Differential Genotype Dependent Inhibition of CYP2C9 in Humans

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ABSTRACT:
The effects of genetic polymorphisms in drug-metabolizing enzymes (e.g., CYP2C9*3) on drug clearance have been well characterized but much less is known about whether these polymorphisms alter susceptibility to drug-drug interactions. Previous in vitro work has demonstrated that genotype-dependent inhibition of CYP2C9 mediated flurbiprofen metabolism, suggesting the possibility of genotype-dependent inhibition interactions in vivo. In the current study, flurbiprofen was used as a probe substrate and fluconazole as a prototypical inhibitor to investigate whether genotype-dependent inhibition of CYP2C9 occurs in vivo. From 189 healthy volunteers who were genotyped for CYP2C9 polymorphisms, 11 control subjects (CYP2C9*1/*1), 9 heterozygous and 2 homozygous for the CYP2C9*3 allele participated in the pharmacokinetic drug interaction study. Subjects received a single 50-mg oral dose of flurbiprofen alone or after administration of either 200 or 400 mg of fluconazole for 7 days using an open, randomized, crossover design. Flurbiprofen and fluconazole plasma concentrations along with flurbiprofen and 4'-hydroxyflurbiprofen urinary excretion were monitored. Flurbiprofen apparent oral clearance differed significantly among the three genotype groups (p < 0.05) at baseline but not after pretreatment with 400 mg of fluconazole for 7 days. Changes in flurbiprofen apparent oral clearance after fluconazole coadministration were gene dose-dependent, with virtually no change occurring in *3/*3 subjects. Analysis of fractional clearances suggested that the fraction metabolized by CYP2C9, as influenced by genotype, determined the degree of drug interaction observed. In summary, the presence of CYP2C9*3 alleles (either one or two alleles) can alter the degree of drug interaction observed upon coadministration of inhibitors.

ABBREVIATIONS: P450, cytochrome P450; AUC, area under the curve; ANOVA, analysis of variance.
total of 189 healthy subjects were enrolled in the genotype screening phase of the study. After obtaining written informed consent, 30 ml of blood was drawn and stored at room temperature for DNA isolation. All subjects were non-smokers and were not taking any medicine at the time of enrollment, except oral contraceptives.

DNA Isolation. The whole blood sample was mixed thoroughly with red blood cell lysis buffer (preheated to 37°C). The mixture was allowed to incubate in the water bath at 37°C for 15 min. The samples were then centrifuged for 15 min at 3000 rpm. The pellets were washed repeatedly with red blood cell lysis buffer until the pellet became white in color. The pellet was then dissolved in 3 ml of white blood cell lysis buffer and incubated at 37°C overnight. To each tube, 10 μl of protease K was added, and the mixture was vortexed and incubated for 24 h at 37°C. Ammonium acetate (1.5 ml of 7.5 M) was added to precipitate the DNA. Proteins were then removed by centrifuging at 3000 rpm for 20 min, and 10 ml of absolute ethanol was added to the supernatant. The mixture was then gently inverted to allow the DNA to precipitate. The precipitated DNA was then suspended in 3 ml of TE buffer (Sigma-Aldrich, St. Louis, MO). Quantitation of DNA was carried out by measuring the sample absorbance at 260 nm.

Determination of CYP2C9 Genotype. DNA samples were diluted to 20 ng/μl, and single nucleotide polymorphisms were determined by TaqMan-based allele discrimination assay kits (Applied Biosystems, Foster City, CA). The genotyping reaction was conducted in 96-well plates, and the reaction components were 1 μl of diluted DNA, 1.25 μl of primer, 12.5 μl of TaqMan universal master mix (Applied Biosystems), and water to a total volume of 25 μl. The thermocycler protocol was as follows: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 92°C for 15 s and 60°C for 1 min. Reactions were analyzed on a Prism model 7500 sequence detection system (Applied Biosystems). The SNP database reference numbers were CYP2C9*2 = rs1799853 and CYP2C9*3 = rs1057910.

