Individual differences in metabolic clearance of S-warfarin efficiently mediated by polymorphic marmoset cytochrome P450 2C19 in livers

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Running title page

Running title: Warfarin clearance by marmoset P450 2C variants

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Abbreviations: AUC, area under the curve; HPLC, high performance liquid chromatography; P450, general term for cytochrome P450 (EC 1.14.14.1); PCR, polymerase chain reaction; \( t_{1/2} \), elimination half-life.
Abstract

Marmoset cytochrome P450 2C19, highly homologous to human P450 2C9 and 2C19, has been identified in common marmosets (Callithrix jacchus), a non-human primate species used in drug metabolism studies. Although genetic variants in human and macaque P450 2C genes account for the inter-individual variability in drug metabolism, genetic variants have not been investigated in the marmoset P450 2C19. In this study, sequencing of P450 2C19 in 24 marmosets identified three variants [p.(Phe7Leu; p.Ser254Leu; p.Ile469Thr)], which showed substantially reduced metabolic capacity of S-warfarin compared to the wild-type group in vivo and in vitro. Although mean plasma concentrations of R-warfarin in marmosets determined after chiral separation were similar between the homozygous mutant and wild-type groups up to 24 h after the intravenous and oral administrations of racemic warfarin, S-warfarin depletion from plasma was significantly faster in the three wild-type marmosets compared to the three homozygous mutant marmosets. These variants, co-segregating in the marmosets analyzed, influenced metabolic activities in 18 marmoset liver microsomes because the homozygotes and heterozygotes showed significantly reduced catalytic activities in liver microsomes toward S-warfarin 7-hydroxylation compared to the wild-type group. Kinetic analysis for S-warfarin 7-hydroxylation indicated that recombinant P450 2C19 Ser254Leu variant would change the metabolic capacity. These results indicated that the interindividual variability of P450 2C-dependent drug metabolism such as S-warfarin clearance is at least partly accounted for by P450 2C19 variants in marmosets, suggesting that polymorphic P450 2C-dependent catalytic function are relatively similar between marmosets and humans.
Introduction

Polymorphic human cytochrome P450 (P450) enzymes have been well characterized and are involved in the oxidative metabolism of a variety of endogenous and exogenous compounds. Individual differences in drug metabolism may be caused predominantly by impaired P450 variants or whole gene deletion, as extensively reported in humans (Ingelman-Sundberg and Sim, 2010). In addition, a nomenclature system for polymorphic human P450 alleles (http://www.cypalleles.ki.se/) has been established. Non-human primates are generally used for preclinical studies in order to predict the pharmacokinetics and toxicity of new drug candidates due to their physiological and genetic similarity to humans. The Old World Monkeys such as cynomolgus monkeys (Macaca fascicularis) are the most frequently used among the non-human primate species. Cynomolgus monkey P450 2C9 (Uno et al., 2015a), 2C19 (Uno et al., 2014a; Utoh et al., 2015), 2C76 (Uno et al., 2009), and 2D6 (Uno et al., 2014b; Uno et al., 2015b) are polymorphic in drug metabolism, similar to human P450s. Furthermore, development of a genotyping tool for a functionally relevant P450 2C19 allele (p.Phe100Asn; p.Ala103Val; p.Ile112Leu) in cynomolgus macaques was recently reported (Uno et al., 2014a; Uno and Yamazaki, 2016). One of the New World Monkeys, the common marmoset (Callithrix jacchus), is used in various research fields such as drug metabolism because of advantageous features including their small body size (Sasaki, 2015). Gene expression of marmoset P450 1A, 2A, 2B, 2C, 2D, 2E, 2J, and 3A in livers was reported (Shimizu et al., 2014), but genetic polymorphisms in marmoset drug metabolism have not been investigated so far.

