European Journal of Clinical Investigation (2003) **33** (Suppl. 2), 23–30

Clinical relevance of genetic polymorphisms in the human *CYP2C9* gene

U. I. Schwarz

Institute of Clinical Pharmacology, Medical Faculty, Technical University, Dresden, Germany

Abstract

Cytochrome P450 (CYP) 2C9 hydroxylates about 16% of drugs in current clinical use. Of special interest are those with a narrow therapeutic index, such as *S*-warfarin, tolbutamide and phenytoin, where impairment in CYP2C9 metabolic activity might cause difficulties in dose adjustment as well as toxicity. Single-nucleotide polymorphisms (SNP) in the *CYP2C9* gene have increasingly been recognized as determinants of the metabolic phenotype that underlies interindividual and ethnic differences. Apart from the wild-type protein CYP2C9*1 at least five allelic variants produce allozymes with reduced or deficient metabolic activity. Among white populations only *CYP2C9*2* and *CYP2C9*3* variants are of significance, with allelic frequencies of 0·08–0·14 and 0·04–0·16, respectively. In contrast, in Africans (African-Americans and Ethiopians) and Asians both variants are much less frequent (0·005–0·04), and *CYP2C9*2* has not yet been detected in Asians. *CYP2C9*4* has been exclusively identified in Japanese patients, and *CYP2C9*5* and **6* were only found among African-Americans with a low allelic frequency of 0·017 and 0·006, respectively. Furthermore in Japanese a *CYP2C9* promotor variant of four linked SNPs was correlated with reduced intrinsic clearance of phenytoin *in vitro*. Subjects who are carriers of one or more variant alleles may be at risk for adverse drug reactions/toxicities when prescribed drugs extensively metabolized by CYP2C9.

Keywords CYP2C9, polymorphisms, warfarin, diclofenac, 5′-flanking region.
Eur J Clin Invest 2003; 33 (Suppl. 2): 23–30

Introduction

Genetically determined differences in drug-metabolizing enzyme activity can lead to a wide interindividual variability in drug response, resulting in altered efficacy or toxicity in the affected individuals [1]. For example, single-nucleotide polymorphisms in the cytochrome P450 (CYP) *2C9* gene produce differences in the metabolism of certain drug substrates that have increasingly been recognized to have clinically significant consequences.

CYP2C9 appears to be the major CYP2C isoform found in the human liver [2–4]. It is one of four known members of the CYP2C subfamily [5], including the CYP isoforms CYP2C8, CYP2C18 and CYP2C19, that share > 82% of amino acid identity [2]. Despite the high sequence similarity, the human CYP2C isoforms exhibit relatively little overlap of substrate specificity [2,4].

CYP2C9 hydroxylates a wide array of mostly acidic substrates in diverse therapeutic categories, about 16% of drugs in current clinical use, including the antidiabetic agents tolbutamide, glibenclamide, glimepiride and glipizide, the anticonvulsant phenytoin, the active *S*-enantiomer of the anticoagulants warfarin, acenocoumarol and phenprocoumon, the antihypertensive drugs losartan and irbesartan, the diuretic torasemide and numerous nonsteroidal anti-inflammatory drugs such as ibuprofen, diclofenac, piroxicam, tenoxicam, mefenamic acid and celecoxib [2,6–14]. Of special interest are drugs with a narrow therapeutic index, such as *S*-warfarin, tolbutamide and phenytoin, because impairment in CYP2C9 metabolic activity might cause difficulties in dose adjustment as well as toxicity [15,16].

Correspondence to: Ute I. Schwarz, MD, Institute of Clinical Pharmacology, Medical Faculty, Technical University, Fiedlerstrasse 27, 01307 Dresden, Germany. Tel: +49-351-458-2823; fax: +49-351-458-4341; e-mail: uschwarz@rcs.urz.tu-dresden.de

CYP2C9 gene structure and allelic variants

The *CYP2C9* gene consists of nine exons encoding a protein of 490 amino acids [2]. Allelic variants are caused by single nucleotide exchanges leading to nonsynonymous amino acid substitutions, differing at only a few residues in the coding region. In addition to the wild-type protein CYP2C9*1 (Arg144, Ile359, Asp360), four allelic variants are known to produce allozymes with reduced intrinsic metabolic activity (see Fig. 1): CYP2C9*2 (Cys144, Ile359, Asp360),

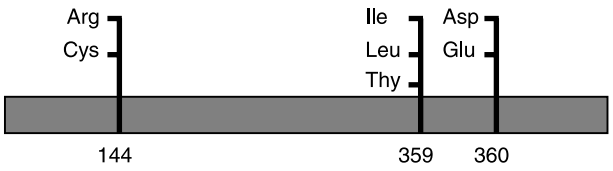

Figure 1 A schematic of sites of polymorphism in the CYP2C9 protein.

CYP2C9*3 (Arg144, Leu359, Asp360), CYP2C9*4 (Arg144, Thr359, Asp360) and CYP2C9*5 (Arg144, Ile359, Glu360) [17–21]. More recently a single basepair deletion polymorphism *CYP2C9*6* (818*del*A) resulting in inactive CYP2C9 protein has been described [22]. An excellent review on *CYP2C9* polymorphisms, including a detailed figure of their location, has recently been published [13].

Sequencing studies indicate that only *CYP2C9*2* and *CYP2C9*3* variants are of particular importance among white populations, where the allelic frequencies are in the range of 0·08–0·14 and 0·04–0·16, respectively, and about 0·5%–2·5% of such individuals are homozygous for the *CYP2C9*2* and *3 allele or heterozygous for the *CYP2C9*2/*3* allele [21,23–28]. In contrast, in Africans (African-Americans and Ethiopians) and Asians both variants are much less frequent (0·005–0·04), and *CYP2C9*2* has not been detected yet in Asians [21,24,26,29,30]. Another new variant, *CYP2C9*4*, has been exclusively identified in Japanese epileptic patients [31], and the recently discovered novel allele *CYP2C9*5* was only found among African-Americans with a low allelic frequency of 0·017 [21]. *CYP2C9*6* was also only detected in African-Americans with an allelic frequency of 0·006. The results of population prevalence according to ethnicity of the *CYP2C9* genotype including the *2, *3 and *5 alleles from the largest genotyping studies are summarized in Table 1.