Drug Interaction Study Design. From the 189 genotyped subjects, 11 CYP2C9*1/*1, 9 CYP2C9*1/*3, and 2 CYP2C9*3/*3 subjects further consented to participate in the drug interaction study. All subjects were healthy as determined by physical examination, medical history, vital signs, and routine biochemical and urinalysis tests. Subjects were instructed to abstain from alcohol for 3 days and caffeine-containing food, chocolate, and beverages for 2 days before the pharmacokinetic study. Female subjects of child-bearing potential underwent a urine pregnancy test.

An open, randomized, crossover design with a 1 week washout was used. Subjects received either 50 mg of flurbiprofen as a tablet (Mylan Pharmaceuticals Inc., Morgantown, WV) alone or 200 or 400 mg of fluconazole as a tablet (Ivax Pharmaceuticals Inc., Miami, FL) for 7 days followed by 50 mg of flurbiprofen on the 7th day. Compliance with fluconazole administration was assessed by pill count as well as examination of subject diaries. Subjects were required to fast overnight before the study day. On the morning of each period, subjects were admitted to the Clinical Research Center and remained there for 12 h, were dismissed, and then returned the next morning for collection of the 24-h blood sample and return of the urine collection. Flurbiprofen was administered in the morning as scheduled, and flurbiprofen administered orally 2 h later. A light snack was allowed 2 h after flurbiprofen dosing.

Plasma and Urine Collection. Blood samples (7 ml) were collected into heparinized tubes at 0 min (before flurbiprofen administration) and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h after the administration of flurbiprofen and immediately centrifuged for 10 min at 3200 rpm, and the resulting plasma was aliquoted and stored at −20°C. Total voided urine was collected before flurbiprofen administration and at intervals of 0 to 12 h and 12 to 24 h after flurbiprofen administration and kept refrigerated throughout the collection period. At the end of each collection interval, total urine volume was recorded and two 25-ml aliquots were stored at −20°C for later analysis.

Analysis of Flurbiprofen Concentrations in Plasma and Flurbiprofen and 4'-Hydroxyflurbiprofen Concentrations in Urine. Flurbiprofen and 4'-hydroxyflurbiprofen concentrations were quantified by high-performance liquid chromatographic according to methods previously developed in our laboratory (Hutzler et al., 2000). Acid hydrolysis, as previously described, was used to cleave glucuronides of flurbiprofen and 4'-hydroxyflurbiprofen, before analysis.

Analysis of Fluconazole in Plasma. Fluconazole plasma concentrations were quantitated by a method described previously (Cociglio et al., 1996).

In Vitro Assay of Genotype-Dependent Inhibition. The ability of fluconazole to inhibit expressed, reconstituted CYP2C9.1, an equimolar mixture of CYP2C9.1/CYP2C9.3, and CYP2C9.3 was determined according to methods previously established in our laboratory (Kumar et al., 2006a). Briefly, fluconazole (0, 10, 50, and 100 μM) was incubated with three concentrations of flurbiprofen (5, 25, and 50 μM) with each of the enzyme preparations listed above and the formation of 4'-hydroxyflurbiprofen was monitored.

Noncompartimental Pharmacokinetic Analysis. Flurbiprofen pharmacokinetic parameters were estimated from plasma concentration-time data by standard noncompartimental methods (WinNonlin v5.2, Pharsight, Palo Alto, CA). The area under the concentration-time curve (AUC0–inf) of flurbiprofen was calculated using the linear trapezoidal rule with extrapolation to infinity. Apparent oral clearance (CL/F) of flurbiprofen was calculated as dose/AUC0–inf. The 4'-hydroxyflurbiprofen formation clearance (CLf,m) was calculated using eq. 1:

\[ \text{CL}_\text{f,m} = \frac{\text{Amt. 4'-OHF formed}_{0-24 \text{ hr}}}{\text{flurbiprofen AUC}_{0-24 \text{ hr}}} \]  

where Amt. 4'-OHF formed0–24 hr is the amount of 4'-hydroxyflurbiprofen metabolite (both as 4'-hydroxyflurbiprofen and its glucuronide conjugate) excreted in the urine during the 24-h collection interval.

