The Effects of Genetic Polymorphisms of CYP2C9 and CYP2C19 on Phenytoin Metabolism in Japanese Adult Patients with Epilepsy: Studies in Stereoselective Hydroxylation and Population Pharmacokinetics


*Division of Pharmaceutical Sciences, †Department of Neuropsychiatry, Faculty of Medicine, and ‡Institute of Health Sciences, Kyushu University, Fukuoka; and §Department of Hospital Pharmacy, Faculty of Medicine, Tottori University, Yonago, Japan

Summary: Purpose: The aim of this study was to clarify the effects of genetic polymorphisms of cytochrome P450 (CYP) 2C9 and 2C19 on the metabolism of phenytoin (PHT). In addition, a population pharmacokinetic analysis was performed.

Methods: The genotype of CYP2C9 (Arg'44/Cys, Ile359/Leu) and CYP2C19 (*1, *2 or *3) in 134 Japanese adult patients with epilepsy treated with PHT were determined, and their serum concentrations of 5-(4-hydroxyphenyl)-5-phenylhydantoin (β-HPPH) enantiomers, being major metabolites of PHT, were measured. A population pharmacokinetic analysis (NONMEM analysis) was performed to evaluate whether genetic polymorphism of CYP2C9/19 affects the clinical use of PHT by using the 336 dose-serum concentration data.

Results: The mean maximal elimination rate (Vmax) was 42% lower in the heterozygote for Leu359 allele in CYP2C9, and the mean Michaelis–Menten constants (Km) in the heterozygous extensive metabolizers and the poor metabolizers of CYP2C19 were 22 and 54%, respectively, higher than those without the mutations in CYP2C9/19 genes. (R)- and (S)-p-HPPH/PHT ratios were lower in patients with mutations in CYP2C9 or CYP2C19 gene than those in patients without mutations.

Conclusions: Although the hydroxylation capacity of PHT was impaired with mutations of CYP2C9/19, the impairment was greater for CYP2C9. In view of the clinical use of PHT, two important conclusions were derived from this population study. First, the serum PHT concentration in patients with the Leu359 allele in CYP2C9 would increase dramatically even at lower daily doses. Second, the patients with CYP2C19 mutations should be treated carefully at higher daily doses of PHT.

Key Words: Phenytoin—CYP2C9—CYP2C19—Genetic polymorphism—NONMEM.

Phenytoin (PHT), a widely prescribed anticonvulsant (AED), is a prochiral compound that is eliminated in humans almost entirely by cytochrome P450 (CYP)-mediated oxidation (1,2). Although the major advantage of its clinical use is that a relation between serum concentration and therapeutic effect has been established, it is sometimes difficult to adjust the dose of PHT to attain a therapeutic drug concentration because of the variability in its pharmacokinetic properties [e.g., capacity limited metabolism (3,4)]. The principal metabolic pathway is formation of 5-(4-hydroxyphenyl)-5-phenylhydantoin (p-HPPH) that exists as (R)- and (S)-enantiomers and accounts for ~90% of total urinary metabolites (5). The results of recent human liver microsomal kinetic (2.6–8), healthy volunteer (9), and patient with epilepsy (3) studies suggest that the human CYP2C subfamily is involved in the hydroxylation of PHT. The genetic polymorphism of the CYP2C subfamily has been well documented in humans and may affect the pharmacokinetics and the pharmacologic responses to certain drugs (10). Among the members of CYP2C subfamily, CYP2C19 plays an important role in the metabolism of a number of drugs, such as (S)-mephentoin (MHT; 11), omeprazole (12), diazepam (DZP; 13), proguanil (14), and imipramine (15). The genetic polymorphism of CYP2C19 has been reported to be caused by point mutations of G to A in exon 5 (CYP2C19*2) (16) and G to A in exon 4 (CYP2C19*3) (17). The genetic mutations (CYP2C19*2/ *2, *3/*3, or *2/*3) lead to a defect in the functional CYP2C19 protein and impair the metabolism of these drugs. Recently we reported that the stereoselective hydroxylation pathway of PHT to form an (R)-p-HPPH
Subjects

