., 1999) and tolbutamide (Kirchheiner *et al*., 2002a) were significantly different from wild-type subjects, with ratios of 0.18 and 0.58, respectively. Compared with wild-type individuals, the clearances of tolbutamide (Kirchheiner *et al*., 2002a) and glyburide (Kirchheiner *et al*., 2002b) were decreased by 50–84% in those carrying the *CYP2C9*3/*3* genotype and by 41–54% in those of *CYP2C9*2/*3* genotype.

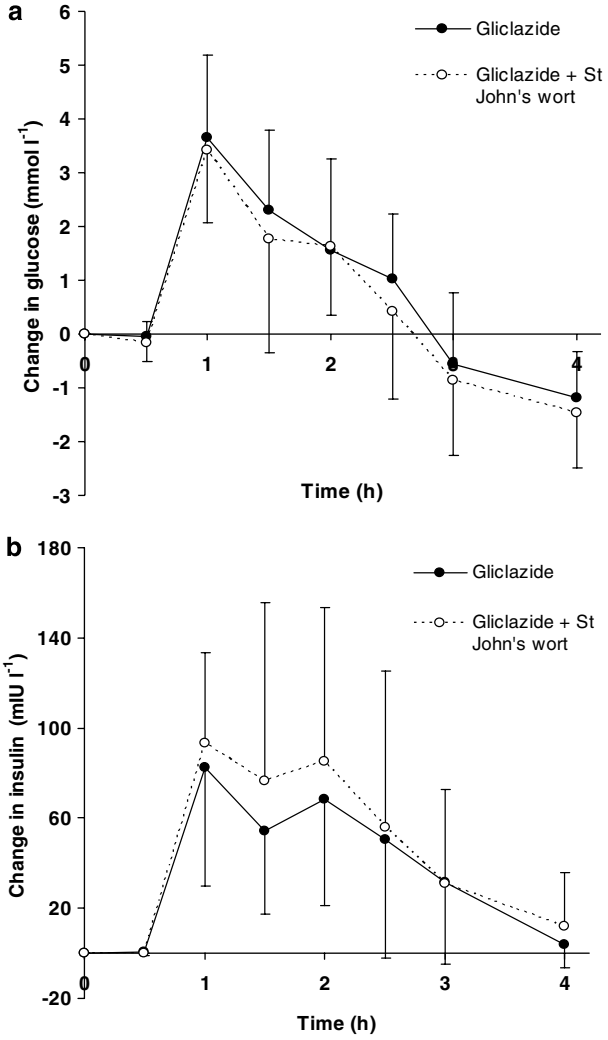

Figure 4 Mean changes in (**a**) glucose concentrations and (**b**) insulin concentration in 21 healthy subjects after single oral dose of 80 mg gliclazide alone and during co-administration with St John's wort (300 mg three times daily for 15 days) (error bars represent s.d.).

Table 3 Genotype frequency and gliclazide CL/F in 21 subjects in gliclazide only phase

CYP2C9 genotype	*Samples* (n)	*Frequency (%)*	*Gliclazide CL/F* (ml h^{-1} kg^{-1})
*1/*1	11	52.4	24.0 (19.0–28.9)
*1/*2 or	6	28.6	18.5 (15.2–21.7)
*2/*2	1	4.8	
*1/*3	3	14.3	25.4 (20.4–30.5)

Abbreviation: CL/F, apparent clearance.
Data shown was not to represent population frequency; data are presented as median and range.