Statistical Analysis. Sample size calculations were based on the study conducted by Zghieb et al. (2007) in which 78% inhibition of 4'-hydroxyflurbiprofen formation clearance was observed after pretreatment with 7 doses of 400 mg of fluconazole. To detect a 30% difference in inhibition between CYP2C9*1/*1 and CYP2C9*1/*3 with power of 90% and 5% chance of type I error, it was estimated that a sample size of at least n = 8 per group would be necessary. One-way ANOVA was applied to compare demographics such as age, body weight, height, and creatinine concentration between the three CYP2C9 genotype groups. Before statistical analysis, flurbiprofen pharmacokinetic parameters (e.g., CL/F, AUC0–inf and half-life) were log-transformed, and two-way repeated-measure ANOVA was then applied. Pairwise multiple comparisons were performed using the Holm-Sidak method. A Friedman repeated-measure ANOVA on ranks was performed on CLf,m, Cmax, and tmax and V/F because of non-normalized distributions. Pairwise comparisons were made using the Tukey-Kramer test. Fluconazole pharmacokinetic parameters (AUC0–24 h, Cmax, and half-life) across groups and treatment periods were compared using one-way analysis of variance with a p value of 0.05 or less regarded as statistically significant. All statistical analyses were conducted with SigmaStat 3.1 (Systat Software, Point Richmond, CA).

Model-Based Pharmacokinetic Analysis. A nonlinear mixed-effects modeling approach was used for both pharmacokinetic parameter estimation and to develop a global model for the flurbiprofen data that included a pharmacokinetic submodel and a drug interaction submodel. All flurbiprofen concentrations from all drug interaction arms in subjects with all three genotypes were simultaneously modeled using NONMEM VI (ICON U.S., Ellicott City, MD) (Beal et al., 2006) implemented with Compaq Visual Fortran v6.6 and PDXPop 2.2a, using a first-order conditional estimation algorithm that allowed for an interaction between the ETA and EPSILON levels of random effects. For the pharmacokinetic submodel, a one-compartment model with first-order absorption and first-order elimination adequately described the flurbiprofen concentration-time profiles. The link between the drug interaction model and the pharmacokinetic model was adapted from the Rowland-Matin equation (Rowland and Matin, 1973). Flurbiprofen clearance was modeled as a function of the genotypic CL2C9, CLnon2C9, the fluconazole concentration (I), and the Ki for the fluconazole-CYP2C9 interaction:

\[ \text{CL} = \frac{\text{CL2C9}}{1 + \frac{\text{I}}{\text{Ki}}} + \text{CLnon2C9} \]  

where CL2C9 represents CYP2C9 metabolic clearance and CLnon2C9 represents non-CYP2C9 clearance. It is important to note that CL2C9 and Ki can both take on different values across different genotypes.

In the population model, the typical values of the uninhibited CL2C9 for each of the three genotypes were estimated as regression parameters. The typical value of CLnon2C9 was also estimated as a regression parameter,
assuming that it is not dependent upon genotype and is shared across all genotypes and inhibitor concentrations. Flurbiprofen volume of distribution, first-order absorption rate constant, and an absorption lag time were also estimated and assumed to be log normally distributed in the population. The between-subject variability for each was expressed as a coefficient of variation. Residual unexplained variability was modeled with a proportional error model and expressed as a coefficient of variation.