In humans, the study of pharmacokinetics using caffeine, S-warfarin, omeprazole, metoprolol, and midazolam, in combination, as probe substrates of P450 1A2, 2C9, 2C19, 2D6, and 3A, respectively, has been reported (Turpault et al., 2009). The well-established
and clinically important anticoagulant drug S-warfarin is a typical human P450 2C9 substrate (Yamazaki and Shimada, 1997). Cynomolgus monkey P450 2C19 has high activity toward R-warfarin 7-hydroxylation (Hosoi et al., 2012), whereas marmoset P450 2C19 efficiently catalyzes S-warfarin 7-hydroxylation (Uehara et al., 2015). Inter-animal variations of R-warfarin clearance in cynomolgus monkeys are accounted for by the monkey P450 2C19 genetic variant [p.(Phe100Asn; p.Ala103Val; p.Ile112Leu)] (Uno et al., 2014a; Utoh et al., 2015). Additionally, the previous study indicated that optical resolution for plasma concentrations of S-warfarin in marmosets rapidly decreased than those of R-warfarin after intravenous administration of racemic warfarin, indicating that S-warfarin is stereo-selectively metabolized in marmoset livers, similar to human livers (Uehara et al., 2016), as mean values. Metabolic clearance of S-warfarin in marmosets in the previous study exhibited larger inter-individual variations than that of R-warfarin (Uehara et al., 2015), suggesting the possible genetic polymorphisms of marmoset P450 2C19. The characterization of polymorphic marmoset P450 enzymes is of use for understanding the catalytic function of marmosets.

In this study, resequencing of the marmoset P450 2C19 gene identified three variants [p.(Phe7Leu; p.Ser254Leu; p.Ile469Thr)] that showed substantially reduced activity compared with wild type P450 2C19 in S-warfarin 7-hydroxylation. We also report on the marmoset P450 2C19 polymorphism in R/S-warfarin 7-hydroxylation catalyzed by 18 marmoset livers in vitro and R/S-warfarin clearance in plasma from 6 marmosets in vivo. Kinetic analysis for S-warfarin 7-hydroxylation suggested that recombinant marmoset P450 2C19 Ser254Leu variant would change the metabolic capacity of marmoset liver P450 2C19.
Materials and Methods

Chemicals and enzymes

S/R-warfarin and 7-hydroxywarfarin were purchased from Sigma-Aldrich (Tokyo, Japan). Oligonucleotides were synthesized by Greiner Japan (Tokyo, Japan). Liver microsomes were prepared from individual tissue samples derived from marmosets as described previously (Uehara et al., 2015). The other chemicals were used in the highest grade commercially available.

Genotyping and plasma preparations

Twenty-four marmosets (males, >2 years old) were obtained from CLEA Japan (Tokyo, Japan). This study was approved by the gene recombination experiment safety management committee and animal ethics committee of the Central Institute for Experimental Animals and was carried out. Animal care was conducted as previously described (Uehara et al., 2015; Uehara et al., 2016) according to the Guidelines for Proper Conduct of Animal Experiments.

Genomic DNA was prepared from skin or liver samples from marmosets using the Puregene DNA Isolation Kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. Direct sequencing of marmoset P450 2C19 exons was carried out. PCR reactions were carried out in a mixture (25 μL) containing 10 ng of genomic DNA, 2.5 pmole of each primer, and 1 unit of AmpliTaq Gold 360 DNA Polymerase (Applied Biosystems, Foster City, CA). The marmoset P450 2C19 gene fragments were amplified using the following conditions: 95°C for 10 min (1 cycle); 15 s at 95°C, 30 s at 60-68°C, and 1-6 min at 72°C (35 cycles); and 7 min at 72°C (1 cycle). Sequencing was performed using ABI
PRISM BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, CA), followed by electrophoresis with the ABI PRISM 3730 DNA Analyzer (Applied Biosystems). The primers used for PCR and sequencing are listed in Table 1. Sequence analyses were conducted using DNASIS Pro (Hitachi Software, Tokyo, Japan) and Sequencer (Gene Codes, Ann Arbor, MI). Genetic variants were determined by comparison of the sequence with the marmoset P450 2C19 cDNA sequence (Uehara et al., 2015).

