Decreased Warfarin Clearance with the *CYP2C9* R150H (*8*) Polymorphism

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Abstract

The cytochrome P450 (CYP) 2C9 R150H (*8) allele occurs commonly in African Americans and is associated with lower warfarin dose requirements. We examined whether the \textit{CYP2C9*8} allele impacts warfarin clearance through a pharmacokinetic study in warfarin-treated African American patients and an \textit{in vitro} kinetic study of \textit{S}-warfarin 7-hydroxylation using cDNA-expressed CYP2C9 enzymes. We observed a 30% reduction in the unbound oral clearance of \textit{S}-warfarin and 25% lower \textit{R}- to \textit{S}-warfarin plasma concentration in patients with the \textit{CYP2C9*8} allele (n=12) compared to \textit{CYP2C9*1} homozygotes (n=26). Consistent with these findings, the \textit{in-vitro} intrinsic clearance of \textit{S}-warfarin was 30% lower with the cDNA-expressed R150H protein compared to the wild-type protein. These data show that the R150H variant of the \textit{CYP2C9*8} allele reduces \textit{S}-warfarin clearance, thus providing clinical and experimental evidence to explain lower warfarin dose requirements with the \textit{CYP2C9*8} allele.
Introduction

Cytochrome P450 (CYP) 2C9 is responsible for the oxidative metabolism of approximately 15% of clinically used drugs, including warfarin.[1] Warfarin is the most commonly prescribed oral anticoagulant worldwide for the prevention of venous thromboembolism and stroke. Warfarin is characterized by a large inter-individual variability in the dose required for optimal anticoagulation and a narrow therapeutic index.[2] The CYP2C9 enzyme metabolizes the more potent $S$-warfarin enantiomer primarily to $S$-7-hydroxywarfarin, while CYP3A4 is the primary enzyme catalyzing $R$-warfarin hydroxylation.[3]

The highly polymorphic $CYP2C9$ gene has over 35 known variant alleles, many of which result in decreased enzyme activity.[4] In particular, it is well documented that the $CYP2C9^*2$ (R144C) and $*3$ (I359L) alleles reduce enzyme activity, $S$-warfarin metabolism, and warfarin dose requirements.[3, 5-10] There is also evidence of increased bleeding risk with the $CYP2C9^*2$ and $*3$ polymorphisms.[11, 12] The warfarin labeling was recently revised to include dosing recommendations based on $CYP2C9$ genotype, with recommendations limited to the $CYP2C9^*2$ and $*3$ variants.[13]

While $CYP2C9^*2$ and $*3$ are the predominant variants in Caucasians, with a prevalence of 30% to 35%, they occur in only about 6% of African Americans.[14, 15] Consequently, the $CYP2C9^*2$ and $*3$ alleles explain less of the variance in warfarin dose among persons of African descent compared to European Caucasians.[16] The $CYP2C9$ R150H (rs7900194) polymorphism, which defines the $CYP2C9^*8$ allele, is nearly twice as common as the
CYP2C9*2 and *3 alleles combined in African Americans, with a frequency of 0.05 to 0.06.[17, 18] Thus, approximately 10% to 12% of African Americans carry the CYP2C9*8 allele. The CYP2C9 D360E (*5), 10601delA (*6), and R335W (*11) alleles also occur almost exclusively in African Americans, albeit at lower frequencies than the CYP2C9*8 allele, and are associated with reduced enzyme activity and clearance of CYP2C9 substrates.[19, 20]