In addition, genetic polymorphisms have been identified in the 5′-flanking region of *CYP2C9* in Japanese individuals. Two different haplotypes, TCGG (wild-type) and CGAA (variant allele), were observed. The allelic pattern of the four linked single-nucleotide polymorphisms (Pattern 2: T1912C, C1886G, G1538A, G982A) was associated with a reduced intrinsic clearance *in vitro* compared with wild-type pattern 1 without those mutations [32]. Interestingly, the promotor variant CGAA was strongly associated with the *CYP2C9*3* allele in exon 7, and this linkage has been finally shown to be the most important haplotype determining the CYP2C9 metabolic capacity among the six observed sequence patterns of the 5′-flanking region.

Substrate specificity

Three of the variants, *CYP2C9*3*, *CYP2C9*4* and *CYP2C9*5* are located in one of six substrate recognition sites (SRS) defined for the *CYP2C9* gene based on a sequence alignment with the bacterial enzyme CYP101 of known crystal structure (SRS 5 spans residues 359–369) [33]. These SRS contribute to the enzyme active site and show more nonsynonymous substitutions than other regions of the CYP2C9 protein.

From studies with site-directed mutagenesis it is apparent that substitution of a few critical residues within such recognition sites is a major determinant of substrate specificity, regioselectivity of hydroxylation and catalytic activity of the

Table 1 Population distribution of CYP2C9 genetic polymorphisms (*2, *3 and *5 allele) according to ethnicity

Ethnicity	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3	*1/*5	*n*	Reference
Caucasian								
American	22·1	8·6	2·1	0	0	0	140	Dickmann *et al.* [21]
British	19·1	9·4	0·5	1·1	0	–	561	Taube *et al.* [45]
	19·0	15·0	3·0	0	1	–	100	Stubbins *et al.* [23]
Israeli	17·9	12·8	0	1·3	0	–	156	Loebstein *et al.* [28]
Italian	15·3	14·0	2·5	1·9	1·3	–	157	Scordo *et al.* [26]
Spanish	15·9	23·5	1·9	8·9	0	–	157	Garcia-Martin *et al.* [27]
Swedish	18·6	11·6	0·5	1·9	0·7	–	430	Yasar *et al.* [25]
Turkish	18·0	17·2	1·0	1·1	0·8	–	499	Aynacioglu *et al.* [83]
African								
African-American	5·0	2·5	0	0	0	3·3	120	Dickmann *et al.* [21]
	2·0	1·0	0	0	0	–	100	Sullivan-Klose *et al.* [24]
Ethiopian	8·7	4·6	0	0	0	–	150	Scordo *et al.* [26]
Asian								
Taiwanese	0	8·2	0	0	0	–	98	Sullivan-Klose *et al.* [24]
Japanese	0	4·1	0	0	0	–		Nasu *et al.* [30]
Chinese	0	3·5	0	0	0	–	115	Wang *et al.* [29]
Korean	0	2·3	0	0	0	–	574	Yoon *et al.* [84]

– Polymorphism not investigated.

Population prevalence of *CYP2C9* genotype as a percentage of total individuals analysed in the study (*n*).

enzyme [34]. Moreover, the first crystallographic structure shown for a mammalian P450 2C5 provides a concordant structural basis for the observed effects of mutations within those predicted SRS regions on enzyme activity in the CYP2 family [35].

In a recent study, recombinant CYP2C9*3 exhibited a reduced intrinsic clearance (V_{max}/K_m) for the seven studied CYP2C9 substrates compared with the wild-type enzyme [36]. A higher K_m value without a change of the V_{max} values was found for tolbutamide methylhydroxylation, *S*-warfarin hydroxylation, torasemide metabolism and diclofenac 4′ hydroxylation, whereas for the hydroxylation of piroxicam, tenoxicam and mefenamic acid only lower V_{max} values were revealed. A similar observation was made for the *CYP2C9*5* polymorphism using a baculovirus expression system, where a 6-fold to 31-fold lower intrinsic clearance for the hydroxylation of diclofenac and *S*-warfarin was observed, respectively, mainly through an increase of K_m values [21]. However, the *CYP2C9*3*-mediated intrinsic clearance reduction for *S*-warfarin hydroxylation was associated with both an increase in K_m and a decrease in V_{max}. Thus, the effect caused by expression of the different *CYP2C9* variants appears to be the result of either altered affinity of the enzyme for the substrate and/or an affected V_{max} with a variable magnitude in impairment of oxidative metabolism that may be substrate dependent. Finally, differences among allozymes of any CYP isoform may variably contribute to the recognition and binding of discrete classes of substrates [37].

Drug substrates of CYP2C9

Oral anticoagulants

Warfarin

More than 80% of the pharmacologically more active *S*-enantiomer of warfarin is eliminated by CYP catalysed biotransformation to 6- and 7-hydroxy *S*-warfarin, whereas CYP2C9 is the main cytochrome P450 responsible for the 6- and 7-hydroxylation pathways [38–40]. Thus, the responsible enzyme activity regulates the steady-state plasma concentration of *S*-warfarin and becomes a critical factor in anticoagulant therapy [41–43].

Kinetic studies with the expressed CYP2C9 variants indicate a significant impairment of *S*-warfarin hydroxylase capacity: the allozymes CYP2C9*2 and CYP2C9*3 showed an approximately 5-fold and 25-fold lower intrinsic clearance (V_{max}/K_{max}) compared with the wild-type gene product, respectively [17,18]. The recently described *CYP2C9*4* allele in Japanese epileptic patients has not been studied yet with respect to warfarin metabolism, but significantly reduced enzyme activity for diclofenac hydroxylation *in vitro* has been demonstrated [20]. The intrinsic clearance for the CYP2C9*5 allozyme found in African-Americans was shown to be 31-fold lower for 7-hydroxylation of *S*-warfarin compared with the wild-type [21].

