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Identification of the human cytochromes P450 catalysing the rate-limiting pathways of gliclazide elimination

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What is already known about this subject
- Gliclazide is a widely used oral hypoglycaemic agent.
- The major metabolites of gliclazide formed *in vivo* have been identified.
- However, the cytochrome P450 enzymes catalysing the rate-limiting pathways of gliclazide elimination are unknown.

What this study adds
- CYP2C9 is the major enzyme involved in the various hydroxylation pathways of gliclazide, although a contribution of CYP2C19 to tolymethylhydroxylation, the major metabolic route, cannot be discounted.
- Factors known to influence CYP2C9 activity will provide the main source of variability in gliclazide pharmacokinetics.

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Aims
To identify the human cytochrome P450 (CYP) enzymes responsible for the formation of the 6β-hydroxy (6β-OHGz), 7β-hydroxy (7β-OHGz) and hydroxymethyl (MeOH-Gz) metabolites of gliclizide (Gz).

Methods
6β-OHGz, 7β-OHGz and MeOH-Gz formation by human liver microsomes and a panel of recombinant human P450s was measured using a high-performance liquid chromatography procedure, and the kinetics of metabolite formation was determined for each pathway. Effects of prototypic CYP enzyme selective inhibitors were characterized for each of the microsomal metabolic pathways.

Results
Microsomes from six human livers converted Gz to its 6β-OHGz, 7β-OHGz, and MeOH-Gz metabolites, with respective mean (± SD) K_m values of 461 ± 139, 404 ± 143 and 334 ± 75 μM and mean V_{max} values of 130 ± 55, 82 ± 31 and 268 ± 115 pmol min^{-1} mg^{-1}, respectively. V_{max}/K_m ratios for the microsomal reactions parallelled relative metabolite formation *in vivo*. Sulfaphenazole inhibited microsomal 6β-OHGz, 7β-OHGz and MeOH-Gz formation by 87, 83 and 64%, respectively, whereas S-mephenytoin caused significant inhibition (48%) of only MeOH-Gz formation. Recombinant CYP2C9, CYP2C18 and CYP2C19 catalysed all hydroxylation pathways, whereas CYP2C8 formed only 6β-OHGz and 7β-OHGz.

Conclusion
Taken together, the results indicate that CYP2C9 is the major contributor to Gz metabolic clearance, although CYP2C19 may also be involved in MeOH-Gz formation (the major metabolic pathway). Factors known to influence CYP2C9 activity will provide the main source of variability in Gz pharmacokinetics.

Oral hypoglycaemic agents remain the cornerstone of the treatment of Type 2 diabetes in patients not responsive to diet, exercise and weight reduction [1]. Of the various oral hypoglycaemic agents available, sulphonylureas and biguanides are considered the first-line treatment. Amongst the sulphonylureas, gliclazide (Gz) is widely used. Almost 4 and 1.2 million prescriptions for Gz were dispensed in the UK (http://www.ic.nhs.uk/pubs/precostanalysis2005/final/file) and Australia (http://www.medicareaustralia.gov.au/providers/health_statistics/statistical_reporting/pbs.htm), respectively, in 2005. Considerable interindividual variability in metabolic clearance is a feature of the sulphonylureas [2, 3] and differences in elimination are believed to contribute to therapeutic outcome and the occurrence of adverse effects [3]. Despite the widespread use of Gz, factors that contribute to pharmacokinetic variability have received less attention than for other sulphonylureas [3].