We recently showed that African Americans with the CYP2C9*8 allele require significantly lower warfarin doses to achieve optimal anticoagulation compared to CYP2C9*I allele homozygotes.[15] Similarly, Scott et al.[17] described a single African American patient with the *8/*8 genotype who had a significantly lower warfarin maintenance dose than expected based on other genotypes and clinical factors. The CYP2C9*8 allele was also correlated with lower warfarin dose requirements in a South African population.[21] These data suggest that the CYP2C9*8 allele reduces warfarin clearance. However, there are limited data on the functional significance of the CYP2C9*8 allele, and existing data are conflicting. Specifically, the R150H variant was reported to increase metabolism of tolbutamide in vitro,[20] reduce metabolism of phenytoin in-vivo,[22] and have no effect on the in-vivo metabolism of losartan.[23] Importantly, there are no functional or pharmacokinetic studies of the CYP2C9*8 allele using warfarin as a phenotyping probe. Information about the extent to which the CYP2C9*8 allele impacts warfarin clearance is important to inform appropriate pharmacogenetic testing and warfarin dosing. In order to systematically investigate the impact of the CYP2C9*8 allele on warfarin clearance as an explanation for its association with lower warfarin dose requirements, we conducted an in vivo pharmacokinetic study in warfarin-treated patients and in
**Results**

Pharmacokinetic study

A total of 38 African Americans on a stable dose of warfarin were enrolled, including 26 with the *CYP2C9*1/*1, 10 with the *CYP2C9* *1/*8, and two with the *CYP2C9*8/*8 genotype. The majority of patients were female (Table 1) and taking warfarin for secondary prevention of venous thromboembolism (81%). Body surface area (BSA) was correlated with unbound oral clearance (CLpo,u) of S-warfarin in our cohort ($P<0.05$). Thus, BSA-adjusted clearance values are reported. No other clinical factor, demographic characteristic, or *VKORC1* genotype was associated with S-warfarin clearance. With the exception of BSA-normalized CLpo,u (S), all data (including warfarin dose) were normally distributed. Warfarin dose requirements were lower in *CYP2C9*8 allele carriers versus noncarriers, as we previously reported.[15] When excluding the two *CYP2C9*8 homozygotes, doses remained lower in those with the 1/*8 genotype ($5.2 \pm 2.0$ mg/day) compared to *CYP2C9*1 allele homozygotes ($P=0.02$). All other characteristics and *VKORC1* genotype distribution were similar between *CYP2C9*8 allele carriers and noncarriers.

Clinical pharmacokinetic data are shown in Table 2. As expected, CLpo (R) was similar between *CYP2C9*8 carriers and *1 allele homozygotes. However, BSA-normalized CLpo,u(S) was 30% lower, and the Css ratio of R- to S-warfarin (i.e., Cpr:S) was 25% lower in *CYP2C9*8 carriers. When excluding the two *CYP2C9*8 homozygotes, BSA-normalized CLpo,u (S) was
28% lower in \( CYP2C9*8 \) heterozygotes compared to the \( CYP2C9*1 \) homozygotes, as shown in Fig. 1.

*In vitro* enzyme kinetic study

Enzyme kinetic profiles for \( S \)-warfarin 7-hydroxylation and diclofenac 4′-hydroxylation in cDNA-expressed wild-type CYP2C9 and its three variants are shown in Fig. 2, and the resulting kinetic parameter estimates are presented in Table 3. In all of the reactions studied, the CYP2C9 R144C (*2) and I359L (*3) proteins showed appreciable decreases in metabolic activity compared with wild-type CYP2C9. Intrinsinc clearance (\( V_{\text{max}}/K_{\text{m}} \)) of both \( S \)-warfarin 7-hydroxylation and diclofenac 4′-hydroxylation were lower for R144C and I359L compared to the wild type protein, consistent with previous studies.[8, 9, 24] The apparent \( K_{\text{m}} \) of \( S \)-warfarin 7-hydroxylation with the R150H variant was similar to that of the wild-type, but the apparent \( V_{\text{max}} \) was significantly lower than that of wild type protein (\( p<0.01 \)), resulting in an overall 30% reduction of the intrinsic clearance compared with the wild-type protein (\( p<0.05 \)). In contrast, the R150H protein catalyzed diclofenac 4′-hydroxylation at rates comparable to, but slightly lower than, those of the wild-type CYP2C9.