Parameters of the drug interaction submodel were based on previous literature (Kunze and Trager, 1996), wherein the average steady-state concentration was reported to be 60 μM after 400 mg/day fluconazole for 6 days. This value was used in the regression equation as $I_0$ for the 400 mg of fluconazole interaction treatment arm. Assuming linear pharmacokinetics of fluconazole, the value for $I_0$ in the 200 mg of fluconazole treatment arm was taken to be 30 μM. This same study reported the average in vivo $K_i$ for the fluconazole/CYP2C9 interaction to be 22 μM. We assumed that all of these subjects were of the predominant *1/*1 genotype. Our laboratory determined the in vitro $K_i$ values to have a *1/*1 to *1/*3 ratio of 1.55; similarly, the *3/*3 to *1/*1 in vitro ratio was 2.00 (see Results). If we assume that the in vitro ratio approximates the in vivo ratio, these ratios were used to estimate the value of $K_i$ in *1/*3 and *3/*3 genotypes from the literature in vivo value.

Because there were only two *3/*3 subjects, a likelihood ratio test was applied to determine whether the information from these two subjects was sufficiently strong to reject the hypothesis that CL2C9 in *3/*3 subjects was no different from the CL2C9 in *1/*3 subjects. Using the maximum likelihood objective function in NONMEM, the difference in objective function values between a full and nested model is approximately $\chi^2$ distributed with degrees of freedom equal to the difference in the number of parameters between the two competing models. A decrease in objective function value of 3.8 or greater indicates that the more complicated model is superior to the nested model (two competing models). A decrease in objective function value of 3.8 or greater of freedom equal to the difference in the number of parameters between the full and nested model is approximately $\chi^2$ distributed with degrees of freedom equal to the difference in the number of parameters between the two competing models. A decrease in objective function value of 3.8 or greater indicates that the more complicated model is superior to the nested model ($p < 0.05$). We also determined whether the model that independently estimated the clearance of each genotypic group in each treatment arm (nine clearance parameters) was significantly different from the model that included all of the assumptions made in the drug interaction submodel (four clearance parameters). With 5 df, a decrease in objective function value of 11.1 results in $\alpha = 0.05$.

Results

After obtaining informed consent, 189 healthy volunteers were genotyped for CYP2C9 alleles. Two subjects were not further evaluated as one subject presented with hypertension and in the other case, phlebotomy was unsuccessful. Of these subjects, 40.2% were male, 72.3% were white, 12.7% were African-American, 8.5% were Asian, two individuals were Pacific-Islander, and three did not self-identify. The median age was 25 years (range 18–61). The allele frequencies for all individuals screened are reported in Table 1. Twenty-two subjects (11 CYP2C9*1/*1, 9 CYP2C9*1/*3, and 2 CYP2C9*3/*3) were then enrolled into the drug interaction study and their demographics are presented in Table 2. Before flurbiprofen administration, one CYP2C9*1/*3 genotype subject withdrew from the study for personal reasons and, hence, was excluded from the analysis. No significant adverse events were reported by any subjects.

Mean flurbiprofen plasma concentration-time profiles before and after pretreatment with either 200 or 400 mg of fluconazole in CYP2C9*1/*1, CYP2C9*1/*3, and CYP2C9*3/*3 genotype individuals are depicted in Fig. 1, A to C, respectively. Pharmacokinetic parameters derived from noncompartmental analysis of individual data are presented in Table 3. With respect to genotype, by comparing the control period versus 7-day pretreatment with 200 mg of fluconazole, statistically significant differences were noted in apparent oral clearance, AUC, and half-life between treatment periods in the CYP2C9*1/*1 and CYP2C9*3/*3 genotype groups ($p < 0.01$). However, no statistically significant differences in oral clearance or AUC were noted in the CYP2C9*3/*3 group, comparing control versus 200 mg of fluconazole.