Gz is extensively metabolized in humans and multiple urinary metabolites have been identified [4, 5]. Like the structurally related 4-methylsulphonylureas tolbutamide [6, 7] and torsemide (used clinically as a diuretic) [8], tolylmethyl hydroxylation (to form MeOH-Gz) followed by oxidation to the corresponding carboxylic acid is the major metabolic pathway. However, Gz is also hydroxylated in the azabicyclooctyl ring with the potential to form seven monohydroxylated metabolites (Figure 1). Of these, four monohydroxy-azabicyclooctyl metabolites (at the 6α-, 6β-, 7α- and 7β- positions) have been identified in human urine [4, 5]. Based on metabolite excretion in urine to 96 h postdose, MeOH-Gz (as this metabolite plus the derived carboxylic acid), 6α-OHGz, 6β-OHGz, 7α-OHGz and 7β-OHGz accounted for 59, 1, 20, 6 and 14% of the recovered dose, respectively [4]. Thus, 6β-, 7β- and tolylmethyl- hydroxylation represent the rate-liming pathways of Gz elimination.

Although the *in vivo* fate of Gz has been elucidated, no systematic investigation has been undertaken to identify the human cytochrome P450 (CYP) enzymes responsible for the various pathways of Gz hydroxylation. The aim of this study was to characterize Gz hydroxylation by human liver microsomes (HLM) and, using CYP enzyme selective inhibitors and recombinant P450s, identify the human enzymes responsible for the formation of the individual hydroxylated metabolites in order to gain insights into factors likely to influence Gz elimination and response.

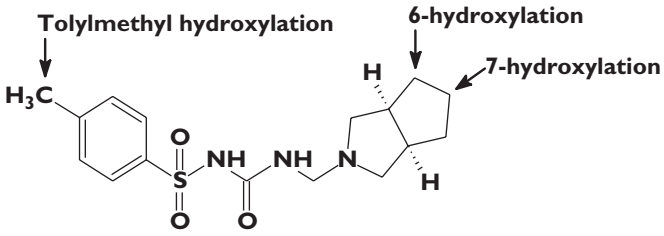

Figure 1
Positions of gliclazide hydroxylation

Materials and methods

Chemicals and reagents

Gliclazide (Gz, 1-(3-azabicyclo[3.3.0]oct-3-yl)-3-(4-methylphenylsulphonyl)urea), 6α-hydroxygliclazide (6α-OHGz; 1-(3-aza-6-hydroxybicyclo[3.3.0]oct-3-yl)-3-(4-methylphenylsulphonyl)urea) as the enantiomeric mixture (1R,5S,6S and 1S,5R,6R), 6β-hydroxygliclazide (6β-OHGz; 1-(3-aza-6-hydroxybicyclo[3.3.0]oct-3-yl)-3-(4-methylphenylsulphonyl)urea) as the enantiomeric mixture (1R,5S,6R and 1S,5R,6S), 7β-hydroxygliclazide (7β-OHGz; (1R,5S,7β)-1-(3-aza-7-hydroxybicyclo[3.3.0]oct-3-yl)-3-(4-methylphenylsulphonyl)urea) and hydroxymethylgliclazide (MeOH-Gz; 1-(3-azabicyclo[3.3.0]oct-3-yl)-3-(4-hydroxymethylphenylsulphonyl)urea) were gifts from Technologie Servier (Melbourne, Australia). Furafylline was obtained from Hoffman La Roche (Basel, Switzerland), whereas coumarin, quinidine hydrochloride monohydrate (S)-(+)-mephenytoin, troleandomycin triacetate, trimethoprim, sulfaphenazole, glucose-6-phosphate, glucose-6-phosphate dehydrogenase and β-nicotinamide adenine dinucleotide phosphate (NADP) were purchased from Sigma-Aldrich (Sydney, Australia). All other chemicals and reagents were of analytical reagent grade.

Human liver microsomes and recombinant CYP enzymes

Human livers (H6, H7, H10, H12, H13 and H40) were obtained from the human liver 'bank' of the Department of Clinical Pharmacology, Flinders Medical Centre. Approval was obtained from the Flinders Medical Centre Clinical Investigation Committee and from the donor next-of-kin for the procurement and use of human liver tissue in xenobiotic metabolism studies. Microsomes were prepared from human livers by differential centrifugation according to Bowalgaha *et al.* [9].