**Discussion**

Our group and others have reported lower warfarin dose requirements among persons of African descent who have a \( CYP2C9*8 \) allele, as defined by the R150H amino acid substitution.[15, 17, 21] These data suggest that, similar to \( CYP2C9*2 \) and \( *3 \) alleles, the
*CYP2C9*8 variant reduces enzyme activity. However, data on the clinical and functional significance of the *CYP2C9*8 allele are inconsistent and even conflicting. Specifically, *in-vitro* data with the *CYP2C9*8 allele show greater activity toward tolbutamine.[20] This is in contrast to clinical pharmacokinetic studies reporting a reduction in phenytoin metabolism and no effect on losartan metabolism with the *CYP2C9*8 allele.[22, 23] To our knowledge, our study is the first to examine the effects on the *CYP2C9*8 variant on warfarin kinetics.

We found that warfarin-treated patients with the *CYP2C9*8 allele have significantly lower body size-adjusted unbound clearance of S-warfarin, a good indicator of the hepatic metabolizing activity, compared to *CYP2C9*1 homozygotes, indicating that the *CYP2C9*8 variant results in a reduction of S-warfarin elimination. In contrast, as expected, there was no significant difference in the clearance of R-warfarin between genotype groups. Because S-warfarin possess more potent anticoagulant activity than the R-enantiomer, [2] and S-warfarin 7-hydroxylation dominates its oral, unbound clearance, [25] our data support and explain our previous observation of lower warfarin dose requirements with the *CYP2C9*8 allele.[15]

Only two patients had the *8/*8 genotype, limiting comparisons between the 3 possible genotypes at the R150H position. However, body size-adjusted unbounded clearance of S-warfarin was significantly lower with the *1/*8 compared to the *1/*1 genotype, suggesting that pharmacokinetic differences by the R150H genotype were not driven by inclusion of the two homozygotes for the variant allele.

Our *in vitro* kinetic data are consistent with our *in-vivo* data and offer experimental evidence that the reduction of S-warfarin clearance results from decreased enzyme activity due to
the R150H substitution. The mechanism underlying the effects of the R150H variant on
S-warfarin 7-hydroxylation remains unclear and requires further investigation. The R150H
variant is located in exon 3 distal to the R144C substitution that defines the CYP2C9*2 allele.
The R144C substitution is located outside the active site of CYP2C9. Nonetheless, it is well
documented that the CYP2C9*2 variant moderately reduces enzyme activity and dose
requirements of warfarin.[5, 6, 8, 10] The reduced catalytic activity of R144C may be related
to an altered interaction between CYP2C9 and cytochrome P450 reductase[9] or differential
uncoupling to shunt products.[26] Given the fairly close proximity of the R144C and R150H
substitutions, R150H may reduce enzyme activity through a mechanism similar to that of the
R144C allele; although this has yet to be determined.

Our clinical and in-vitro findings with warfarin are consistent with previous findings of
reductions in phenytoin metabolism with the CYP2C9*8 allele.[22] However, our in-vitro data
indicate that R150H catalyzes diclofenac 4’-hydroxylation at rates comparable to those of the
wild-type, a result different from that with warfarin. The magnitude of reduction in enzyme
activity with different CYP2C9 variants is reported to be highly substrate-dependent.[27] For
example, similar to our findings, Dickmann et al.[19] showed that the CYP2C9*3 and *5 variants
have a greater impact on S-warfarin 7-hydroxylation than on diclofenac 4’-hydroxylation. Our
disparate in-vitro findings with warfarin and diclofenac, together with conflicting data on
CYP2C9*8 effects on the metabolism of other substrates, support substrate-dependent activity of
the R150H allele. Kumar et al.[28] suggest that substrates bind to different regions (or
orientation) within the large active site of CYP2C9, and this may provide a potential explanation
for substrate-dependent effects of CYP2C9 variants on enzyme activity. In the absence of confirmatory data on the substrate specificity of the CYP2C9*8 variant, our disparate findings with warfarin and diclofenac should serve as a caution against generalizing our findings with warfarin and the CYP2C9*8 allele to other substrates.