hundred eighteen patients had received other AEDs [e.g.,
Table 1. No patients had hepatic or renal failure. One
Japan). The details of these patients are summarized in
epilepsy treated at the Department of Neuropsychiatry of
granule of PHT (Aleviatin; Dainippon, Co. Ltd., Osaka,
routinely treated with oral administration of the tablet or
Kyushu University Hospital. These patients had been
were receiving long-term PHT therapy.

CYP2C9, another major enzyme in the human liver, catalyzes a wide range of therapeutic agents, including
tolbutamide (18), warfarin (19), and PHT (7). Several
amino acid variants of CYP2C9 have been reported at
Arg [Arg144/Cys (CYP2C9*2), Tyr359/Cys, Ile359/Leu
(CYP2C9*3) and Gly417/Asp (19). The existence of the
allelic variants of CYP2C9 influences catalytic activity
toward various substrates. Veronese et al. (8) indicated that the amino acid substitution, Leu359 → Ile, increased
the rate of PHT hydroxylation by complementary DNA
(CDNA) expressed CYP2C9 in COS cells (8). However,
it is not clear to what extent these substitutions affect
functional CYP2C9 activity in vivo.

In this study, we examined the effect of CYP2C9/19
mutations on the stereoselective hydroxylation of PHT
and performed a population pharmacokinetic analysis in
medium-sized Japanese adult patients with epilepsy who
were receiving long-term PHT therapy.

METHODS

Subjects

The subjects were 134 Japanese adult patients with
epilepsy treated at the Department of Neuropsychiatry of
Kyushu University Hospital. These patients had been
routinely treated with oral administration of the tablet or
granule of PHT (Aleviatin; Dainippon, Co. Ltd., Osaka,
Japan). The details of these patients are summarized in
Table 1. No patients had hepatic or renal failure. One
hundred eighteen patients had received other AEDs [e.g.,
carbamazepine (CBZ), phenobarbital (PB), or valproic
acid (VPA)] concurrent with PHT. It was confirmed that
the patients had not changed their PHT doses or their
comedications for ≥1 month before the study. They were
informed both verbally and in writing of the experimen-
tal procedure and purpose of this study. Each patient
gave written consent to participation in this study, which
was approved by the local ethics committee. All patients
were divided into four groups based on their genotypes:
G1, G2, G3 and G4 were homozygotes for the wild type
of CYP2C19, heterozygous EMs of CYP2C19, PMs of
CYP2C19, and EMs of CYP2C19 with heterozygous for
Leu359 allele in CYP2C9, respectively (see Table 2).

Genotyping procedures for CYP2C9 and CYP2C19

Blood samples (10 ml) were obtained from all pa-
tients, and genomic DNA was isolated from peripheral
lymphocytes with an extraction kit (GENOMIX; Talent,
Trieste, Italy). The CYP2C9*1 (Arg144/Ile559) gene and
two mutant alleles, CYP2C9*2 (Cys144) in exon 3 and
CYP2C9*3 (Leu559) in exon 7, were identified according
to the methods of Wang et al. (20), with minor modifi-
cations (21). The CYP2C9*1 gene and two mutant al-
leles associated with the PM of (S)-mephenytoin,
CYP2C19*2 in exon 5 and CYP2C19*3 in exon 4, were
identified according to the methods of de Morais et al.
(16,17), with minor modifications (21).