Two marmosets were subjected to oral and intravenous administration of a five-drug composite of caffeine, warfarin, omeprazole, metoprolol, and midazolam (cassette dosing) at doses of 0.20 mg kg\(^{-1}\) each (Utoh et al., 2016). After dosing, approximately 0.3 mL of blood was collected with a sodium heparin primed syringe at each sampling time point (0.5, 1, 2, 4, 7, and 24 h). Blood samples were centrifuged and isolated plasma was stored at −80°C until analysis. The plasma samples taken from these two marmosets, along with the previous plasma samples from four marmosets (Uehara et al., 2015; Uehara et al., 2016) in the spirit of minimizing animal usage, were analyzed for \(R-/S\)-warfarin using LC-MS/MS (10A System, Shimadzu, Kyoto, Japan; API4000, AB Sciex, Framingham, MA) as described previously (Utoh et al., 2015). Briefly, \(R-/S\)-warfarin was separately determined using a Chiralcel OD-RH column (Daicel, Tokyo, Japan) with 40% (v/v) 0.1% formic acid solution and 60% (v/v) acetonitrile for 6 min. The mass transitions (\(m/z\)) monitored were 307 \(\rightarrow\) 250 for \(R-/S\)-warfarin.

Pharmacokinetic parameters were derived from the respective plasma concentrations versus time curves using non-compartmental analysis in Phoenix WinNonlin® 6.1 (Pharsight, Mountain View, CA). The warfarin doses divided by 2 for intravenous and oral administration were used for the calculations of the pharmacokinetic parameters of \(R\)- and \(S\)-warfarin.
Metabolic capacity of recombinant marmoset P450 2C19 proteins

Wild-type and variant marmoset P450 2C19 proteins were co-expressed with NADHP-P450 reductase by heterologous expression systems in *Escherichia coli* DH5α by the methods described previously (Uehara et al., 2015). For functional characterization of two marmoset P450 2C19 variants, c.761C>T and c.1406T>C, the mutation was introduced to the expression plasmid of wild-type P450 2C19 (Uehara et al., 2015) by site-directed mutagenesis using the QuikChang XL II kit (Stratagene, La Jolla, CA) according to the manufacturer’s protocol. The third variant c.19T>C was excluded from the analysis because its position overlapped with the *N*-terminal modification site (Gillam et al., 1993; Dong et al., 1996) that has been incorporated into the expression vector to enhance protein expression. The primer pairs used were 5′-AGAACACCAAGAATTGGTAGACATTAACA-3′ and 5′-TGTTAATGTCTACCAATTCTTGGTGTTC-3′ for c.761C>T; and 5′-CAAAAACCTTGACACCACTCCAGTTGTCA-3′ and 5′-TGACAACTGGAGTGGTGTCAAGGTTTTTG-3′ for c.1406T>C. Sequences of the entire inserts were confirmed by sequencing with an ABI PRISM 3730 DNA Analyzer (Applied Biosystems). PCR products were subcloned into pCW vectors containing human NADPH-P450 reductase cDNA as described previously (Uehara et al., 2015). The membrane fractions were prepared from *E. coli* expressing P450 2C19 proteins, and the concentrations of P450 protein and NADPH-P450 reductase were measured as described previously (Uehara et al., 2015). Similar expression levels of recombinant P450 2C19 wild-type and Ser254Leu variant proteins were also confirmed using anti-human P450 2C9 antibodies (Corning Life Sciences, Woburn, MA) in the immunoblotting system described previously (Uehara et al., 2015). S/R-Warfarin 7-hydroxylation activities at substrate concentrations of 10 and 100 µM, unless otherwise specified, by recombinant marmoset P450
2C proteins and marmoset liver microsomes were measured as described previously (Uehara et al., 2015).