Flurbiprofen oral clearance, AUC, and half-life differed significantly between baseline and 400 mg of fluconazole pretreatment in subjects with either the CYP2C9*1/*1 or CYP2C9*1/*3 genotypes ($p < 0.01$). These differences remained, when the parameters for the 200 and 400 mg doses of fluconazole were compared. However, no statistically significant differences in the above parameters among any of the periods (baseline versus 200 mg of fluconazole versus 400 mg of fluconazole) were observed in the CYP2C9*3/*3 genotype group. Finally, no statistically significant differences were noted in $C_{\text{max}}$, $T_{\text{max}}$, and $V/F$ across periods in any of the three genotype groups.

Figure 2 is an interaction plot of median oral clearance (25th and 75th percentile) for each of the three genotype groups during the three periods (baseline and 200 mg or 400 mg of fluconazole). At baseline, median oral clearance in CYP2C9*1/*1 individuals differed significantly ($p < 0.05$) from that in the CYP2C9*1/*3 and CYP2C9*3/*3 individuals. After 200 mg of fluconazole, individual oral clearances in all three genotype groups decreased, but the median oral clearance in CYP2C9*1/*1 individuals was not statistically different ($p = 0.084$) from that of CYP2C9*1/*3 individuals. However, median oral clearance in the CYP2C9*1/*1 group was statistically different ($p = 0.015$) from that for CYP2C9*3/*3 individuals after 200 mg of fluconazole. Finally, no differences were noted ($p > 0.05$) in flurbiprofen median oral clearance across all genotypes after 400 mg of fluconazole pretreatment. In the CYP2C9*3/*3 individuals, no significant decrease in apparent oral clearance was observed at either of the fluconazole doses. Similar trends were seen in the AUC$_{0-\text{inf}}$, and this was reflected by a 2.4- and 3.2-fold increase in flurbiprofen AUC$_{0-\text{inf}}$ in CYP2C9*1/*3 and CYP2C9*3/*3 groups, but almost no change was observed in the CYP2C9*3/*3 group following coadministration of 400 mg of fluconazole for 7 days (Fig. 3).

The 4'-hydroxyflurbiprofen formation clearances (CL$_{\text{max}}$) for the three periods are presented in Table 3. At both baseline and 200 mg of fluconazole coadministration, the formation clearance in individuals with either one or two *3 alleles varied significantly from that for wild-type individuals. However, after 400 mg of fluconazole coadministration, only individuals with two *3 alleles differed statistically from those carrying only the *1 allele.

Mean plasma concentration-time profiles of fluconazole after seven doses of 200 and 400 mg in the three genotype groups are shown in Fig. 4, and the pharmacokinetics parameters are summarized in Table 4. No statistically significant differences were noted in AUC$_{0-\text{24h}}$, half-life, or $C_{\text{max}}$ for a given dose of fluconazole among the three genotype groups.

In vitro studies of fluconazole inhibition of flurbiprofen metabolism were conducted to provide the framework for the in vitro–in vivo correlations and model-based analysis (see below). In expressed enzyme preparations of CYP2C9.1 (simulate wild-type *1/*1 individuals), the $K_i$ value was 11 μM for fluconazole inhibition of flurbiprofen 4’-hydroxylation. In an equimolar mixture of CYP2C9.1/CYP2C9.3 (simulate heterozygote *1/*3 individuals), the fluconazole $K_i$ was 17 μM. Finally, with the CYP2C9.3 protein (simulate homozygote *3/*3
creatinine concentrations modeled in the drug interaction submodel as $1/(1 + IK)$. These values of flurbiprofen clearance and the magnitude of the drug interaction are all consistent with the noncompartmental results. Diagnostic plots of weighted residuals versus time or predicted concentration were randomly distributed around zero and suggested no reason to reject the model. The plot of observed versus predicted concentration indicated a good fit of the observed data under the model. The attempt to combine CL2C9 in the *3/*3 subjects with the CL2C9 in *1/*3 subjects as a single parameter resulted in a significantly inferior model ($p < 0.05$, $\chi^2$, df = 1) as indicated by an increase in the objective

individuals) the flucnazole $K_i$ was 23 $\mu$M. Thus, the $K_i$ of flucnazole inhibition was altered in a gene dose-dependent fashion.