Serum-concentration measurements

The PHT concentration was routinely measured by
the fluorescence polarization immunoassay (FPIA) method.
The coefficient of variation of the assay was <10%. The
concentration of p-HPPH enantiomers in serum were
measured in duplicate by high-performance liquid chro-
matography (HPLC), according to the methods of Eto et
al. (22), with minor modifications. For HPLC analysis,
0.6 ml of 75 mM KH2PO4-K2HPO4 buffer (pH 6.8) contain-
ing allobarbital as an internal standard was added to
0.5 ml serum. As the amount of free p-HPPH enanti-
omers in the human serum sample was too low for detec-
tion, the p-HPPH conjugate was hydrolyzed before the
assay. After treatment using p-HPPH glucronide with
200 U P-glucuronidase at 37°C for 30 min, 1.0 ml of the
mixture was poured into an Extrelute-I column (E. Merck).
After 10 min, the column was eluted with 3.0 ml tert-
butyl-methyl ether. The eluate was dried and dis-
solved in 150 µl of 50% methanol, and 30-µl aliquots
were injected into the chromatograph. A Shimadzu LC-
10AS system (Shimadzu, Kyoto, Japan) equipped with a
LC-10AS pump and a UV detector (SPD-10A) was used.
The column (50 x 4 mm L.D.) was a YMC-Pack FL-ODS
with a 5-µm particle diameter (YMC, Kyoto, Japan). The
column temperature was ambient. The mobile phase was
a mixture of 11.2 mM β-cyclodextrin in 20 mM KH2PO4
and 8% methanol. The flow rate was 1.2 ml/min, and the
eluates were monitored at 210 nm. The sensitivity of the
p-HPPH assay was 10 ng/ml. The coefficient of the in-
traassay variation in (R)- and (S)-p-HPPH were 3.1 and
1.9%, those of interassay variation were 4.3 and 3.5%,
respectively. Recoveries of each enantiomer ranged from
90 to 100%.

Population pharmacokinetic analysis

For estimation of the pharmacokinetic parameters of
PHT among 134 patients, 202 serum PHT concentration
data at steady state (C∞) after repetitive dosing were
collected retrospectively for each patient in addition to
TABLE 2. CYP2C9/19 genotypes, the daily doses and serum concentrations of phenytoin in 134 patients with epilepsy

<table>
<thead>
<tr>
<th>Group</th>
<th>CYP2C9</th>
<th>CYP2C19</th>
<th>No.</th>
<th>Dose (mg/day/kg)</th>
<th>C_{ss} (µg/ml)</th>
<th>C_{ss}/dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>*1/*1</td>
<td>*1/*1</td>
<td>52</td>
<td>3.58 ± 1.48</td>
<td>6.6 ± 4.8</td>
<td>1.7 ± 0.8</td>
</tr>
<tr>
<td>G2</td>
<td>*1/*1</td>
<td>*1/*2</td>
<td>47</td>
<td>3.50 ± 1.33</td>
<td>9.1 ± 7.3</td>
<td>2.3 ± 1.2*</td>
</tr>
<tr>
<td>G3</td>
<td>*1/*1</td>
<td>*2/*2</td>
<td>17</td>
<td>2.95 ± 1.36</td>
<td>7.1 ± 5.6</td>
<td>2.2 ± 0.9</td>
</tr>
<tr>
<td>G4</td>
<td>*1/*3</td>
<td>*1/*1</td>
<td>15</td>
<td>2.09 ± 0.17</td>
<td>4.7 ± 2.0</td>
<td>2.2 ± 0.8</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD.
*1, wild-type allele.
*Compared with G1 group, p = 0.018.

the initial 134 data. All the 336 daily dose–C_{ss} pairs were used for the population analysis.

Analysis was performed with the NONMEM program developed by Beal and Sheiner (23) (Version IV, level I.1) on the Hewlett Packard computer (HP Apollo 9000, model 712/60; Palo Alto, CA). This program uses the method of extended least squares to estimate population pharmacokinetic parameters.