Recombinant human CYP enzymes and rat NADPH-cytochrome P450 reductase were coexpressed in *Escherichia coli* following published methods; CYP1A2 according to Polasek *et al.* [10], CYP2A6, CYP2D6 and CYP2E1 as described by Parikh *et al.* [11], CYP2C8 and CYP2C9 by the method of Boye *et al.* [12] and CYP2C18 and CYP2C19 as reported by Kinobe *et al.* [13] and Cuttle *et al.* [14], respectively.

Kinetic and inhibitor studies

Incubation mixtures, in a total volume of 0.5 ml, contained HLM (0.5 mg of microsomal protein) or recombinant CYP [10 pmol, except for CYP2C8 (20 pmol)], Gz (50–750 μM), acetonitrile (1% v/v) and NADPH generating system (1 mM NADP, 100 mM glucose-6-phosphate, 1 IU glucose-6 phosphate dehydrogenase and 5 mM $MgCl_2$) in phosphate buffer (0.1 M, pH 7.4). Incubations were performed in air at 37°C in a shaking water bath. After a 5-min preincubation, reactions were initiated by the addition of NADPH generating system. Reactions were terminated after 90 min (HLM) or 60 min (recombinant enzymes) by the addition of perchloric acid (5 μl, 11.6 M). Mixtures were vortex mixed, cooled on ice and then centrifuged at 3000 *g* for 10 min to precipitate microsomal protein. Sodium hydroxide (2 M, 5 μl) was added to a 200-μl aliquot of the supernatant fraction to raise the pH to 6 before injection onto the high-performance liquid chromatography (HPLC) column for analysis.

6α-OHGz, 6β-OHGz, 7β-OHGz, 7β-OHGz and MeOH-Gz formation was quantified by reversed phase HPLC using an Agilent 1100 series HPLC system (Agilent Technologies, Sydney, Australia), comprising a quaternary solvent delivery module with in-line degasser, autoinjector and variable-wavelength ultraviolet/visible detector. The HPLC was fitted with a Beckman ODS column (250 × 4 mm i.d., 5 μm particle size) (Beckman, Fullerton, CA, USA). The mobile phase consisted of an acetate buffer (5 mM, pH 4.3) containing 20% v/v acetonitrile (A) and acetonitrile (B) gradient delivered at a flow rate of 1.5 ml min^{-1}: initial conditions were 96% A:4% B held for 3 min, changing to 76% A:24% B over 7 min then held for 2 min, changing to 40% A:60% B over 1 min, which was held for 0.5 min before returning to starting conditions. Gz and its hydroxy metabolites were detected at 235 nm. Retention times for 6α-OHGz, 6β-OHGz, 7β-OHGz, 7β-OHGz, MeOH-Gz and Gz, determined by reference to authentic standards, were 8.1, 8.5, 9.2, 10.2 and 15.5 min, respectively. Concentrations of the individual metabolites present in incubations were determined by comparison of the peak areas with calibration curves constructed over the concentration range 0.2–10 μM for each metabolite. Calibration curves were linear with r^2 values >0.99. The lower limit of quantification was 0.045 μM (equivalent to rates of product formation of 0.50 pmol min^{-1} mg^{-1} for incubations with HLM as the enzyme source and 0.025 pmol min^{-1} per pmol CYP for incubations with recombinant CYP enzymes).

Incubation conditions were optimized to ensure linearity with respect to protein concentration and time; substrate depletion was <10%. Overall assay within-day precision was assessed at a single Gz concentration (400 μM) by measuring 6β-OHGz, 7β-OHGz and MeOH-Gz formation (see Results) in nine separate incubations with the same batch of HLM. The within-day coefficients of variation were 3.8, 3.2 and 4.1% for 6β-OHGz, 7β-OHGz and MeOH-Gz, respectively. Inhibition studies with CYP enzyme selective inhibitors were performed at a Gz concentration of 500 μM using pooled HLM (equal protein amounts from each liver). Concentrations of inhibitors used were: furafylline (10 μM, CYP1A2), coumarin (2.5 μM, CYP2A6), trimethoprim (100 μM, CYP2C8), sulfaphenazole (2.5 μM, CYP2C9) (S)-(+)-mephenytoin (100 μM, CYP2C19), quinidine (2.5 μM, CYP2D6) and troleandomycin (50 μM, CYP3A). The mechanism-based inhibitors furafylline and troleandomycin were preincubated with HLM for 10 min in the presence of NADPH generating system prior to the addition of Gz.