One-way analysis of variance (ANOVA) with Dunnett’s post test to compare kinetic parameters among the genotype groups and kinetic analyses with Michaelis-Menten equations were carried out using Prism (Graphpad Software, La Jolla, CA).
Results

In our previous research (Uehara et al., 2015), the metabolic clearance of S-warfarin in four marmosets had a large inter-individual variation, suggesting possible genetic polymorphisms. Thus, these four marmosets, along with two additional marmosets, were genotyped by direct sequencing. The analysis found novel P450 2C19 variants [p.(Phe7Leu; p.Ser254Leu; p.Ile469Thr)] in this study. In order to study the pharmacokinetics of S- and R-warfarin in marmosets, plasma concentrations of S-/R-warfarin (determined after chiral separation) after single intravenous and oral administration to the six male marmosets at doses of 0.20 and 1.0 mg racemic warfarin kg\(^{-1}\) each, and are summarized in Fig. 1. Consequently, three wild-type and three homozygous marmosets for P450 2C19 [p.(Phe7Leu; p.Ser254Leu; p.Ile469Thr)] were evaluated in the current study (Fig. 1). Plasma concentrations of R-warfarin (determined after chiral separation) were similar between the homozygous and wild-type groups up to 24 h after the intravenous (Fig. 1B) and oral (Fig. 1D) administrations of warfarin under the present conditions. However, S-warfarin depletion from plasma was faster in the wild-type group compared to the homozygous group after the intravenous (Fig. 1A) and oral (Fig. 1C) administrations of racemic warfarin.

Calculated mean pharmacokinetic parameters for S- and R-warfarin in the three wild-type and three homozygous marmosets are summarized in Tables 2 and 3. No significant effects of the genotype were seen on the kinetic parameters of in vivo pharmacokinetics of R-warfarin in the wild-type and homozygous mutant groups (Table 2). For the same animal groups, in contrast, significant effects of the genotype \((p < 0.05)\) were observed on the elimination half-lives \((t_{1/2})\), area under the curves (AUC), and clearance values of S-warfarin (Table 2). Similarly, kinetic parameters for R-warfarin orally administered were not
different between the wild-type and homozygous groups (Table 3); however, mean values of $t_{1/2}$ and AUC in the homozygous group were significantly higher than those of the wild-type group (Table 3).

$R/S$-Warfarin 7-hydroxylation activities mediated by eighteen marmoset liver microsomes were determined in vitro. One-way ANOVA revealed no significant effects of the genotype on $R$-warfarin 7-hydroxylation activities among the wild-type, heterozygous, or homozygous groups (Fig. 2A). Among the same animal groups, in contrast, significant effects of the genotype ($p < 0.05$) were observed on $S$-warfarin 7-hydroxylation activities at substrate concentrations of 10 µM (Fig. 2B) and 100 µM (Fig. 2C). Mean values of $S$-warfarin 7-hydroxylation activities at 10 µM in the heterozygous and homozygous mutant groups were significantly lower than that of the wild-type group as shown by one-way ANOVA post tests (Fig. 2B); the mean $S$-warfarin 7-hydroxylation activities (at 100 µM) of the homozygous group was significantly lower than that of the wild-type group (Fig. 2C).

Recombinant marmoset P450 2C19 wild-type enzyme showed a similar affinity ($K_m$, 14 ± 1 µM) but higher $V_{max}$ (334 ± 5 min$^{-1}$) rates for $S$-warfarin 7-hydroxylation as compared to P450 2C19 Ser254Leu variant protein ($K_m$, 12 ± 2 µM; $V_{max}$, 61 ± 1 min$^{-1}$) (Fig. 3). Because marmoset P450 2C19 Ile469Thr variant protein in bacterial membrane preparations did not show any CO-difference spectra or immunoreactive bands with commercial anti-human P450 2C9 antibodies, its catalytic function could not be evaluated under the current conditions.
Discussion