The Michaelis–Menten model was used to describe the pharmacokinetics of phenytoin.

\[ R_{ij} = \frac{V_{max_i} \cdot C_{ss_j}}{[F_i] \cdot (K_{m_j} + C_{ss_j})} \]  

where \( R_{ij} \) is the daily dose of PHT for the \( i \)th patient (mg/day/kg), \( V_{max_i} \) is the \( i \)th maximal elimination rate (mg/day/kg) for the \( j \)th patient, \( K_{m_j} \) is the \( j \)th Michaelis–Menten constant (µg/ml) for the \( j \)th patient, \( C_{ss_j} \) is the \( j \)th steady-state concentration (µg/ml) for the \( j \)th patient, and \( F_i \) is the bioavailability of PHT for the \( i \)th dosage form administered to the \( j \)th patient. We used \( F_i \) at a constant value of 1 because oral bioavailability of PHT is almost 100% with tablets or granules. Patient’s weight, medication (monotherapy or polytherapy), and the genotypes of the CYP2C9/19 were used to estimate the population mean value for \( V_{max} \) (Eq. 2) and for \( K_{m} \) (Eq. 3) based on our previous studies in Japanese patients with epilepsy (24).

\[ \hat{V}_{max_j} = V_{max,G1} \cdot (WGT_j/60)^{\theta_{pw}} \cdot G T_{ij}^{V_{max}}, \ CO_{ij}^{V_{max}} \]  

\[ \hat{K}_{m_j} = K_{m,G1} \cdot G T_{ij}^{K_{m}}, \ CO_{ij}^{K_{m}} \]  

\[ GT_{ij}^{V_{max}} = \Theta_{G2ij}^{V_{max}} \text{ for G2; } \Theta_{G3ij}^{V_{max}} \text{ for G3; } \Theta_{G4ij}^{V_{max}} \text{ for G4} \]  

\[ GT_{ij}^{K_{m}} = \Theta_{G2ij}^{K_{m}} \text{ for G2; } \Theta_{G3ij}^{K_{m}} \text{ for G3; } \Theta_{G4ij}^{K_{m}} \text{ for G4} \]  

\[ CO_{ij}^{V_{max}} = 1 \text{ for monotherapy; } \Theta_{CO}^{V_{max}} \text{ for polytherapy} \]  

\[ CO_{ij}^{K_{m}} = 1 \text{ for monotherapy; } \Theta_{CO}^{K_{m}} \text{ for polytherapy} \]  

Where \( \hat{V}_{max} \) and \( \hat{K}_{m} \) are the \( i \)th predicted parameters for the \( j \)th individual, respectively. \( V_{max,G1} \) and \( K_{m,G1} \) are the parameter values for the ‘‘standard’’ patient (60-kg adult patient in G1 group); \( WGT_j \) is the \( j \)th body weight of the \( j \)th individual in kg; \( \theta_{pw} \) is the power of weight for size adjustment; \( GT_{ij}^{V_{max}} \) or \( GT_{ij}^{K_{m}} \) are indicator variables for each genotype; \( \Theta_{GN}^{V_{max}} \) and \( \Theta_{GN}^{K_{m}} \) (N = 2–4) are the parameters for \( V_{max} \) and \( K_{m} \) for each genotype, respectively; \( CO_{ij}^{V_{max}} \) or \( CO_{ij}^{K_{m}} \) are indicator variables that have a value of unity if the \( j \)th patient is treated with PHT alone, and \( \Theta_{CO}^{V_{max}} \) or \( \Theta_{CO}^{K_{m}} \) otherwise, respectively; \( \hat{R}_{ij} \) is the predicted daily dosage of PHT for the \( i \)th \( C_{ss} \)-pair in the \( j \)th patient (mg/day/kg); \( e_{ij} \) is the intraindividual error and independently distributed statistical errors with mean zero and variance \( \omega_{e_{ij}}^{2} \); \( \eta_{ij}^{V_{max}} \) and \( \eta_{ij}^{K_{m}} \) are the interindividual errors and independently distributed statistical errors with mean zero and variances \( \omega_{\eta}^{2} \) and \( \omega_{\eta}^{2} \).