In order to more clearly elucidate the effects of the CYP2C9*8 allele on warfarin clearance, we excluded patients taking potent CYP2C9 inducers or inhibitors or with significant liver disease. As such, we cannot draw any conclusions about the effect of the CYP2C9*8 allele on warfarin clearance in these types of patients. We also excluded patients with the CYP2C9*8 allele and another CYP2C9 variant (e.g. *2/*8). The CYP2C9*1/*2 and *2/*2 genotypes are reported to reduce S-warfarin clearance by approximately 40% and 70%, respectively, whereas we observed a 28% reduction in clearance with the CYP2C9*1/*8 genotype. Thus, one might expect a reduction in clearance within the intermediate range of 40% to 70% with the *2/*8 genotype. Another limitation to our study is that we limited our analysis to the CYP2C9*8 allele. The less common CYP2C9*6 and *11 alleles also occur almost exclusively in African Americans, and their effects on warfarin clearance remain to be determined.

In conclusion, our pharmacokinetic and in vitro data indicate that the R150H substitution defining the CYP2C9*8 allele results in an intermediate reduction of S-warfarin metabolism. The present findings shed light on the mechanism underlying reduced warfarin dose requirements with the CYP2C9*8 allele. Ultimately, consideration of CYP2C9*8 genotype, in addition to CYP2C9*2, CYP2C9*3, and other variants that result in reduced warfarin clearance
may improve the accuracy of warfarin dosing and potentially reduce the risk for supratherapeutic anticoagulation and bleeding across racial groups.

**Methods**

Clinical pharmacokinetic study

*Patients*

Warfarin-treated African American patients with the *CYP2C9* R150H variant were identified among patients genotyped as part of previous warfarin pharmacogenetic studies.[15, 18] Inclusion criteria for the current study were treatment with a fixed maintenance dose of warfarin for at least 2 weeks and an international normalized ratio (INR) within 0.1 units of the target range by point-of-care testing using the ProTime® monitor (ITC, Edison NJ, USA).

Exclusion criteria were concomitant treatment with moderate to potent inducers or inhibitors of *CYP2C9*-mediated warfarin metabolism (e.g. phenytoin, carbamazepine, rifampin, amiodarone, metronidazole, sulfonamides), documented history of hepatic disease or recent (within the previous 6 months) serum transaminase levels greater than 2 times the upper limit of normal, and nonadherence to warfarin within the previous 2 weeks. Of 26 patients from our clinic who were previously genotyped for the R150H allele (6 homozygotes and 20 heterozygotes),[15, 18] 10 were no longer followed in the clinic, 4 had another *CYP2C9* variant (e.g. *5 or *11) or were taking a *CYP2C9* inducer (e.g. carbamazepine) and were excluded, and the remaining 12 were enrolled. Additional patients with the *CYP2C9*1/*1 genotype, based on absence of the *CYP2C9*2, *3, *5, *6, *8, or *11 allele, who met the same eligibility criteria and were of similar
age and gender as R150H carriers were consecutively enrolled as they presented to the Antithrombosis Clinic for their regularly scheduled visit.

**Procedures**

Patients were approached about study participation during a regularly scheduled visit to the pharmacist-managed anticoagulation clinic at the University of Illinois at Chicago. After obtaining written informed consent, a venous blood sample was collected 12 to 16 hours after the last warfarin dose for determination of warfarin enantiomer concentrations and confirmation of \textit{CYP2C9} genotype. Clinical data were collected via subject interview and review of the medical record. Creatinine clearance was estimated using the Cockcroft-Gault equation with ideal body weight.\[29\] The study protocol was approved by the University of Illinois at Chicago Institutional Review Board and conducted according to the Declaration of Helsinki.