In vivo, a homozygous individual for the *CYP2C9*3* allele was found to have a reduction in clearance of *S*-warfarin and an exacerbated response to warfarin, thus the *CYP2C9*3/*3* genotype was clearly shown to be linked to warfarin sensitivity [15]. A strong association has been demonstrated between the presence of one or more *CYP2C9*2* and *CYP2C9*3* variant alleles and low-dose requirements for warfarin in a large number of published studies, whereas in most cases the dose is lowest if *CYP2C9*3* is present [28,44–46]. Moreover, the higher sensitivity for warfarin in two studies was correlated with a higher incidence of major bleeding complications [44,46]. A recent study also demonstrated a clear relationship between *S*-warfarin clearance and *CYP2C9* genotype [47]. Therefore, there seems to be a reasonable correlation between the observed *in vitro* activities of these variant forms of CYP2C9 and the *in vivo* consequences for metabolic clearance of warfarin. In addition to the *CYP2C9* genotype, age and environmental factors were also discussed to be a possible cause for a decline in warfarin daily dose [28]. However, all published studies to date have been retrospective, therefore prospective case-control and cohort studies with a large patient number may provide additional information on the value of *CYP2C9* genotyping to warfarin treatment and safety.

Acenocoumarol and phenprocoumon

The main alternatives to warfarin, the coumarinic derivatives acenocoumarol and phenprocoumon, are widely used in certain European countries [12]. The chemical structure of these racemates is closely related to warfarin, and both *S*-Enantiomers are substrates of CYP2C9 [48,49]. Besides CYP2C19 and CYP1A2, a role of CYP2C9 has also been demonstrated for the more active *R*-acenocoumarol [48]. In the case of acenocoumarol there is increasing clinical evidence that the *CYP2C9*3* allele only is related to a low-dose requirement for this drug, a higher frequency of over-anticoagulation and an unstable anticoagulant response [50–52], and even one copy of *CYP2C9*3* might profoundly reduce the oral drug clearance [53]. In the case of phenprocoumon no relationship of dosage and *CYP2C9* genotype has been established yet. A more complex situation needs to be assumed because it is a potent inhibitor of its own metabolism [49].

Although there is accumulating evidence on the relationship between the *CYP2C9* genotype and dose requirements, an individual's anticoagulant dose will likely depend on a complex interaction between genetic and environmental factors. Genotyping for the more common *CYP2C9* alleles may therefore be an aid to successful anticoagulation and the extent of clinical monitoring needed [12].

Oral antidiabetics

Tolbutamide

In humans tolbutamide is metabolized almost exclusively by methylhydroxylation that accounts for 85% of tolbutamide clearance; the initial and rate-limiting step [54,55]. There is evidence both *in vitro* and *in vivo* that CYP2C9 solely mediates the tolbutamide hydroxylation, and the antidiabetic

agent is therefore accepted widely as a prototypic substrate for the assessment of hepatic CYP2C9 activity [37,56].

A poor metabolizer phenotype in the metabolism of tolbutamide and a possible genetic regulation was first reported in 1979 [57]. Pharmacokinetics of tolbutamide in 50 nondiabetic subjects, including twins, showed an almost 9-fold variation of the elimination rate constant; the corresponding range of half-lives was 2·9–25·0 h. According to these data about 30% of the population was estimated to be a 'poor metabolizer'; however, the true genotypes had not yet been defined. In a later *in vivo* study genotyping of an individual with a tolbutamide half-life of 37 h confirmed homozygosity for the rare *CYP2C9*3* allele that carries the Ile359Leu mutation [58], and was subsequently identified to cause this phenotype [24,59]. The expressed recombinant CYP2C9*3 had been shown to exhibit lower intrinsic clearance (V_{max}/K_m) for tolbutamide methylhydroxylation than did the wild-type, caused by a higher K_m value without a change of the V_{max} values. It was proposed that the tolbutamide poor metabolizer status could be attributed to homozygosity for the *CYP2C9*3* allele.

In a recent study the relationship between *CYP2C9* genotype and tolbutamide plasma clearance (CL/F) phenotype in 23 healthy subjects expressing all six *CYP2C9* allele combinations was reported [60]. Tolbutamide oral clearances were: 0·97 (**1/*1*), 0·88 (**1/*2*), 0·75 (**2/*2*), 0·56 (**1/*3*), 0·45 (**2/*3*) and 0·16 (**3/*3*) L h^{-1}. On the basis of this genotype–phenotype analysis the authors proposed a classification of tolbutamide extensive (*CYP2C9* genotypes **1/*1*; **1/*2*; **2/*2*), intermediate (**1/*3*; **2/*3*) and slow metabolizer (**3/*3*) phenotypes. According to this classification, intermediate and slow metabolizers may be predicted to comprise approximately 12% and 1%, respectively. Consistent with those data are two recently published studies: in Korean subjects genotyped for *CYP2C9* a 2-fold higher area under the concentration–time curve (AUC) of tolbutamide for *CYP2C9*1/*3* heterozygotes compared with subjects homozygous for the wild-type allele was reported [61], and in 15 Caucasian subjects (*CYP2C9*1* heterozygotes and wild-type subjects, each group $n = 5$) a 1·5-fold and 1·9-fold increase in tolbutamide AUC as well as a 29% and 48% reduction of tolbutamide clearance were revealed, respectively, in subjects expressing **1/*2* and **1/*3* genotypes vs. **1/*1* carriers [62]. However, the clinical consequences of *CYP2C9* polymorphisms for the treatment with oral hypoglycaemic agents such as tolbutamide, remain unclear [63]. To evaluate the pharmacodynamic effect nondiabetic healthy subjects were monitored for blood/serum glucose (and plasma insulin [60]) following tolbutamide administration (500 mg, p.o.) with or without a challenge with glucose or dextrose in the three prospective studies mentioned above [60–62]. No relationship of glucose or insulin concentrations and *CYP2C9* genotype was reported by Lee *et al.* and Kirchheiner *et al.* [60,62], moreover, hypoglycaemia has not been observed, even without additional carbohydrate administration after tolbutamide. In contrast, in Koreans the enhancement in serum glucose increase relative to baseline was significantly lower in *CYP2C9*1/*3* heterozygotes compared with homozygotes for the wild-type allele [61]. Further data are necessary to support the approach of dosage adjustment on the basis of genotype in diabetic patients, and pharmacodynamic monitoring remains the rational option for tolbutamide treatment [63].