In a study by Holstein *et al*. (2005), the clinical implications of *CYP2C9* genotype in people receiving sulphonylurea agents were examined. It was found that the *CYP2C9*2/*3* and *CYP2C9*3/*3* genotypes were associated with a higher risk of experiencing severe hypoglycaemia (Holstein *et al*.,

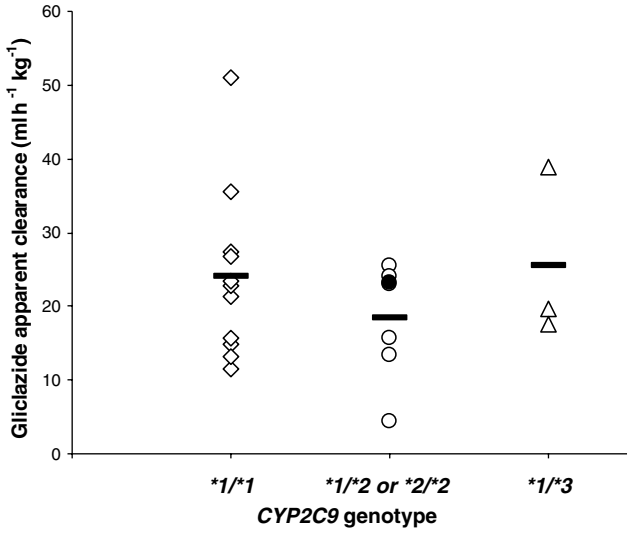

Figure 5 Gliclazide apparent clearance (CL/F) of three groups of *CYP2C9* genotypes in gliclazide only phase. ('—' indicates mean in each group, the solid circle represents a subject with *CYP2C9*2/*2* genotype).

2005). However, these genotypes were not found in the present study population, which is compatible with the reported frequencies in Caucasians of the *CYP2C9*2* and *CYP2C9*3* alleles of 0.014 and 0.04, respectively (Lee *et al*., 2002); thus, the impact of these genotypes on gliclazide pharmacokinetics could not be evaluated.

The apparent differences in the impact of the *CYP2C9* genotype on the clearance of drug substrates may be attributable in part to contributions from other CYPs. Thus, evidence has been presented that CYP2C19 can also oxidize tolbutamide, at least *in vitro*, although the *in vivo* significance is uncertain (Lasker *et al*., 1998; Wester *et al*., 2000). CYP2C9 is believed to be the principal enzyme involved in gliclazide elimination by tolylmethyl hydroxylation, but CYP2C19 may also contribute (Elliot *et al*., 2007). Accordingly, the potential contributions of *CYP2C19* allelic variants to the pharmacokinetics of oral hypoglycaemic agents such as tolbutamide and gliclazide cannot be excluded, and this might explain the lack of concordance between *CYP2C9* genotype and gliclazide clearance. A recent study investigated the relative influence of CYP2C9 and CYP2C19 genetic polymorphisms on the pharmacokinetics of gliclazide in healthy Chinese subjects (Zhang *et al*., 2007). These researchers suggest a greater role for CYP2C19 polymorphisms than has been reported previously.

The major aim of the present study was to evaluate the impact of St John's wort treatment on gliclazide pharmacokinetics and pharmacodynamics. Co-administration of St John's wort over a 15-day period significantly decreased gliclazide $AUC_{0-\infty}$ and $t_{1/2}$, which is consistent with increased CL/F. Park *et al*. (2003) reported a 70% reduction of gliclazide AUC and fourfold increase of CL/F of gliclazide after 6 days of rifampicin treatment. Induction of CYP2C9 is the most likely mechanism responsible for the effects of rifampicin and St John's wort. However, it is noteworthy that altered gliclazide pharmacokinetics in response to co-administered St John's wort was somewhat similar in subjects carrying *CYP2C9* variant alleles.

Despite the observed pharmacokinetic changes for gliclazide, there was no apparent change in the incremental AUC_{0-4} for either glucose or insulin after St John's wort treatment. This finding is in contrast to the observations of Park *et al*. (2003) who investigated the interaction between rifampicin and gliclazide. The reason for the discrepancy is unclear, but may relate to differences in the magnitude of the induction between St John's wort and rifampicin. The extent of induction of gliclazide clearance and the decrease in AUC produced by St John's wort may have been insufficient to alter the pharmacodynamic response in individuals with normal insulin regulation. However, there may have been additional environmental factors, such as diet (Shaw, 2006), that could have contributed to the minimal impact on pharmacodynamics in the present study.