To test the hypothesis whether the fit of the model to the data was significantly different, we used the extended sum of squares (ESS), which was the minimum objective function determined in the NONMEM fitting routine. Assuming that the interindividual and intraindividual variances were normally distributed, then the difference in ESS was distributed as a \( \chi^2 \) with degrees of freedom equal to the number of parameters that were fixed to the hypothesized values. A difference of 3.841 with a degree of freedom of 1 was used as statistical significance (p < 0.05).

Statistics

Differences in values between various genotypes were estimated statistically by using analysis of variance (ANOVA). In all statistical tests, p < 0.05 was considered statistically significant.
RESULTS

The genotype results of CYP2C9 and CYP2C19 are shown in Table 2. No patients had the CYP2C9 mutation for the Cys144 allele, and 52 (38.8%) patients had no mutations in CYP2C9 or CYP2C19 (G1, CYP2C9*1/*1 and CYP2C19*1/*1). A total of three (2.2%) patients were heterozygous for the Leu359 allele (G4, CYP2C9*1/*3), but there were no homozygotes for the Leu359 allele. Thus the allele frequency of CYP2C9*3 was 0.0112. On the other hand, 64 (47.8%) patients were heterozygous for the CYP2C19 mutation (G2, *1/*2 or *1/*3), and 15 patients were homozygous or compound heterozygous for the mutations in CYP2C19. Thus the frequency of PMs of CYP2C19 in this study was 11.2%. The allele frequencies of the CYP2C19*2 and *3 were 0.250 and 0.108, respectively.

Table 3 shows the final estimates of the population pharmacokinetic parameters of PHT. $V_{\text{max}}$(G1) and $K_m$(G1) were estimated to be 6.07 mg/day/kg and 4.0 $\mu$g/ml in the standard patient. NONMEM estimates indicated that a nonlinear function of weight $[(\text{WGT}/60)^{-0.416}]$ as the optimal adjustment of $V_{\text{max}}$ for body size is preferable to a linear function. The values of $\theta_{G2}V_{\text{max}}$, $\theta_{G3}V_{\text{max}}$, and $\theta_{G4}K_m$ were also fixed because of no statistically significant differences in ESS compared with $V_{\text{max}}$(G1) and $K_m$(G1). $V_{\text{max}}$ in G4 and $K_m$ in G2 and G3 were estimated to be 0.582 $V_{\text{max}}$(G1) mg/day/kg, 1.22 $K_m$(G1) $\mu$g/ml and 1.54 $K_m$(G1), respectively. Thus the mean value of $V_{\text{max}}$ for G4 was 42% lower, and those of $K_m$ for G2 and G3 were 22 and 54% higher than those for G1, respectively. Figure 1 shows the relation between daily dose and serum concentration with respect to four CYP2C9/19 genotype groups: the dose-C$_{\text{cr}}$ relation was observed. The differences in the predicted serum concentrations among CYP2C19 genotypes (G1–G3) become larger as the dose increases. For example, at 5 mg/day/kg dose of PHT, predicted serum concentrations are 18.7 $\mu$g/ml for G1, 22.8 for G2, and 28.8 for G3 (arrows). G1, homozygotes for wild type of CYP2C19; G2, heterozygous extensive metabolizers (EMs) of CYP2C19; G3, poor metabolizers (PMs) of CYP2C19; G4, EMs of CYP2C9 with heterozygosity for Leu359 in CYP2C9.