Glibenclamide and glimepiride

Genetic polymorphisms of *CYP2C9* affected pharmacokinetics of the two sulfonylurea antidiabetic drugs glibenclamide and glimepiride in healthy volunteers [6,7]. In individuals heterozygous for the *CYP2C9*3* allele the glibenclamide *AUC* was 280% higher [7], and in *CYP2C9*3* homozygotes the oral clearance was reduced by more than 50% in relation to the wild-type carriers [6]. Furthermore, in heterozygotes for *CYP2C9*3* the glimepiride AUC was also significantly increased by 267% of the respective values in subjects with the **1/*1* genotype [7]. In both studies blood glucose responses to glibenclamide and glimepiride were not significantly affected, whereas the insulin secretion after glibenclamide ingestion was higher in subjects with the **3/*3* genotype compared with the other genotypes [6].

Anticonvulsants

Phenytoin

The major pathway of phenytoin metabolism is 4′ hydroxylation, which accounts for approximately 80%–90% of the drug elimination and is mainly mediated by CYP2C9 according to *in vitro* and *in vivo* studies [64,65]. There is evidence that the CYP2C9 activity is rate-limiting in phenytoin metabolic clearance [37].

Site-directed mutagenesis revealed that phenytoin hydroxylase activity of CYP2C9*2 and *3 variants was lower compared with the wild-type enzyme [66]. *In vivo*, a modest decrease in the maximal rate of phenytoin elimination (~30%) was observed in six Japanese epileptic patients heterozygous for *CYP2C9*3* compared with the 38 patients homozygous for the *CYP2C9*1* allele [67]. Furthermore, pharmacokinetics of phenytoin in an individual homozygous for the *CYP2C9*3* allele showed a more than 4-fold increase in *AUC* and a 21% decrease in phenytoin clearance compared with 24 healthy subjects genotyped as wild-type [10]. Genotyping data indicates that about 4%–16% of Caucasians are heterozygous and less than 1% homozygous for the *CYP2C9*3* allele [24], in agreement with the estimated frequencies of phenytoin and tolbutamide poor metabolizer phenotypes [68,69]. Nonetheless, because phenytoin is metabolized almost exclusively by CYP2C9, this rare polymorphism can lead to toxicity and difficult dosage adjustment of phenytoin in the affected individuals. Indeed, in a recent case report concerning a heterozygous *CYP2C9*3* allele carrier, excessive phenytoin concentrations were described despite the modest daily dose taken, leading to signs of central nervous system intoxication [16]. Furthermore, a strong association between *CYP2C9* genotype and phenytoin maintenance dose requirement was shown in 60 epileptic patients on long-term phenytoin

therapy [70]. Those patients, having at least one variant *CYP2C9* allele **2* and **3*, required a significant lower dose of phenytoin than wild-type individuals to reach an effective serum concentration; there were no patients homozygous for the *CYP2C9*3* allele present in this study. Finally, the recently identified loss of function mutation in *CYP2C9* (**6*) was associated with a marked increase in half-life and clinical toxicity to phenytoin in a subject homozygous for this polymorphism [22].

Since phenytoin has a narrow therapeutic index, dosage adjustment based on *CYP2C9* genotype, especially at the beginning of therapy, would be of value in order to lower the risk of concentration-dependent drug intoxications in variant carriers.

Nonsteroidal anti-inflammatory drugs

Diclofenac and ibuprofen

Diclofenac 4′-hydroxylation is mainly mediated by CYP2C9 *in vitro* [71]. The *in vitro* effects of *CYP2C9* variant alleles on the extent of the reduction in 4′-hydroxylation were less pronounced than those observed with other CYP2C9 substrates such as warfarin, tolbutamide and phenytoin [36]. Moreover, the absence of clinically significant effects in the CYP2C9-mediated metabolism of diclofenac has been reported in several *in vivo* studies with a lack of pharmacokinetic as well as pharmacodynamic changes. A prospective evaluation of diclofenac pharmacokinetics in 12 subjects revealed no significant differences between *CYP2C9*3* heterozygotes and wild-type subjects regarding any parameter after a single 50 mg oral diclofenac dose [72]. Three similar investigations in healthy volunteers with all combinations of the *CYP2C9* variants **2* and **3* confirmed the lack of genotypic influence on diclofenac disposition [73–75]. In additon, Kirchheiner *et al.* also investigated the formation of thromboxane B2 and prostaglandin E2, reflecting constitutively expressed cyclooxygenase 1 (COX-1) and inducible cyclooxygenase 2 (COX-2) activity, respectively, as a surrogate parameter for pharmacodynamics of diclofenac [75]. Marked diclofenac-mediated inhibition of COX-1 and COX-2 activity was detected to be independent of *CYP2C9* genotype.

The widely used nonsteroidal anti-inflammatory drug ibuprofen is another nonselective inhibitor of COX-1 and COX-2. *In vitro* data indicated that CYP2C9 might be the major *S*-ibuprofen hydroxylase, whereas *R*-ibuprofen appeared to be metabolized by CYP2C8 [71,76]. One recent study investigated the impact of *CYP2C9* polymorphisms in 21 healthy volunteers on ibuprofen pharmacokinetics and inhibition of COX-1 and COX-2 following an oral dose of 600 mg ibuprofen [77]. *S*-ibuprofen oral clearance was associated with the *CYP2C9* genotype: 3·25, 2·38 and 1·52 L h^{-1} in carriers of *CYP2C9 *1/*1*, **1/*3* and **3/*3*, respectively, whereas the *CYP2C9* variant **2* had no significant effect. Moreover, in carriers of the *CYP2C9*3* allele pharmacodynamics of ibuprofen measured as the inhibition of thromboxane B2 as well as prostaglandin E2, were strongly dependent on the genotype.

Celecoxib

Methyl hydroxylation of celecoxib, a novel COX-2 inhibitor, has been shown to be primarily catalyzed by human liver microsomal CYP2C9 *in vitro* (approximately 72%–92%), although CYP3A4 also plays a role (approximately 0%–27%) [14]. Benner *et al.* reported no association between the *CYP2C9* variants **2* and **3* and the celecoxib steady-state disposition after 400 mg oral celecoxib daily for 15 days in 25 healthy subjects [74].