Kinetic constants for the various pathways of Gz hydroxylation were determined by fitting untransformed experimental data to the Michaelis–Menten equation using EnzFitter (Biosoft, Cambridge, UK), a nonlinear least squares fitting program. It should be noted that there was no deviation from hyperbolic kinetics for any of the hydroxylation pathways characterized. Data points represent the mean of duplicate determinations (<10% variance).

Results

Microsomes from all six human livers studied hydroxylated Gz. The principal metabolites detected were 6β-OHGz, 7β-OHGz and MeOH-Gz. A small peak corresponding to 6α-OHGz was observed only at the highest Gz concentration (750 μM), while 7α-OHGz was not detected across the substrate concentration range employed. Formation of hydroxylated Gz metabolites was not observed in the absence of NADPH generating system. 6β-OHGz, 7β-OHGz and MeOH-Gz formation exhibited Michaelis–Menten kinetics with microsomes from the six livers (Figure 2). It should be noted that the poor solubility of Gz limited the concentration range studied (50–750 μM) and all incubations contained 1% by volume acetonitrile to assist solubilization of substrate. Mean apparent K_m (± SD, with 95% CIs in parentheses) values for 6β-OHGz, 7β-OHGz and MeOH-Gz were 461 ± 139 μM (356–620 μM), 404 ± 143 μM (269–576 μM) and 334 ± 75 μM (270–422 μM), respectively. The mean V_{max} (± SD, with 95% CIs in parentheses) values for 6β-OHGz, 7β-OHGz and MeOH-Gz were 130 ± 55 pmol min^{-1} mg^{-1} (70–195 pmol min^{-1} mg^{-1}), 82 ± 31 pmol min^{-1} mg^{-1} (48–