![FIG. 1. Relation between daily dose and serum concentration of phenytoin (PHT) with respect to the four CYP2C9/19 genotype groups. The points show observed concentrations for daily doses, corrected to match the "standard" patient; daily dose/60 x (weight/60)$^{1.17}$. The solid lines are simulated PHT concentrations for a patient, assuming 60 kg in weight based on the NONMEM analysis. The differences in the predicted serum concentrations among CYP2C19 genotypes (G1–G3) become larger as the dose increases. For example, at 5 mg/day/kg dose of PHT, predicted serum concentrations are 18.7 $\mu$g/ml for G1, 22.8 for G2, and 28.8 for G3 (arrows). G1, homozygotes for wild type of CYP2C19; G2, heterozygous extensive metabolizers (EMs) of CYP2C19; G3, poor metabolizers (PMs) of CYP2C19; G4, EMs of CYP2C9 with heterozygosity for Leu359 in CYP2C9.](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{max}}$(G1) (mg/day/kg)</td>
<td>6.07</td>
<td>5.59 to 6.55</td>
</tr>
<tr>
<td>$K_m$(G1) ($\mu$g/ml)</td>
<td>4.0</td>
<td>3.19 to 4.81</td>
</tr>
<tr>
<td>$\theta_{G2}V_{\text{max}}$</td>
<td>-0.416</td>
<td>-0.565 to -0.267</td>
</tr>
<tr>
<td>$\theta_{G3}V_{\text{max}}$</td>
<td>0.582</td>
<td>0.548 to 0.616</td>
</tr>
<tr>
<td>$\theta_{G2}K_m$</td>
<td>1.22</td>
<td>1.03 to 1.41</td>
</tr>
<tr>
<td>$\theta_{G3}K_m$</td>
<td>1.54</td>
<td>1.21 to 1.87</td>
</tr>
<tr>
<td>$\omega_{G2}$ (%)</td>
<td>11.1</td>
<td>6.5 to 14.4</td>
</tr>
<tr>
<td>$\omega_{G3}$ (%)</td>
<td>22.8</td>
<td>12.4 to 29.8</td>
</tr>
<tr>
<td>$\sigma_{\text{cr}}$(mg/day/kg)</td>
<td>0.348</td>
<td>0.295 to 0.393</td>
</tr>
</tbody>
</table>

CI, confidence interval.
CYP2C9/19 POLYMORPHISMS AND PHT METABOLISM

![Graph](image)

**FIG. 2.** Mean values and standard errors of (R)- and (S)-p-HPPH/PHT ratio in the four CYP2C9/19 genotype groups (G1–G4). *Significant difference between the two values (p = 0.017).

of PHT was lower in patients with the mutations in CYP2C9/19 genes.

**DISCUSSION**

In this study, we examined the effect of genetic polymorphism of CYP2C9 and CYP2C19 on the pharmacokinetics of PHT by using 336 dose-\(C_{as}\) data obtained from 134 Japanese adults patients with epilepsy by a NONMEM analysis. In addition, to verify the observations from the NONMEM analysis, serum (R)- and (S)-p-HPPH concentrations were measured. The findings indicated that the genetic polymorphism of CYP2C9/19 plays an important role in the pharmacokinetic variability of PHT among patients with epilepsy; the mean \(V_{max}\) value was 42% lower in the heterozygote for Leu359, and the mean \(K_m\) values in the heterozygous EMS and PMs of CYP2C19 were 22 and 54%, respectively, higher than those in patients without mutations in CYP2C9/19 genes. Furthermore, both enantiomer/PHT ratios were lower in patients with mutations in CYP2C9 or CYP2C19 genes than those in patients without mutations, suggesting that the hydroxylation capacity of PHT is clearly impaired in patients with mutations in CYP2C9/19 genes.