This lack of genotypic influence on celecoxib and diclofenac disposition in contrast to ibuprofen might demonstrate substrate-specific effects, which need further investigation.

Antihypertensive drugs

Losartan

The selective angiotensin II receptor antagonist losartan undergoes primarily CYP2C9-mediated oxidative metabolism to its active metabolite E3174 [78], whereas CYP3A4 also appears to have a limited role in this metabolic reaction.

An identified losartan poor metabolizer converted less than 1% of the administered losartan dose to the active metabolite E3174, and genotyping subsequently revealed a subject with homozygous *CYP2C9*3* allele [79]. This individual also demonstrated reduced metabolite levels of tolbutamide and phenytoin and a normal erythromycin breath test, revealing functionally deficient CYP2C9 and normal CYP3A4 activity. These findings confirmed the predominant role of CYP2C9 in the conversion of losartan to E3174, moreover providing evidence regarding the potential clinical implications of the *CYP2C9* polymorphisms in losartan metabolism. More recently, one study investigated the pharmacokinetics of losartan in relation to the *CYP2C9* genotypes on healthy volunteers [9]. The authors report a 30-fold, 3-fold and 2-fold increase of the ratio of losartan *AUC* to the metabolite E3174 *AUC* in subjects with the *CYP2C9*3/*3*, **2/*3* and **1/*3* genotype, respectively. Furthermore, the *CYP2C9*3* allele was shown to be associated with a decreased formation of the metabolite in plasma and urine. However, the influence of *CYP2C9* genotypes on clinical use of losartan is not known yet.

Irbesartan

There is *in vitro* evidence that CYP2C9 also plays a major role in the metabolism of another selective angiotensin II receptor antagonist irbesartan [80,81]. *In vivo* effects of *CYP2C9* polymorphisms on pharmacokinetics and the clinical use of irbesartan are currently under investigation [13]. In an *in vivo* study a diastolic blood pressure (DBP) response in 49 patients with essential hypertension given irbesartan for 12 weeks in relation to the *CYP2C9* genotype was described: 7·5% and 14·4% reduction in DBP in patients with *CYP2C9*1/*1* ($n = 33$) and **1/*2* ($n = 12$) compared with baseline, respectively [82]. However, DBP in the three patients heterozygous for the **3* allele differed not markedly from baseline (approximately 1·8% difference).

Other substrates

Limited, preliminary data describing the potential effects of *CYP2C9* genotype on the oxidative metabolism of other substrates *in vivo* have also been reported. After a single 10 mg glipizide dose, a single healthy subject homozygous for *CYP2C9*3* was reported to have a 2-fold increase in half-life, a 5·5-fold reduction of oral clearance and a 5·5-fold increase in the *AUC* as well as profound symptoms of hypoglycaemia compared with 23 control individuals [10]. In addition to the data reported with tolbutamide, these findings suggest a potentially significant effect of the *CYP2C9*3* variant on the pharmacokinetics and serious adverse event rate of oral hypoglycaemic drugs that are substrates of CYP2C9. For the diuretic torasemide, *in vivo* effects of the *CYP2C9* genotype on pharmacokinetics and clinical use are not known yet but are currently under investigation.

Conclusion

CYP2C9 ranks among the most important drug metabolizing CYP isoforms present in the human liver. It hydroxylates a wide array of clinically used drugs. Modulation of CYP2C9 activity by genetic factors, stimulatory and inhibitory interactions and age effects, cause a wide interindividual variability for elimination and/or dosage requirement. Of special interest are compounds with a narrow therapeutic index, such as warfarin, tolbutamide and phenytoin, and individualization of dose is clearly necessary. Genetic polymorphisms, especially the homozygous appearance of the variant allele *CYP2C9*3*, can lead to a marked impairment in CYP2C9 metabolic activity in the affected individuals, and is likely to be associated with effects on efficacy, dosage adjustment and severe toxicity. Furthermore, differences in incidence of variant alleles among various ethnic populations may contribute to the outcome and risks to certain drug therapies.

Acknowledgements

This work was supported in part by United States Public Health Service Grant GM31304.