**\textit{CYP2C9 genotyping}**

Genomic DNA was isolated from whole blood using a Puregene kit (Qiagen, Valencia, CA). The \textit{CYP2C9} R144C (*2), I359L (*3), D360E (*5), 10601delA (*6), and R335W (*11) polymorphisms and the \textit{VKORC1} -1639G>A (rs9923231) genotype were determined by PCR and pyrosequencing, and R150H (*8) was determined by PCR and capillary sequencing, as previously described.\[30-32\]

**Pharmacokinetic data analysis**

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Total and free concentrations of warfarin enantiomers were determined by a chiral HPLC-based method as previously described.[33, 34] The oral clearance (CLpo) of both enantiomers and unbound oral clearance (CLpo,u) of S-warfarin were calculated according to equations 1 and 2:[34]

\[
CLpo = \frac{D}{2\tau} / C_{ss} \tag{1}
\]

\[
CLpo,u (S) = \frac{D}{2\tau} / C_{u,ss} \tag{2}
\]

in which D is the daily dose of racemic warfarin, \(\tau\) is the dosing interval, \(C_{ss}\) is the average total plasma concentration of S- or R-warfarin at steady state, and \(C_{u,ss}\) is the average unbound plasma concentration of S-warfarin at steady state.. This formula assumes, based on experimental data, that the oral bioavailability of racemic warfarin is complete, and that warfarin plasma concentration measured 12 to 16 hours after dosing at steady state is very close to the \(C_{ss}\).[34] Pharmacokinetic parameters were corrected for BSA based on a correlation between BSA and CLpo,u in our cohort and previous evidence that physiologic parameters relevant to drug metabolism and elimination (e.g., renal and hepatic function) are proportional to body surface area.[35]

*In-vitro* kinetic studies

**Chemicals**

S-warfarin, 7-hydroxywarfarin, diclofenac, 4'-hydroxyclofenac, mebendazole, indomethacin, isocitric acid, isocitric acid dehydrogenase, and NADP+ were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of HPLC grade or of the highest
grade commercially available.

Cell Culture

HepG2 cells from ATCC (Manassas, VA) were cultured in complete DMEM supplemented with 10% fetal bovine serum (Gemini, Woodland, CA, USA), 2 mM L-glutamine, 100 U penicillin/ml, 100 μg streptomycin/ml, and 1% MEM nonessential amino acids.

Enzyme kinetics assays

To examine the effect of the R150H allele on CYP2C9 activity, a kinetic study of S-warfarin 7-hydroxylation was conducted using cDNA-expressed wild-type or variant CYP2C9 proteins. The CYP2C9 R144C (*2) and I359L (*3) proteins were included as positive controls. Diclofenac, a well-established substrate of CYP2C9, was included as a control substrate.

cDNA-expressed CYP2C9*1 (wild type), *2 (R144C), *3 (I359L), and *8 (R150H) coexpressing human NADPH-P450 reductase (CPR) and human cytochrome b5 (b5) were purchased from BD Biosciences Corp. (Woburn, MA, USA). The P450 contents were provided by the manufacturer in the datasheets. A typical incubation mixture (200 μl total volume) contained recombinant CYP2C9*1, *2, *3, or *8 (final concentration: 8 pmol/incubation for S-warfarin and 4 pmol/incubation for diclofenac), 100 mM Tris-HCl buffer (pH 7.4), NADPH-generating system (5 mM isocitric acid, 0.2 unit/ml isocitric acid dehydrogenase, 5 mM magnesium chloride, 1 mM NADP+), and a range of concentrations of substrates of CYP2C9, including S-warfarin (0.2-200 μM), or diclofenac (0.2-200 μM). After pre-incubation at 37°C for 5 min, the reactions were
started by addition of NADP+ and incubated at 37°C for 20 min for S-warfarin, or 10 min for diclofenac. The reactions were terminated by addition of 200 μl acetonitrile containing internal standard, followed by centrifugation at 16,100 ×g for 10 min to obtain the supernatant. Aliquots were then analyzed by LC/MS/MS (Applied Biosystems, 3200 Qtrap) equipped with an electrospray ion source.