Marmoset cytochrome P450 2C19, highly homologous to human P450 2C9 and 2C19, were identified in marmosets (Uehara et al., 2015), non-human primate species used in drug metabolism studies. Although genetic variants in human and macaque P450 2C genes account for the inter-individual variability in drug oxidation activities (Uno et al., 2014a; Utoh et al., 2015), genetic variants have not been investigated in marmoset P450 2C19. The pharmacokinetics of the human P450 probes caffeine, warfarin, omeprazole, metoprolol, and midazolam (Turpault et al., 2009) were investigated in mice transplanted with human hepatocytes, marmosets, monkeys, dogs, and minipigs at doses of 1.0 mg/kg to clarify the similarities and occasional species differences (Mogi et al., 2012; Koyanagi et al., 2015; Uehara et al., 2016; Utoh et al., 2016). Racemic warfarin was metabolized to 7- and 6-hydroxywarfarin in marmosets in vivo in a similar manner to humans (Uehara et al., 2016). The similar stereo-selectivity for warfarin 7-hydroxylation between marmosets and humans were supported by drug-metabolizing properties of marmoset P450 2C19 and human P450 2C9 (Uehara et al., 2015; Hosoi et al., 2012; Yamazaki and Shimada, 1997). In this study, the metabolic clearance of S-warfarin with a large inter-individual variation was mediated by polymorphic marmoset P450 2C19 enzymes involved in drug oxidations (Fig. 1). In contrast, R-warfarin clearance in marmosets in vivo was not associated with marmoset P450 2C19 genetic variants (Figs. 1B and 1D) because this reaction is catalyzed by other P450 forms such as P450 2C18 in marmoset liver microsomes (Uehara et al., 2015).

It is well-known that human P450 genetic variants (http://www.cypalleles.ki.se/) may cause inter-individual differences in pharmacokinetics or toxicokinetics of drugs. Whole-genome sequencing has indicated the genetic divergence of marmosets (Sato et al., 2015). Indeed, in the current study, genetic variants of marmoset P450 2C19 [p.(Phe7Leu; p.Ser254Leu; p.Ile469Thr)] were found by direct sequencing of 18 marmoset genomes (Fig.
2). Kinetic analysis for S-warfarin 7-hydroxylation showed that recombinant marmoset P450 2C19 Ser254Leu variant would change the metabolic capacity of marmoset P450 2C19 in liver microsomes (Fig. 3). Although p.Phe7Leu, p.Ser254Leu, and p.Ile469Thr were co-segregating in the marmosets analyzed in this study, the effects of p.Ser254Leu on recombinant marmoset P450 2C19 protein appeared to sufficiently account for substantial reduction of S-warfarin 7-hydroxylation activities in the heterozygotes and/or homozygotes \textit{in vitro} and \textit{in vivo} for the compound variant allele of [p.(Phe7Leu; p.Ser254Leu; p.Ile469Thr)]. The P450 2C19 variant Ser254Leu (c.761C>T) is located at 14 amino acids after the end of substrate recognition site 4 (Uehara et al., 2015). The Ser254 residue of marmoset P450 2C19 is highly conserved among 7 P450 isoforms including marmoset P450 2C18, 2C58, and 2C76 and human 2C8, 2C9, and 2C19, implying this position as relatively important area for catalytic function even exiting outside the substrate recognition sites (Uehara et al., 2015). In contrast, another P450 2C19 Ile469Thr (c.1406T>C) residue exists just before the substrate recognition site 6 (Uehara et al., 2015), but is not conserved among these P450 forms, because human P450 2C8, 2C9, and 2C19 forms have the common Thr residue at the corresponding positions. It could not be completely ruled out the deleting effects of this Ile469Thr mutation, but presumably altering effects of this variant would be assumed under the present findings. Taken together, the novel genetic polymorphisms of marmoset P450 2C19 gene would be expected to alter P450 2C19 function and could account for the inter-animal variability in P450 2C-dependent S-warfarin clearance and oxidation in marmosets.