References

1 Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science* 1999;**286**:487–91.
2 Goldstein JA, de Morais SM. Biochemistry and molecular biology of the human CYP2C subfamily. *Pharmacogenetics* 1994;**4**:285–99.
3 Richardson TH, Griffin KJ, Jung F, Raucy JL, Johnson EF. Targeted antipeptide antibodies to cytochrome P450 2C18 based on epitope mapping of an inhibitory monoclonal antibody to P450 2C51. *Arch Biochem Biophys* 1997;**338**:157–64.
4 Wrighton SA, Stevens JC. The human hepatic cytochromes P450 involved in drug metabolism. *Crit Rev Toxicol* 1992;**22**:1–21.
5 Ged C, Umbenhauer DR, Bellew TM, Bork RW, Srivastava PK, Shinriki N *et al.* Characterization of cDNAs, mRNAs, and proteins related to human liver microsomal cytochrome P-450 (S)-mephenytoin 4′-hydroxylase. *Biochemistry* 1988;**27**:6929–40.
6 Kirchheiner J, Brockmoller J, Meineke I, Bauer S, Rohde W, Meisel C *et al.* Impact of CYP2C9 amino acid polymorphisms on glyburide kinetics and on the insulin and glucose response in healthy volunteers. *Clin Pharmacol Ther* 2002;**71**:286–96.
7 Niemi M, Cascorbi I, Timm R, Kroemer HK, Neuvonen PJ, Kivisto KT. Glyburide and glimepiride pharmacokinetics in subjects with different CYP2C9 genotypes. *Clin Pharmacol Ther* 2002;**72**:326–32.
8 Lewis DF. The CYP2 family: models, mutants and interactions. *Xenobiotica* 1998;**28**:617–61.
9 Yasar U, Forslund-Bergengren C, Tybring G, Dorado P, Llerena A, Sjoqvist F *et al.* Pharmacokinetics of losartan and its metabolite E-3174 in relation to the CYP2C9 genotype. *Clin Pharmacol Ther* 2002;**71**:89–98.
10 Kidd RS, Straughn AB, Meyer MC, Blaisdell J, Goldstein JA, Dalton JT. Pharmacokinetics of chlorpheniramine, phenytoin, glipizide and nifedipine in an individual homozygous for the CYP2C9*3 allele. *Pharmacogenetics* 1999;**9**:71–80.
11 Miners JO, Coulter S, Birkett DJ, Goldstein JA. Torsemide metabolism by CYP2C9 variants and other human CYP2C subfamily enzymes. *Pharmacogenetics* 2000;**10**:267–70.
12 Daly AK, King BP. Pharmacogenetics of oral anticoagulants. *Pharmacogenetics* 2003;**13**:247–52.
13 Lee CR, Goldstein JA, Pieper JA. Cytochrome P450 2C9 polymorphisms: a comprehensive review of the in-vitro and human data. *Pharmacogenetics* 2002;**12**:251–63.
14 Tang C, Shou M, Mei Q, Rushmore TH, Rodrigues AD. Major role of human liver microsomal cytochrome P450 2C9 (CYP2C9) in the oxidative metabolism of celecoxib, a novel cyclooxygenase-II inhibitor. *J Pharmacol Exp Ther* 2000;**293**:453–9.
15 Steward DJ, Haining RL, Henne KR, Davis G, Rushmore TH, Trager WF *et al.* Genetic association between sensitivity to warfarin and expression of CYP2C9*3. *Pharmacogenetics* 1997;**7**:361–7.
16 Ninomiya H, Mamiya K, Matsuo S, Ieiri I, Higuchi S, Tashiro N. Genetic polymorphism of the CYP2C subfamily and excessive serum phenytoin concentration with central nervous system intoxication. *Ther Drug Monit* 2000;**22**:230–2.
17 Rettie AE, Wienkers LC, Gonzalez FJ, Trager WF, Korzekwa KR. Impaired (S)-warfarin metabolism catalysed by the R144C allelic variant of CYP2C9. *Pharmacogenetics* 1994;**4**:39–42.
18 Haining RL, Hunter AP, Veronese ME, Trager WF, Rettie AE. Allelic variants of human cytochrome P450 2C9. baculovirus-mediated expression, purification, structural characterization, substrate stereoselectivity, and prochiral selectivity of the wild-type and I359L mutant forms. *Arch Biochem Biophys* 1996;**333**:447–58.
19 Crespi CL, Miller VP. The R144C change in the CYP2C9*2 allele alters interaction of the cytochrome P450 with NADPH. cytochrome P450 oxidoreductase. *Pharmacogenetics* 1997;**7**:203–10.
20 Ieiri I, Tainaka H, Morita T, Hadama A, Mamiya K, Hayashibara M *et al.* Catalytic activity of three variants (Ile, Leu, and Thr) at amino acid residue 359 in human CYP2C9