Chromatographic separation was achieved with a Waters XterraTM MS C18 column (2.1 × 50 mm, 3.5 μm; Agilent Technologies, Santa Clara CA, USA). The mobile phase for S-warfarin consisted of 5 mM ammonium acetate buffer, pH 4.6 (A) and acetonitrile (B). For diclofenac, the mobile phase was 0.1% formic acid in water (A) and acetonitrile (B). Initial mobile-phase composition was 20% mobile phase B. The proportion of mobile phase B was increased to 90% over 2 min, then held constant for 1 min before returning to the starting composition. The system was operated in negative ion mode. 7-Hydroxywarfarin was detected by examining an ion pair of 323.1/176.9, and mebendazole was used as an internal standard (ion pair of 294.0/262.0). 4'-hydroxydiclofenac was detected by examining an ion pair of 310.1/266.1, and indomethacin was used as an internal standard (ion pair of 356.2/312.3). The metabolites were quantified by comparing the ratio of ion currents obtained for the metabolites and internal standards calibration curve. The apparent enzyme kinetic parameters Michaelis-Menten constant (Km) and maximum velocity of substrate conversion (Vmax) were determined by using nonlinear regression (GraphPad Prism 5 software, La Jolla, CA, USA).
For the clinical study, continuous data were tested for normality using the Kolmogorov-Smirnov normality test and log-transformed to obtain normality if necessary prior to analysis. Clinical characteristics and pharmacokinetic parameters were compared between CYP2C9*8 allele carriers and CYP2C9*1 allele homozygotes by the two-sided Student’s unpaired t-test. Including at least 10 patients in each genotype group was estimated to provide 80% power to detect a 0.20 difference in R:S-warfarin plasma concentration between groups, assuming a SD of 0.15.[7] Comparisons among the in vitro data from different variants of CYP2C9 were made using one-way ANOVA followed by the Student’s t-test. The threshold for statistical significance was set at p<0.05.
Acknowledgements

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Conflict of interest

Dr. Cavallari is co-inventor for U.S. Utility Patent Application No. 12/572,908 entitled, CYP2C9*8 alleles correlate with decreased warfarin metabolism and increased warfarin sensitivity. Published: May 27, 2010; Pub. No. US 2010/0130599. The authors declared no other conflict of interest.
References


8 Yamazaki, H., Inoue, K., Shimada, T. Roles of two allelic variants (Arg144Cys and


Figure Legends

Figure 1. Mean ± SEM body-size adjusted unbound, oral clearance of S-warfarin according to CYP2C9 R150H (*8) genotype. P value by one-sided unpaired t-test of log-transformed data.

Figure 2. Kinetics of S-warfarin 7-hydroxylation (A) and diclofenac 4’-hydroxylation (B) in cDNA-expressed wild-type CYP2C9 and its three variants. All data points shown represent the mean of three independent experiments in duplicate measurements.
Table 1. Patient characteristics of CYP2C9*8 carriers and CYP2C9*1 homozygotes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>*1/*8 or *8/*8 (n=12)</th>
<th>*1/*1 (n=26)</th>
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<tr>
<td>Age (yrs)</td>
<td>57 ± 12</td>
<td>54 ± 12</td>
</tr>
<tr>
<td>Female</td>
<td>9 (75)</td>
<td>24 (92)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>105 ± 37</td>
<td>99 ± 28</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>2.11 ± 0.33</td>
<td>2.02 ± 0.28</td>
</tr>
<tr>
<td>Target INR</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>9 (75)</td>
<td>3 (25)</td>
</tr>
<tr>
<td></td>
<td>24 (92)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>INR on enrollment</td>
<td>2.7 ± 0.4</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>Estimated CrCl (ml/min)</td>
<td>57 ± 27</td>
<td>62 ± 23</td>
</tr>
<tr>
<td>Warfarin dose (mg/d)</td>
<td>5.4 ± 1.9</td>
<td>7.4 ± 3.2*</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>2 (17)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>0</td>
<td>1 (4)</td>
</tr>
<tr>
<td>VKORC1 -1639 G&gt;A Genotype</td>
<td>GG</td>
<td>AG</td>
</tr>
<tr>
<td></td>
<td>9 (75)</td>
<td>3 (25)</td>
</tr>
<tr>
<td></td>
<td>23 (88)</td>
<td>3 (12)</td>
</tr>
</tbody>
</table>

Values are presented as No (%) or mean ± SD.