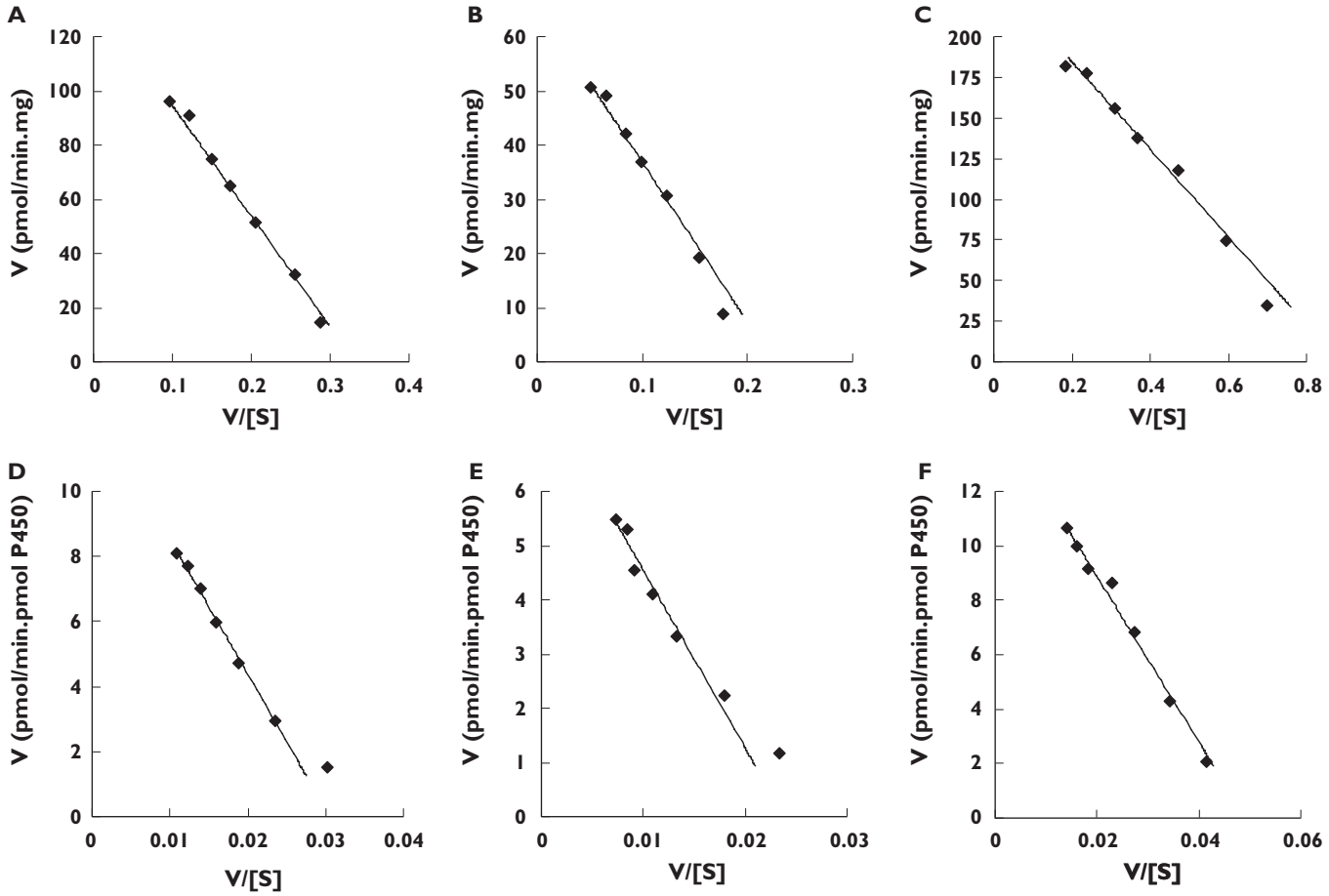

Figure 2

Eadie–Hofstee plots for gliclazide 6β-hydroxylation (A), 7β-hydroxylation (B) and tolylmethyl hydroxylation (C) by microsomes from liver H40, and gliclazide tolylmethyl hydroxylation by CYP2C9 (D), CYP2C18 (E) and CYP2C19 (F)

119 pmol min^{-1} mg^{-1}) and 268 ± 115 pmol min^{-1} mg^{-1} (135–369 pmol min^{-1} mg^{-1}), respectively. Based on intrinsic clearance (V_{max}/K_m) values, the predominant pathway is the formation of MeOH-Gz (through oxidation of the tolylmethyl group); the V_{max}/K_m ratio for MeOH-Gz (0.81 μl min^{-1} mg^{-1}) formation was 2.8 and 3.9 times higher than the V_{max}/K_m ratios for 6β-OHGz (0.29 μl min^{-1} mg^{-1}) and 7β-OHGz (0.21 μl min^{-1} mg^{-1}), respectively.

The effect of CYP selective inhibitors on the formation of Gz metabolites by HLM is shown in Table 1. Sulfaphenazole, a selective inhibitor of CYP2C9, decreased MeOH-Gz formation by approximately two-thirds and the 6β-OHGz and 7β-OHGz pathways by >80%. (S)-(+)-mephenytoin inhibited the OHMe-Gz pathway by 48%, but exhibited only 11 and 17% inhibition of 6β-OHGz and 7β-OHGz formation, respectively. Interestingly, activation of MeOH-Gz formation (by 16–27%) occurred in the presence of coumarin, trimethoprim and quinidine, although these compounds caused a comparatively minor reduction in the formation of 6β-OHGz and 7β-OHGz. Similarly, furafylline and troleandomycin had a minor effect on Gz hydroxylation.