gene and simultaneous detection using single-strand conformation polymorphism analysis. *Ther Drug Monit* 2000;**22**:237–44.
21 Dickmann LJ, Rettie AE, Kneller MB, Kim RB, Wood AJJ, Stein M *et al.* Identification and functional characterization of a new CYP2C9 variant (CYP2C9*5) selectively expressed among African Americans. *Mol Pharm* 2001;**60**:317–23.
22 Kidd RS, Curry TB, Gallagher S, Edeki T, Blaisdell J, Goldstein JA. Identification of a null allele of CYP2C9 in an African-American exhibiting toxicity to phenytoin. *Pharmacogenetics* 2001;**11**:803–8.
23 Stubbins MJ, Harries LW, Smith G, Tarbit MH, Wolf CR. Genetic analysis of the human cytochrome P450 CYP2C9 locus. *Pharmacogenetics* 1996;**6**:429–39.
24 Sullivan-Klose TH, Ghanayem BI, Bell DA, Zhang ZY, Kaminsky LS, Shenfield GM *et al.* The role of the CYP2C9-Leu359 allelic variant in the tolbutamide polymorphism. *Pharmacogenetics* 1996;**6**:341–9.
25 Yasar M, Eliasson E, Dahl ML, Johansson I, Ingelman-Sundberg M, Sjoqvist F. Validation of methods for CYP2C9 genotyping: frequencies of mutant alleles in a Swedish population. *Biochem Biophys Res Commun* 1999;**254**:628–31.
26 Scordo MG, Aklillu E, Yasar U, Dahl ML, Spina E, Ingelman-Sundberg M. Genetic polymorphism of cytochrome P450 2C9 in a Caucasian and a black African population. *Br J Clin Pharmacol* 2001;**52**:447–50.
27 Garcia-Martin E, Martinez C, Ladero JM, Gamito FJ, Agundez JA. High frequency of mutations related to impaired CYP2C9 metabolism in a Caucasian population. *Eur J Clin Pharmacol* 2001;**57**:47–9.
28 Loebstein R, Yonath H, Peleg D, Almog S, Rotenberg M, Lubetsky A *et al.* Interindividual variability in sensitivity to warfarin – Nature or nurture? *Clin Pharmacol Ther* 2001;**70**:159–64.
29 Wang SL, Huang J, Lai MD, Tsai JJ. Detection of CYP2C9 polymorphism based on the polymerase chain reaction in Chinese. *Pharmacogenetics* 1995;**5**:37–42.
30 Nasu K, Kubota T, Ishizaki T. Genetic analysis of CYP2C9 polymorphism in a Japanese population. *Pharmacogenetics* 1997;**7**:405–9.
31 Imai J, Ieiri I, Mamiya K, Miyahara S, Furuumi H, Nanba E *et al.* Polymorphism of the cytochrome P450 (CYP) 2C9 gene in Japanese epileptic patients: genetic analysis of the CYP2C9 locus. *Pharmacogenetics* 2000;**10**:85–9.
32 Shintani M, Ieiri I, Inoue K, Mamiya K, Ninomiya H, Tashiro N *et al.* Genetic polymorphisms and functional characterization of the 5′-flanking region of the human CYP2C9 gene: in vitro and in vivo studies. *Clin Pharmacol Ther* 2001;**70**:175–82.
33 Gotoh O. Substrate recognition sites in cytochrome P450 family 2 (CYP2) proteins inferred from comparative analyses of amino acid and coding nucleotide sequences. *J Biol Chem* 1992;**267**:83–90.
34 Negishi M, Iwasaki M, Juvonen RO, Sueyoshi T, Darden TA, Pedersen LG. Structural flexibility and functional versatility of cytochrome P450 and rapid evolution. *Mutat Res* 1996;**350**:43–50.
35 Williams PA, Cosme J, Sridhar V, Johnson EF, McRee DE. Mammalian microsomal cytochrome P450 monooxygenase: structural adaptations for membrane binding and functional diversity. *Mol Cell* 2000;**5**:121–31.
36 Takanashi K, Tainaka H, Kobayashi K, Yasumori T, Hosakawa M, Chiba K. CYP2C9 Ile359 and Leu359 variants: enzyme kinetic study with seven substrates. *Pharmacogenetics* 2000;**10**:95–104.
37 Miners JO, Birkett DJ. Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. *Br J Clin Pharmacol* 1998;**45**:525–38.
38 Kaminsky LS, Zhang ZY. Human P450 metabolism of warfarin. *Pharmacol Ther* 1997;**73**:67–74.
39 Toon S, Low LK, Gibaldi M, Trager WF, O'Reilly RA, Motley CH *et al.* The warfarin–sulfinpyrazone interaction: stereochemical considerations. *Clin Pharmacol Ther* 1986;**39**:15–24.
40 Takahashi H, Echizen H. Pharmacogenetics of warfarin elimination and its clinical implications. *Clin Pharmacokinet* 2001;**40**:587–603.
41 Hall SD, Hamman MA, Rettie AE, Wienkers LC, Trager WF, Vandenbranden M *et al.* Relationships between the levels of cytochrome P4502C9 and its prototypic catalytic activities in human liver microsomes. *Drug Metab Dispos* 1994;**22**:975–8.
42 Rettie AE, Korzekwa KR, Kunze KL, Lawrence RF, Eddy AC, Aoyama T *et al.* Hydroxylation of warfarin by human cDNA-expressed cytochrome P-450: a role for P-4502C9 in the etiology of (S) –warfarin–drug interactions. *Chem Res Toxicol* 1992;**5**:54–9.
43 Kunze KL, Wienkers LC, Thummel KE, Trager WF. Warfarin-fluconazole. I. Inhibition of the human cytochrome P450-dependent metabolism of warfarin by fluconazole: in vitro studies. *Drug Metab Dispos* 1996;**24**:414–21.
44 Aithal GP, Day CP, Kesteven PJ, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet* 1999;**353**:717–9.
45 Taube J, Halsall D, Baglin T. Influence of cytochrome P-450 CYP2C9 polymorphisms on warfarin sensitivity and risk of over-anticoagulation in patients on long-term treatment. *Blood* 2000;**96**:1816–9.
46 Higashi MK, Veenstra DL, Kondo LM, Wittkowsky AK, Srinouanprachanh SL, Farin FM *et al.* Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA* 2002;**287**:1690–8.
47 Scordo MG, Pengo V, Spina E, Dahl ML, Gusella M, Padrini R. Influence of CYP2C9 and CYP2C19 genetic polymorphisms on warfarin maintenance dose and metabolic clearance. *Clin Pharmacol Ther* 2002;**72**:702–10.
48 Thijssen HH, Flinois JP, Beaune PH. Cytochrome P4502C9 is the principal catalyst of racemic acenocoumarol hydroxylation reactions in human liver microsomes. *Drug Metab Dispos* 2000;**28**:1284–90.
49 He M, Korzekwa KR, Jones JP, Rettie AE, Trager WF. Structural forms of phenprocoumon and warfarin that are metabolized at the active site of CYP2C9. *Arch Biochem Biophys* 1999;**372**:16–28.
50 Thijssen HH, Verkooijen IW, Frank HL. The possession of the CYP2C9*3 allele is associated with low dose requirement of acenocoumarol. *Pharmacogenetics* 2000;**10**:757–60.
51 Verstuyft C, Morin S, Robert A, Loriot MA, Beaune P, Jaillon P *et al.* Early acenocoumarol overanticoagulation among cytochrome P450 2C9 poor metabolizers. *Pharmacogenetics* 2001;**11**:735–7.
52 Tassies D, Freire C, Pijoan J, Maragall S, Monteagudo J, Ordinas A *et al.* Pharmacogenetics of acenocoumarol: cytochrome P450 CYP2C9 polymorphisms influence dose requirements and stability of anticoagulation. *Haematologica* 2002;**87**:1185–91.
53 Thijssen HH, Drittij MJ, Vervoort LM, de Vries-Hanje JC. Altered pharmacokinetics of R- and S-acenocoumarol in a subject heterozygous for CYP2C9*3. *Clin Pharmacol Ther* 2001;**70**:292–8.