BSA, body surface area; INR, international normalized ratio; CrCl, creatinine clearance

*P=0.02
Table 2. Clinical pharmacokinetic parameters of R- and S-warfarin in CYP2C9*8 carriers and CYP2C9*1 homozygotes

<table>
<thead>
<tr>
<th>Kinetic parameter</th>
<th>*1/*8 or *8/*8</th>
<th>*1/*1</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLpo (R) (ml/min)</td>
<td>1.94 ± 0.79</td>
<td>1.89 ± 0.58</td>
<td>0.850</td>
</tr>
<tr>
<td>BSA-normalized CLpo (R) (ml/min/m²)</td>
<td>0.90 ± 0.22</td>
<td>0.94 ± 0.27</td>
<td>0.626</td>
</tr>
<tr>
<td>CLpo (S) (ml/min)</td>
<td>2.39 ± 0.90</td>
<td>3.27 ± 1.70</td>
<td>0.044</td>
</tr>
<tr>
<td>BSA-normalized CLpo (S)</td>
<td>1.12 ± 0.36</td>
<td>1.63 ± 0.86</td>
<td>0.015</td>
</tr>
<tr>
<td>CLpo,u (S) (ml/min)</td>
<td>182 ± 69</td>
<td>248 ± 142</td>
<td>0.062</td>
</tr>
<tr>
<td>BSA-normalized CLpo,u (S) (ml/min/m²)</td>
<td>85 ± 27</td>
<td>122 ± 66</td>
<td>0.033*</td>
</tr>
<tr>
<td>Cp (R)/(S)</td>
<td>1.29 ± 0.42</td>
<td>1.73 ± 0.69</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD.

* P value for log-transformed data
Table 3. Kinetic parameters for S-warfarin 7-hydroxylation and diclofenac 4′-hydroxylation in cDNA-expressed wild-type CYP2C9 and its variants.

<table>
<thead>
<tr>
<th></th>
<th>CYP2C9*1</th>
<th>CYP2C9*2</th>
<th>CYP2C9*3</th>
<th>CYP2C9*8</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-warfarin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vmax (pmol/min/nmol P450)</td>
<td>310 ±10</td>
<td>88±16**</td>
<td>32±4**</td>
<td>207±30**</td>
</tr>
<tr>
<td>Km (μM)</td>
<td>3.3±0.1</td>
<td>2.0±0.3**</td>
<td>9.1±1.0**</td>
<td>3.3±0.5</td>
</tr>
<tr>
<td>Vmax/Km (μl/min/nmol P450)</td>
<td>92.5±1.3</td>
<td>43.8±3.0**</td>
<td>3.6±0.1**</td>
<td>63.8±7.7*</td>
</tr>
<tr>
<td>Diclofenac</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vmax (pmol/min/pmol P450)</td>
<td>91±12</td>
<td>44±3**</td>
<td>41±3**</td>
<td>55±6**</td>
</tr>
<tr>
<td>Km (μM)</td>
<td>11.1±0.4</td>
<td>11.0±2.4</td>
<td>14.4±1.4*</td>
<td>7.4±0.4**</td>
</tr>
<tr>
<td>Vmax/Km (μl/min/pmol P450)</td>
<td>8.2±1.1</td>
<td>4.1±0.8**</td>
<td>2.9±0.4**</td>
<td>7.3±0.7</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD of three independent experiments in duplicate.

*P < 0.05; **P < 0.01 vs. CYP2C9*1