The extent to which gliclazide clearance was influenced by co-administered St John's wort varied considerably between subjects. Pronounced increases in CL/F were observed in a few subjects showing efficient induction of CYP2C9, but the majority of individuals exhibited relatively small changes, whereas in four subjects, gliclazide clearance decreased somewhat after treatment with St John's wort. The adherence to St John's wort treatment was established by regular telephone contact and confirmed by interview, which corroborated the compliance records submitted immediately after completion of the study. Therefore, we speculated that such variation in response might be related to differences in the inducibility of the *CYP2C9* gene between individuals.

The mechanism underlying the St John's wort–gliclazide interaction has not been established unequivocally. However, St John's wort has been shown to induce the *CYP3A4* gene and to increase the clearance of many CYP3A4 substrates, for example, indinavir (Piscitelli *et al*., 2000), simvastatin (Sugimoto *et al*., 2001), cyclosporin (Bauer *et al*., 2003) and midazolam (Dresser *et al*., 2003). Hyperforin, a major constituent of St John's wort, is a high-affinity ligand for the PXR (pregnane X-receptor), which is a nuclear receptor that regulates the induction of a battery of drug-metabolizing enzymes and transporters, including CYPs 2C9, 2C19, 2C8, 3A4 and MDR (multidrug resistance gene)1 (P-glycoprotein, P-gp) (Moore *et al*., 2000; Chen *et al*., 2004). Interestingly, the extent of the interaction between St John's wort and the CYP3A4 substrate cyclosporin has been shown to be related to hyperforin content (Mai *et al*., 2004). In the present study, a hyperforin-rich St John's wort extract LI 160 (Kira) was used (Wurglics *et al*., 2001), which is most likely to have mediated the induction of CYP2C9 and led to altered gliclazide pharmacokinetics observed in this study. Although CYP2C9 appears to be the major enzyme involved in gliclazide metabolism, the contributions of CYP2C19 and possibly CYP2C18 cannot be excluded (Elliot *et al*., 2007). Therefore, in addition to the CYP2C9 induction by St John's wort, the induction of CYP2C19 and possibly CYP2C18 may contribute to the observed decrease in gliclazide AUC.

The effect of St John's wort on CYP2C9 induction has been studied with several drugs. Our previous studies demonstrated that St John's wort constituents induced the metabolism of *S*-warfarin, another CYP2C9 substrate (Jiang *et al*.,

2004). Wang *et al*. (2001) studied the prototypic sulphonylurea, tolbutamide, under conditions of short-term and long-term St John's wort co-administration but, somewhat surprisingly, neither of these treatments influenced the pharmacokinetic parameters of tolbutamide (Wang *et al*., 2001). However, the hyperforin content of the St John's wort preparation used by these researchers was not clearly defined, so that comparisons with the present study are not straightforward.

Despite the finding that allelic variants of *CYP2C9* contribute to gliclazide pharmacokinetic differences, there are additional pharmacodynamic factors that could account for interindividual differences in glucose and insulin concentrations in response to gliclazide treatment. These factors include polymorphisms in genes encoding the pancreatic sulphonylurea receptor (SUR1 or ABCC8) and its endogenous ligand α-endosulphine (ENSA), potassium channels and other regulators of insulin secretion (Goksel *et al*., 1998; Elbein *et al*., 2001; Wang *et al*., 2004). Further study on variations of those genes involved in pharmacodynamic differences may help to better understand the findings of the present study.

St John's wort significantly decreased gliclazide $AUC_{0-\infty}$, which was probably due to induction of CYP2C9 and/or CYP2C19. Variant alleles of *CYP2C9* did not appear to mediate differences in gliclazide clearance or glucose-lowering effects. Further investigations in people with diabetes will improve our understanding of the impact of pharmacogenetics on gliclazide pharmacokinetics and provide further insight into its optimal clinical usage and the effects of herbal remedies on the pharmacokinetics and pharmacodynamics of prescription medicines.

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Conflict of interest

The authors state no conflict of interest.

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