54 Thomas RC, Ikeda GJ. The metabolic fate of tolbutamide in man and in the rat. *J Med Chem* 1966;**9**:507–10.
55 Nelson E, O'Reilly I. Kinetics of carboxytolbutamide excretion following tolbutamide and carboxytolbutamide administration. *J Pharmacol Exp Ther* 1961;**132**:103–9.
56 Lee CR, Pieper JA, Frye RF, Hinderliter AL, Blaisdell JA, Goldstein JA. Tolbutamide, flurbiprofen, and losartan as probes of CYP2C9 activity in humans. *J Clin Pharmacol* 2003;**43**:84–91.
57 Scott J, Poffenbarger PL. Pharmacogenetics of tolbutamide metabolism in humans. *Diabetes* 1979;**28**:41–51.
58 Miners JO, Wing LM, Birkett DJ. Normal metabolism of debrisoquine and theophylline in a slow tolbutamide metaboliser. *Aust N Z J Medical* 1985;**15**:348–9.
59 Bhasker CR, Miners JO, Coulter S, Birkett DJ. Allelic and functional variability of cytochrome P4502C9. *Pharmacogenetics* 1997;**7**:51–8.
60 Kirchheiner J, Bauer S, Meineke I, Rohde W, Prang V, Meisel C *et al.* Impact of CYP2C9 and CYP2C19 polymorphisms on tolbutamide kinetics and the insulin and glucose response in healthy volunteers. *Pharmacogenetics* 2002;**12**:101–9.
61 Shon JH, Yoon YR, Kim KA, Lim YC, Lee KJ, Park JY *et al.* Effects of CYP2C19 and CYP2C9 genetic polymorphisms on the disposition of and blood glucose lowering response to tolbutamide in humans. *Pharmacogenetics* 2002;**12**:111–9.
62 Lee CR, Pieper JA, Hinderliter AL, Blaisdell JA, Goldstein JA. Evaluation of cytochrome P4502C9 metabolic activity with tolbutamide in CYP2C9*1 heterozygotes. *Clin Pharmacol Ther* 2002;**72**:562–71.
63 Miners J. CYP2C9 polymorphism. impact on tolbutamide pharmacokinetics and response. *Pharmacogenetics* 2002;**12**:91–2.
64 Doecke CJ, Veronese ME, Pond SM, Miners JO, Birkett DJ, Sansom LN *et al.* Relationship between phenytoin and tolbutamide hydroxylations in human liver microsomes. *Br J Clin Pharmacol* 1991;**31**:125–30.
65 Dickinson RG, Hooper WD, Patterson M, Eadie MJ, Maguire B. Extent of urinary excretion of p-hydroxyphenytoin in healthy subjects given phenytoin. *Ther Drug Monit* 1985;**7**:283–9.
66 Veronese ME, Doecke CJ, Mackenzie PI, McManus ME, Miners JO, Rees DL *et al.* Site-directed mutation studies of human liver cytochrome P-450 isoenzymes in the CYP2C subfamily. *Biochem J* 1993;**289**:533–8.
67 Odani A, Hashimoto Y, Otsuki Y, Uwai Y, Hattori H, Furusho K *et al.* Genetic polymorphism of the CYP2C subfamily and its effect on the pharmacokinetics of phenytoin in Japanese patients with epilepsy. *Clin Pharmacol Ther* 1997;**62**:287–92.
68 Veronese ME, Miners JO, Rees DL, Birkett DJ. Tolbutamide hydroxylation in humans: lack of bimodality in 106 healthy subjects. *Pharmacogenetics* 1993;**3**:86–93.
69 Inaba T. Phenytoin. pharmacogenetic polymorphism of 4′-hydroxylation. *Pharmacol Ther* 1990;**46**:341–7.
70 van der Weide J, Steijns LS, van Weelden MJ, de Haan K. The effect of genetic polymorphism of cytochrome P450 CYP2C9 on phenytoin dose requirement. *Pharmacogenetics* 2001;**11**:287–91.
71 Leemann T, Transon C, Dayer P. Cytochrome P450TB (CYP2C): a major monooxygenase catalyzing diclofenac 4′-hydroxylation in human liver. *Life Sci* 1993;**52**:29–34.
72 Shimamoto J, Ieiri I, Urae A, Kimura M, Irie S, Kubota T *et al.* Lack of differences in diclofenac (a substrate for CYP2C9) pharmacokinetics in healthy volunteers with respect to the single CYP2C9*3 allele. *Eur J Clin Pharmacol* 2000;**56**:65–8.
73 Yasar U, Eliasson E, Forslund-Bergengren C, Tybring G, Gadd M, Sjoqvist F *et al.* The role of CYP2C9 genotype in the metabolism of diclofenac in vivo and in vitro. *Eur J Clin Pharmacol* 2001;**57**:729–35.
74 Brenner SS, Herrlinger C, Dilger K, Murdter TE, Hofmann U, Marx C *et al.* Influence of age and cytochrome P450 2C9 genotype on the steady-state disposition of diclofenac and celecoxib. *Clin Pharmacokinet* 2003;**42**:283–92.
75 Kirchheiner J, Meineke I, Steinbach N, Meisel C, Roots I, Brockmoller J. Pharmacokinetics of diclofenac and inhibition of cyclooxygenases 1 and 2: no relationship to the CYP2C9 genetic polymorphism in humans. *Br J Clin Pharmacol* 2003;**55**:51–61.
76 Hamman MA, Thompson GA, Hall SD. Regioselective and stereoselective metabolism of ibuprofen by human cytochrome P4502C. *Biochem Pharmacol* 1997;**54**:33–41.
77 Kirchheiner J, Meineke I, Freytag G, Meisel C, Roots I, Brockmoller J. Enantiospecific effects of cytochrome P450 2C9 amino acid variants on ibuprofen pharmacokinetics and on the inhibition of cyclooxygenases 1 and 2. *Clin Pharmacol Ther* 2002;**72**:62–75.
78 Stearns RA, Chakravarty PK, Chen R, Chiu SH. Biotransformation of losartan to its active carboxylic acid metabolite in human liver microsomes. Role of cytochrome P4502C and 3A subfamily members. *Drug Metab Dispos* 1995;**23**:207–15.
79 McCrea JB, Cribb A, Rushmore T, Osborne B, Gillen L, Lo MW *et al.* Phenotypic and genotypic investigations of a healthy volunteer deficient in the conversion of losartan to its active metabolite E-3174. *Clin Pharmacol Ther* 1999;**65**:348–52.
80 Taavitsainen P, Kiukaanniemi K, Pelkonen O. In vitro inhibition screening of human hepatic P450 enzymes by five angiotensin-II receptor antagonists. *Eur J Clin Pharmacol* 2000;**56**:135–40.
81 Bourrie M, Meunier V, Berger Y, Fabre G. Role of cytochrome P-4502C9 in irbesartan oxidation by human liver microsomes. *Drug Metab Dispos* 1999;**27**:288–96.
82 Hallberg P, Karlsson J, Kurland L, Lind L, Kahan T, Malmqvist K *et al.* The CYP2C9 genotype predicts the blood pressure response to irbesartan: results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation vs Atenolol (SILVHIA) trial. *J Hypertens* 2002;**20**:2089–93.
83 Aynacioglu AS, Brockmoller J, Bauer S, Sachse C, Guzelbey P, Ongen Z *et al.* Frequency of cytochrome P450 CYP2C9 variants in a Turkish population and functional relevance for phenytoin. *Br J Clin Pharmacol* 1999;**48**:409–15.
84 Yoon YR, Shon JH, Kim MK, Lim YC, Lee HR, Park JY *et al.* Frequency of cytochrome P450 2C9 mutant alleles in a Korean population. *Br J Clin Pharmacol* 2001;**51**:277–80.