A panel of recombinant P450s was screened to determine the capacity of the major drug-metabolizing CYP enzymes to catalyse Gz hydroxylation at a substrate concentration of 500 μM. Results are shown in Figure 3. All members of the CYP2C subfamily hydroxylated Gz. Of the remaining P450s, only CYP2D6 hydroxylated Gz (at the 6β position). Kinetic parameters were determined with recombinant CYP2C8, CYP2C9, CYP2C18 and CYP2C19 as the enzyme sources (Table 2 and Figure 2). MeOH-Gz formation was the predominant metabolic pathway for CYP2C9, CYP2C18 and CYP2C19. However, CYP2C8 formed only 6β-OHGz and 7β-OHGz. The largest differences in K_m occurred for 6β-hydroxylation, with a threefold difference between CYP2C8 and CYP2C19. Generally, however,

Table 1
Effect of CYP enzyme selective inhibitors on the hydroxylation of gliclazide by pooled human liver microsomes

Inhibitor and enzyme		Inhibitor concentration (μM)	Percent control activity 6β-OHGz	7β-OHGz	MeOH-Gz
Furafylline	(CYP1A2)	10	92	94	93
Coumarin	(CYP2A6)	2.5	88	81	116
Trimethoprim	(CYP2C8)	100	89	90	118
Sulfaphenazole	(CYP2C9)	2.5	13	17	36
(S)-(+)-mephenytoin	(CYP2C19)	100	89	83	52
Quinidine	(CYP2D6)	2.5	80	73	127
Troleandomycin	(CYP3A4)	50	93	93	83

Data represent mean of duplicate estimations using pooled human liver microsomes (see 'Kinetic and inhibitor studies').

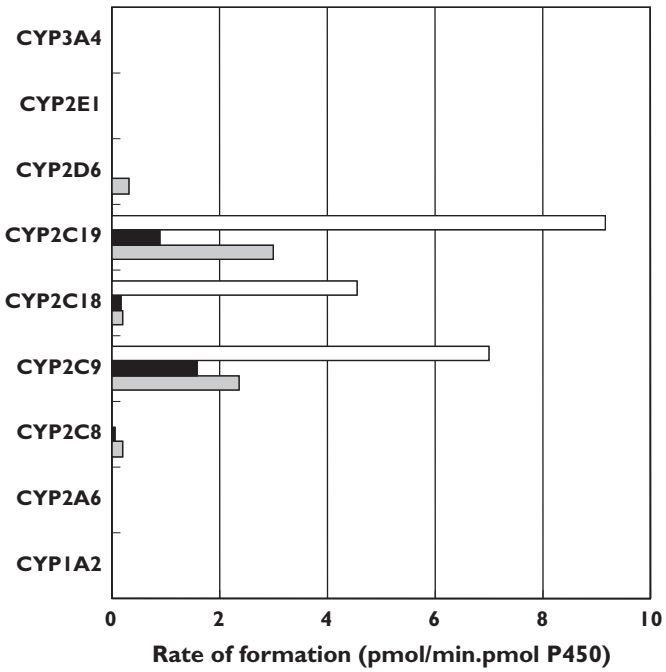

Figure 3
Formation of 6β-, 7β- and tolylmethyl- hydroxy gliclazide by recombinant human CYP enzymes (at a substrate concentration of 500 μM). MeOHGz (□); 7β-OHGz (■); 6β-OHGz (▩)

differences in intrinsic clearances (i.e. V_{max}/K_m) between and within metabolic pathways arose predominately from variability in V_{max}.

Discussion

Based on metabolite recovery data, tolylmethyl hydroxylation (to form MeOH-Gz) and subsequent oxidation to the corresponding aromatic carboxylic acid, is responsible for approximately 60% of Gz metabolic clearance in humans [4]. Hydroxylation in the azabicyclooctyl ring, at the 6α-, 6β-, 7α- and 7β- positions, accounts for the remainder of Gz metabolism *in vivo*, although 6α- and 7α- hydroxylation represent minor biotransformation pathways (≤6% of the recovered dose) [4]. Consistent with the *in vivo* observations, human liver microsomes formed MeOH-Gz, 6β-OHGz and 7β-OHGz. V_{max}/K_m ratios for the microsomal reactions paralleled relative urinary metabolite ratios *in vivo*.