Results from the model-based analysis (eq. 2) of the drug interaction are presented in Table 5. Only the values of the uninhibited clearance, CL2C9, for each genotype were estimated. The clearances as the result of the drug interaction (i.e., the reduced clearances) were modeled in the drug interaction submodel as $1/(1 + IK)$. These values of flurbiprofen clearance and the magnitude of the drug interaction are all consistent with the noncompartmental results. Diagnostic plots of weighted residuals versus time or predicted concentration were randomly distributed around zero and suggested no reason to reject the model. The plot of observed versus predicted concentration indicated a good fit of the observed data under the model. The attempt to combine CL2C9 in the *3/*3 subjects with the CL2C9 in *1/*3 subjects as a single parameter resulted in a significantly inferior model ($p < 0.05$, $\chi^2$, df = 1) as indicated by an increase in the objective

![fig1](image1.png)

**Figure 1.** Mean ± S.D. plasma flurbiprofen concentration-time profiles after oral administration of flurbiprofen tablet (50 mg) alone (●) and after seven doses of fluconazole: 200 mg (○) and 400 mg (▲) in 11 CYP2C9*1/*1 subjects (A), 8 CYP2C9*1/*3 subjects (B), and 2 CYP2C9*3/*3 subjects (C).

**Table 2**

Demographics of subjects participating in drug interaction study (n = 21)

<table>
<thead>
<tr>
<th>Demographics</th>
<th>CYP2C9*1/*1</th>
<th>CYP2C9*1/*3</th>
<th>CYP2C9*3/*3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. subjects</td>
<td>11</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Age (years)</td>
<td>25 (19–36)</td>
<td>23 (19–28)</td>
<td>25 (29)</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>4/7</td>
<td>3/5</td>
<td>20</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>73.7 (51–108)</td>
<td>66.9 (49–84)</td>
<td>77 (75)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166 (154–193)</td>
<td>167 (160–189)</td>
<td>177 (179)</td>
</tr>
<tr>
<td>Creatinine concentration</td>
<td>0.87 (0.7–1.27)</td>
<td>0.86 (0.76–1.03)</td>
<td>(1, 1.1)</td>
</tr>
</tbody>
</table>

**Table 3**

Noncompartmental pharmacokinetic parameters of flurbiprofen before (baseline) and after pretreatment with 200 or 400 mg of fluconazole for 7 days

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Units</th>
<th>CYP2C9*1/*1 (n = 11)</th>
<th>CYP2C9*1/*3 (n = 8)</th>
<th>CYP2C9*3/*3 (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL/F</td>
<td>Liters $\cdot$ hour$^{-1}$</td>
<td>1.6 (1.2–2.2)</td>
<td>0.9 (0.5–2)</td>
<td>(0.4, 0.6)</td>
</tr>
<tr>
<td>$\text{AUC}^{0-\text{tinf}}$</td>
<td>micrograms $\cdot$ milliliter$^{-1}$ $\cdot$ hour</td>
<td>30.8 (23.2–42.7)</td>
<td>53.7 (24.6–112)</td>
<td>(85.8, 119)</td>
</tr>
<tr>
<td>$T_{1/2}$</td>
<td>Hours</td>
<td>4.8 (4.2–6.1)</td>
<td>7.2 (5.5–7.5)</td>
<td>(9.7, 13.8)</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>micrograms $\cdot$ milliliter$^{-1}$</td>
<td>7.6 (4.1–9.5)</td>
<td>8.9 (4.2–10.7)</td>
<td>(8.9, 4.4)</td>
</tr>
<tr>
<td>$T_{\text{max}}$</td>
<td>Hours</td>
<td>1.5 (0.5–2.0)</td>
<td>2.0 (1.5–6.1)</td>
<td>(2.0, 2.0)</td>
</tr>
<tr>
<td>$V/F$</td>
<td>Liters</td>
<td>10.8 (7.8–13.8)</td>
<td>9.2 (3.9–22)</td>
<td>(8.2, 8.4)</td>
</tr>
<tr>
<td>$\text{CL}_{1\text{m}}$</td>
<td>Liters $\cdot$ hour$^{-1}$</td>
<td>0.67 (0.43–0.95)</td>
<td>0.35 (0.15–0.86)</td>
<td>(0.05, 0.05)</td>
</tr>
</tbody>
</table>