Based on our recent paper describing roles of marmoset P450 2C19 in warfarin oxidation (Uehara et al., 2015), in this present study, the novel marmoset P450 2C19 variant [p.(Phe7Leu; p.Ser254Leu; p.Ile469Thr)] was identified and associated with substantially reduced activity compared to wild type P450 2C19 in terms of S-warfarin 7-hydroxylation. In
conclusion, the present results demonstrate that polymorphic marmoset P450 2C19 has functional characteristics toward the typical substrate S-warfarin, similar to human P450 2C9, decontaminating that polymorphic P450 2C-dependent drug oxidation activities show relative similarities between marmosets and humans.
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Authorship contribution

Participated in research design: Uehara, Uno, and Yamazaki

Conducted experiments: Uehara, Uno, Inoue, Kawano, and Toda

Contributed new reagents or analytic tools: Inoue and Sasaki

Performed data analysis: Uehara, Uno, Shimizu, Utoh, and Yamazaki

Wrote or contributed to the writing of the manuscript: Uehara, Uno, and Yamazaki
References


Uno Y, Uehara S, Kohara S, Osada N, Murayama N and Yamazaki H (2015b) CYP2D44


Footnotes

Shotaro Uehara and Yasuhiro Uno equally contributed.

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Legends for figures

Fig. 1. Plasma concentrations of S- and R- warfarin after intravenous and oral administration of racemic warfarin in marmosets.

Plasma concentrations of S- (A,C) and R- (B,D) warfarin after intravenous (0.2 mg/kg, A,B) and oral (1.0 mg/kg, C,D) administration of racemic warfarin were measured in six marmosets genotyped for \textit{P450 2C19} [p.(Phe7Leu; p.Ser254Leu; p.Ile469Thr)]. The individual values observed in wild-type and mutant-type \textit{P450 2C19} [p.(Phe7Leu; p.Ser254Leu; p.Ile469Thr)] are marked by solid and broken lines, respectively. Results of R-/S-warfarin plasma concentrations from four marmosets (with circles and triangles) only after intravenous administration of warfarin were partly reported in our previous paper (Uehara et al., 2015).

Fig. 2. S- and R-warfarin 7-hydroxylation activities mediated by liver microsomes from marmosets genotyped for \textit{P450 2C19}.

Four wild-type, six heterozygous, and eight homozygous marmosets for \textit{P450 2C19} [p.(Phe7Leu; p.Ser254Leu; p.Ile469Thr)] were used. Individual (plots) and mean (bars) from 18 marmosets were analyzed (*\( p < 0.05 \), one-way ANOVA with Dunnett’s post tests, as compared to the wild-type group).

Fig. 3. Kinetic analyses for S-warfarin 7-hydroxylation activities mediated by recombinant marmoset \textit{P450 2C19}.

Functional activities of recombinant marmoset P450 2C19 for wild-type \((K_m, 14 \pm 1 \mu\text{M}; V_{\text{max}}, 334 \pm 5 \text{ min}^{-1})\) and Ser254Leu variant \((K_m, 12 \pm 2 \mu\text{M}; V_{\text{max}}, 61 \pm 1 \text{ min}^{-1})\) proteins in S-warfarin 7-hydroxylation were determined by nonlinear regression analysis.
Table 1

The primers used for amplification of marmoset *P450 2C19*.

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<th>Exon</th>
<th>F</th>
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<td>TTAGCAATACCTGGGCTCCA</td>
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<td></td>
<td>R</td>
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F, forward primer; R, reverse primer; S, sequence primer.
Table 2. Pharmacokinetic parameters of R-/S-warfarin after intravenous administration of racemic warfarin at a dose of 1.0 mg/kg body weight.

<table>
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<th>Genotype</th>
<th>t_{1/2} (h)</th>
<th>C₀ (ng/mL)</th>
<th>AUC_{inf} (ng·h/mL)</th>
<th>Clearance (mL/h/kg)</th>
<th>Vss (mL/kg)</th>
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<tr>
<td><strong>wt/wt</strong></td>
<td>7.9 ± 0.9</td>
<td>452 ± 52</td>
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<td>29.4 ± 1.2</td>
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<tr>
<td><strong>mut/mut</strong></td>
<td>15.2 ± 3.2*</td>
<td>446 ± 117</td>
<td>7590 ± 1850*</td>
<td>13.8 ± 3.6*</td>
<td>272 ± 34</td>
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<td><strong>R-Warfarin</strong></td>
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<tr>
<td><strong>wt/wt</strong></td>
<td>11.0 ± 1.1</td>
<td>423 ± 42</td>
<td>5920 ± 205</td>
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</tbody>
</table>