Inhibition of the human liver microsomal metabolism of a compound by CYP enzyme selective inhibitors is considered to provide the most reliable indication of the relative contributions of P450s to a metabolic pathway [15]. The effect of the prototypic CYP2C9 inhibitor sulfaphenazole [6, 7] demonstrates that this enzyme is responsible for half to two-thirds of MeOH-Gz formation, while inhibition by (S)-(+)-mephenytoin indicates that CYP2C19 accounts for the remainder of tolylmethyl hydroxylation. Based on sulfaphenazole inhibition, CYP2C9 is responsible for >80% of Gz 6β- and 7β-hydroxylation. Activity screening and kinetic experiments with recombinant P450s support a major contribution of CYP2C9 and CYP2C19 to hepatic Gz tolylmethyl hydroxylation. Of the hepatically expressed enzymes (i.e. CYP2C8, CYP2C9 and CYP2C19), highest V_{max}/K_m values were observed with CYP2C9 and CYP2C19. In contrast to the 'gold standard' microsomal inhibition data, however, derived kinetic constants obtained for recombinant P450s suggest significant involvement of CYP2C19 in Gz 6β- and 7β- hydroxylation. Similar observations have been reported for chlorpropamide 2-hydroxylation [16]; comparable activities were obtained with recombinant CYP2C9 and

Table 2
Derived kinetic parameters for gliclazide hydroxylation by recombinant CYP2C enzymes

	6β-OHGz			7β-OHGz			MeOH-Gz			Pathway ratio*		
Enzyme	K_m	V_{max}	V_{max}/K_m	K_m	V_{max}	V_{max}/K_m	K_m	V_{max}	V_{max}/K_m	6β-OHGz	7β-OHGz	MeOH-Gz
CYP2C8	984	0.63	0.0006	346	0.06	0.0002	NA†	NA	NA	1.0	0.3	0
CYP2C9	471	4.5	0.0096	327	2.6	0.0080	415	13	0.0306	1.0	0.8	3.2
CYP2C18	794	0.53	0.0007	524	0.37	0.0007	328	7.8	0.0239	1.0	1.1	35.8
CYP2C19	321	5.0	0.0155	434	1.7	0.0039	304	15	0.0493	1.0	0.2	3.2

*Units: K_m, μM; V_{max}, pmol min^{-1} pmol CYP^{-1}; V_{max}/K_m, μl min^{-1} pmol CYP^{-1}. *Based on V_{max}/K_m ratios. †No measurable activity.*

CYP2C19 despite near complete inhibition of the human liver microsomal reaction by sulfaphenazole and the absence of an effect of *CYP2C19* genotype on chlorpropamide clearance and response *in vivo*. These examples highlight the difficulties associated with data interpretation when reaction phenotyping is conducted with recombinant P450s alone. However, K_m values for the various pathways of Gz hydroxylation by recombinant CYP2C enzymes were comparable in value, consistent with the monophasic kinetics observed for the human liver microsomal reactions.