**Note:**

- $P < 0.05$ between CYP2C9*1/*1 and CYP2C9*1/*3 or CYP2C9*3/*3.
- $\dagger P < 0.05$ between CYP2C9*1/*3 and CYP2C9*3/*3.
function value of 5.2 units. When nine CL2C9 typical values were estimated (one for each genotype and level of drug interaction), the objective function value decreased by 4.8 units compared with the model incorporating the in vivo and in vitro drug interaction model (not statistically significant ($p < 0.05$, $\chi^2$, df = 5).

**Discussion**

The effects of genetic polymorphisms on rates of drug metabolism are well known, but how these polymorphisms impact susceptibility to drug-drug interactions is less clear. In particular, whether reduced function proteins (e.g., CYP2C9.3) are inhibited to the same extent in vivo as wild-type proteins (e.g., CYP2C9.1) has not been determined. This knowledge is potentially clinically important because differential dosage adjustments may be needed in individuals with the CYP2C9*3 genotype compared with the more prevalent wild-type-expressing individuals, when a known interacting drug is coadministered. Results of the present in vivo interaction study demonstrated that genotype-dependent inhibition of flurbiprofen oral clearance occurs when it is coadministered with fluconazole in subjects with none, one, or two CYP2C9*3 alleles.

CYP2C9 exclusively catalyzes the oxidative metabolism of flurbiprofen to its 4'-hydroxy (primary) oxidative metabolite (Tracy et al., 1995, 1996), and this reaction has been demonstrated to be a reliable probe of CYP2C9 activity in vivo (Lee et al., 2003b; Zgheib et al., 2007). Differences in flurbiprofen pharmacokinetics have been evaluated previously in individuals genotyped for CYP2C9*1/*2 and CYP2C9*1/*3, and reduced oral clearance was noted in individuals with both genotypes compared with individuals with the CYP2C9*1/*1 genotype (Lee et al., 2003a). Fluconazole is a prototypical CYP2C9 inhibitor, with nearly complete bioavailability and less than 10% of a dose being metabolized (Shiba et al., 1990). Greenblatt et al. (2006) reported a reduction of flurbiprofen oral clearance, after two doses of 200 mg of fluconazole, to approximately 55% of the baseline value (Greenblatt et al., 2006), similar to the current results in wild-type individuals. In another study, the formation clearance of 4'-hydroxyflurbiprofen (as an indicator of CYP2C9 activity) decreased by 69 and 78% from baseline after single and seven doses of 200 mg of fluconazole, respectively (Zgheib et al., 2007), again similar to the current results. However, in neither study were the subjects’ genotypes known.

In vitro studies from our laboratory (Kumar et al., 2006b) reported genotype-dependent inhibition of CYP2C9, and in the current work we report a 2-fold difference in fluconazole $K_i$ values (with flurbiprofen as a substrate) between the CYP2C9.1 and CYP2C9.3 proteins, suggesting that genotype-dependent inhibition in vivo was possible. Pretreatment with seven doses of either 200 or 400 mg of fluconazole, to approximately 55% of the baseline value (Greenblatt et al., 2006), similar to the current results in wild-type individuals. In another study, the formation clearance of 4'-hydroxyflurbiprofen (as an indicator of CYP2C9 activity) decreased by 69 and 78% from baseline after single and seven doses of 200 mg of fluconazole, respectively (Zgheib et al., 2007), again similar to the current results. However, in neither study were the subjects’ genotypes known.