Three wild-type marmosets and three homozygotes for P450 2C19 [p.(Phe7Leu; p.Ser254Leu; p.Ile469Thr)] were used. Results are expressed as mean values (± SD) obtained from 6 marmosets (***p < 0.01 and *p < 0.05; unpaired t test as compared with the wt/wt group). Half life (t_{1/2}), extrapolated plasma concentration at time 0 (C₀), area under the concentration-time curve from the time of dosing up to infinity with extrapolation of the terminal phase (AUC_{inf}), and steady state volume of distribution (Vss) are shown.
Table 3. Pharmacokinetic parameters of \( R-/S \)-warfarin after oral administration of racemic warfarin at a dose of 1.0 mg/kg body weight.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>( t_{1/2} ) h</th>
<th>( t_{\text{max}} ) h</th>
<th>( C_{\text{max}} ) ng/mL</th>
<th>AUC(_{0-24, \text{p.o.}}) ng·h/mL</th>
<th>AUC(_{\text{inf, p.o.}}) ng·h/mL</th>
<th>AUC(<em>{0-24, \text{p.o.}} / \text{AUC}</em>{\text{inf, p.o.}}) %</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>( S )-Warfarin</td>
<td>( \text{wt/wt} )</td>
<td>5.4 ± 0.4</td>
<td>0.5 ± 0.0</td>
<td>2590 ± 333</td>
<td>19100 ± 5410</td>
<td>19900 ± 5840</td>
<td>112 ± 27</td>
</tr>
<tr>
<td></td>
<td>( \text{mut/mut} )</td>
<td>17.1 ± 7.8**</td>
<td>1.2 ± 0.8</td>
<td>3210 ± 721</td>
<td>44400 ± 4830**</td>
<td>69800 ± 11100**</td>
<td>124 ± 42</td>
</tr>
<tr>
<td>( R )-Warfarin</td>
<td>( \text{wt/wt} )</td>
<td>9.8 ± 1.0</td>
<td>0.5 ± 0.0</td>
<td>2160 ± 269</td>
<td>25800 ± 4330</td>
<td>31400 ± 5660</td>
<td>87 ± 12</td>
</tr>
<tr>
<td></td>
<td>( \text{mut/mut} )</td>
<td>12.0 ± 3.3</td>
<td>1.2 ± 0.8</td>
<td>2650 ± 712</td>
<td>31300 ± 4990</td>
<td>41000 ± 1670</td>
<td>89 ± 27</td>
</tr>
</tbody>
</table>

Detailed information is as shown in the legend for Table 2. Half life (\( t_{1/2} \)), time to reach peak concentrations (\( t_{\text{max}} \)), maximum (or peak) concentration (\( C_{\text{max}} \)), area under the concentration-time curve from the time of dosing up to 24 h (AUC\(_{0-24, \text{p.o.}}\)) and infinity with extrapolation of the terminal phase (AUC\(_{\text{inf, p.o.}}\)), and bioavailability are shown.
Fig. 1

Intravenous administration

[Graph A] [Graph B]

Oral administration

[Graph C] [Graph D]

[S-Warfarin] ng/mL vs. Time after administration, h
Fig. 2

R-Warfarin 7-hydroxylation, pmol/min/mg protein

[S], 10 μM

Wild
Homozygote
Heterozygote

S-Warfarin 7-hydroxylation, pmol/min/mg protein

[S], 10 μM

Wild
Homozygote
Heterozygote

S-Warfarin 7-hydroxylation, pmol/min/mg protein

[S], 100 μM

Wild
Homozygote
Heterozygote

*
Fig. 3: S-Warfarin 7-hydroxylation, nmol/min/nmol P450.