CYP2C9 exhibits genetic polymorphism and the six livers used in this study were previously genotyped [17] for the most common variant alleles, namely *CYP2C9*2* and *CYP2C9*3*. None carried the Arg144Cys mutation associated with *CYP2C9*2* and one was heterozygous for *CYP2C9*3*. *CYP2C9*3* encodes a protein with leucine at residue 359, compared with isoleucine in the wild-type enzyme [17, 18]. The leucine-359 variant is associated with lower activity towards all known CYP2C9 substrates [2, 3, 17–20]. Consistent with a major involvement of CYP2C9 in the Gz hydroxylations, V_{max}/K_m values observed for 6β-OHGz, 7β-OHGz and MeOH-Gz formation by microsomes from the *CYP2C9*3* heterozygous liver were four- to fivefold lower than the mean V_{max}/K_m values for the corresponding pathways obtained using microsomes from the five *CYP2C9*1* homozygous livers. The predominant involvement of CYP2C9 in the Gz hydroxylations is also consistent with the known major contribution of this enzyme to the metabolism of other sulphonylureas, and a previous report linking MeOH-Gz formation to the tolbutamide hydroxylase activity of rat liver microsomes [21]. Reaction phenotyping studies *in vitro* and/or investigation of the effects of polymorphic variants on drug disposition *in vivo* indicate that CYP2C9 activity is the principal determinant of the metabolic clearance for tolbutamide [6, 7, 22, 23], chlorpropamide [16], glipizide [24], glimepiride [25] and glibenclamide [25–27]. Similarly, CYP2C9 is responsible for the tolylmethyl hydroxylation of the structurally related sulphonylurea torsemide [8, 28], used clinically as a diuretic. Although CYP2C19 has been implicated in tolbutamide and chlorpropamide hydroxylation *in vitro* [16, 29], phenotype- and genotype-based clinical studies exclude a significant contribution of this enzyme to drug elimination *in vivo* [26, 30]. However, the concordance between (S)-(+)-mephenytoin inhibition of human liver microsomal Gz tolylmethyl hydroxylation and MeOH-Gz formation by recombinant CYP2C19 observed here suggests a potentially significant role for CYP2C19 in Gz metabolic clearance, given that tolylmethyl hydroxylation represents the major elimination pathway *in vivo*. Although the contribution of CYP2B6 to Gz hydroxylation was not investigated here, this enzyme appears to have a minor role in drug metabolism and the sulfaphenazole and (S)-(+)-mephenytoin inhibition data preclude significant involvement of another P450 in hepatic Gz hydroxylation.

Regioselectivity was observed for the various pathways of Gz hydroxylation by the individual CYP2C enzymes. The relative formation of 6β- and 7β-OHGz (based on V_{max}/K_m ratios) differed between CYP2C9 and CYP2C19, while CYP2C18 preferentially formed MeOH-Gz. Unlike other CYP2C enzymes, CYP2C8 did not catalyse Gz tolylmethyl hydroxylation. Previous work has similarly shown an approximately 60-fold difference in torsemide tolylmethyl hydroxylation by CYP2C8 and CYP2C9 [28].

Of further interest was the observation that the V_{max}/K_m ratio for Gz tolylmethyl hydroxylation by CYP2C18 was similar to the ratios determined for CYP2C9 and CYP2C19. In contrast to CYP 2C8, 2C9 and 2C19, CYP2C18 appears to be expressed only to a

minor extent in liver [31, 32]. However, it is the most abundant CYP2C enzyme in skin and lung and is also found in brain, duodenum, kidney, mammary gland and uterus [33–35]. CYP2C18 has previously been shown to metabolize torsemide, phenytoin, diclofenac, tienilic acid and tolbutamide, other predominantly CYP2C9 substrates [13, 36–39]. Thus, there appears to be overlapping substrate selectivity between the two enzymes. At this stage the functional significance of CYP2C18-catalysed biotransformation in extrahepatic tissues remains unknown, but a role in phenytoin hypersensitivity has been postulated [13].

In summary, CYP2C9 is the principal enzyme involved in the 6β-, 7β- and tolylmethyl- hydroxylation of Gz. Numerous factors are known to affect CYP2C9 activity, including genetic polymorphism and drug–drug interactions [2, 19, 20], and these may contribute to dose variability and the development of adverse effects during therapy with sulphonylureas [3]. Indeed, an association between the incidence of severe hypoglycaemia and CYP2C9 poor metabolizer genotypes has been reported in patients receiving sulphonylureas [40]. CYP2C19, another polymorphic enzyme, may also contribute to Gz tolylmethyl hydroxylation, introducing an additional potential source of variability in Gz pharmacokinetics.

Competing interests: None declared.

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