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In vitro studies from our laboratory (Kumar et al., 2006b) reported genotype-dependent inhibition of CYP2C9, and in the current work we report a 2-fold difference in fluconazole $K_i$ values (with flurbiprofen as a substrate) between the CYP2C9.1 and CYP2C9.3 proteins, suggesting that genotype-dependent inhibition in vivo was possible. Pretreatment with seven doses of either 200 or 400 mg of fluconazole, to approximately 55% of the baseline value (Greenblatt et al., 2006), similar to the current results in wild-type individuals. In another study, the formation clearance of 4'-hydroxyflurbiprofen (as an indicator of CYP2C9 activity) decreased by 69 and 78% from baseline after single and seven doses of 200 mg of fluconazole, respectively (Zgheib et al., 2007), again similar to the current results. However, in neither study were the subjects’ genotypes known.
interaction analysis were consistent with those from the noncompartmental analysis. It is particularly noteworthy that the clearances of flurbiprofen at two different levels of fluconazole interaction were closely predicted from a drug interaction model. For example, in the CYP2C9*1/*3 group, the uninhibited clearance was estimated to be 0.428 liter/h under the drug interaction model. Values estimated in the 200 mg of fluconazole drug interaction arm, the uninhibited clearance was 0.5 liter/h; this difference is minimal in these homozygotic variant individuals. This finding has important implications for prediction of drug-drug interactions because one must consider both genotype and fraction metabolized by a given pathway when predicting these interactions. Further studies are needed to test this hypothesis with drugs that have greater and lesser fractions metabolized by a given pathway in individuals with variant genotypes.

Some studies have reported the impact of genetic polymorphisms in CYP2C19 and CYP2D6 on drug-drug interactions in humans. Yasui-Furukori et al. (2004a,b) studied the degree of inhibition of omeprazole and lansoprazole metabolism after coadministration of the CYP2C19 inhibitor fluvoxamine, in subjects genotyped for CYP2C19 polymorphisms. This study, in concurrence with similar studies by others (Uno et al., 2006), concluded that extensive metabolizers experience a greater extent of CYP2C19 inhibition upon coadministration of inhibitors compared with CYP2C19 poor metabolizers. Similarly, studies have been conducted in extensive versus poor metabolizers of CYP2D6 and the extent of drug-drug interactions was compared. These studies have consistently reported a greater degree of inhibition of wild-type CYP2D6 enzyme compared with variant forms of CYP2D6 (Hamelin et al., 2000; Lessard et al., 2001; Llerena et al., 2001; Lindh et al., 2003). The variant alleles of CYP2C19, as well as those of CYP2D6, in the above studies cause either a splicing defect or a frame shift resulting in either premature termination of translation or a truncated protein. Thus, these genotype-dependent inhibition results for CYP2C19 and CYP2D6 polymorphisms identified in these studies are not surprising, given that the polymorphisms result in the expression of inactive proteins, such that no residual activity is present to be inhibited. The present findings suggest that despite CYP2C9.3 protein maintaining ~20 to 30% residual activity compared with the CYP2C9.1 enzyme, with respect to fluconazole inhibition, the CYP2C9.3 enzyme in vivo behaves as if it were inactive in that the contribution to overall clearance of the drug is so minor that inhibition has little effect on overall clearance.

These findings of genotype-dependent inhibition in an enzyme with residual activity have potentially important clinical implications. If
one were to reduce the dose of a target drug based on interaction potential of the inhibitor without considering genotype, the net result could be underdosing of the patient and potentially suboptimal therapy. Thus, if these results are more generally applicable, one may also need to consider both genotype status and fraction metabolized for drugs metabolized by polymorphic enzymes in situations in which combinations of drugs that are known to interact must be used. Additional studies are underway to determine whether this phenomenon in CYP2C9*3 subjects occurs with other substrates or is limited to the studied combination of flurbiprofen and flunixin.

References