In a previous study, we assessed the role of (R)- and (S)-p-HPPH in the pathogenesis of gingival hyperplasia, and a bimodal distribution was found in the serum log (R)/(S) enantiomeric ratio, which suggested that stereoselective hydroxylation of PHT is polymorphically distributed (25). Therefore to elucidate the mechanism of this polymorphic distribution, we conducted a panel study in which a single dose of PHT was administered to six healthy Japanese volunteers and demonstrated that the cumulative urinary excretion of (R)-p-HPPH at 36 h was 3.5-fold lower, and that of (S)-p-HPPH was 1.3-fold lower in genetically identified PMs of CYP2C19 than in EMS (9). The role of CYP2C9 in the hydroxylation of PHT to total \((S + R)\)-p-HPPH was elucidated by Veronese et al. (8), who indicated that cDNA expressed CYP2C9 containing the Leu359 \(\rightarrow\) Ile substitution increases the rate of PHT hydroxylation by up to fivefold. Evidence collected in vivo and in vitro suggests that CYP2C9 and CYP2C19 are involved in the hydroxylation of PHT and is consistent with our findings in this study.

Although the hydroxylation capacity of PHT was impaired with mutations of CYP2C9/19 genes, the impairment was greater for CYP2C9. Recently Bajpai et al. (2) evaluated the role of the CYP2C subfamily in the stereoselective hydroxylation of PHT by using cDNA expressed and human liver microsomes and showed preferential (R)-p-HPPH formation by CYP2C19 and preference (S)-p-HPPH formation by CYP2C9. The observations shown in Fig. 2 might support this hypothesis. In humans, (R)-p-HPPH is the minor and (S)-p-HPPH is the major enantiomer of p-HPPH formed. Thus the formation of (S)-p-HPPH might be the major determinant of the disposition of PHT. In this study, although no patient had the Cys144 allele, the frequency of the Cys144 allele in white Americans, white British, and African-Americans were 0.08, 0.125, and 0.01, respectively (26,27). The Cys144 allele is also low in other Asian groups [e.g., Chinese Taiwanese (26)]. Previous in vivo and in vitro studies have shown that the amino acid exchange for Arg144 \(\rightarrow\) Cys and Ile359 \(\rightarrow\) Leu is associated with an impaired metabolic capacity of substrates of CYP2C9 including PHT, tolbutamide, and warfarin (8, 28–31). Consequently a genotype test by simple polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) for CYP2C9 may be a useful tool in dosage adjustment and in evaluating serum concentrations of PHT.

In addition to CYP2C9, the effect of genetic polymorphism of CYP2C19 on the disposition kinetics of PHT was observed. As shown in Table 3, the mean \(K_m\) value in PMs of CYP2C19 was 54% higher than that in homozygous EMS, whereas the heterozygous EMS had a
value intermediate between these two values. These results suggest a gene–dose effect for CYP2C19. Furthermore, the mean (R)- and (S)-p-HPHP/PHT ratios were synchronized with the mean Michaelis–Menten constants (Fig. 2), supporting this hypothesis. The gene–dose effects were suspected in the disposition kinetics of the substrates of CYP2C19, such as (S)-mephenytoin (32) and omeprazole (33). Therefore, the possibility of identification of heterozygous individuals by genotyping allows an estimation of the pharmacokinetics of phenytoin in EMs.

Recently Odani et al. (28) estimated pharmacokinetic parameters of PHT by means of an empiric bayesian analysis among 44 Japanese patients with epilepsy with respect to the genetic polymorphisms of CYP2C9/19. They reported that the $V_{\max}$ of PHT in the heterozygote for Leu$^{359}$ was 33% lower than that in the homozygote for Ile$^{359}$. Also, the $V_{\max}$ in PMs of CYP2C19 was slightly lower than that in EMs without mutation. In view of the clinical use of PHT, at least two important conclusions are derived from the population studies. First, the serum PHT concentration in patients with Leu$^{359}$ allele would increase dramatically even at the lower daily dose. Second, the differences in serum PHT concentrations among patients with various CYP2C19 genotypes will become larger as the daily dose increases. The fact that the impact of mutations in CYP2C19 is greater at higher PHT doses is consistent with the greater contribution of this enzyme at higher plasma concentrations; the $K_m$ of CYP2C19 is much higher than that of CYP2C9 (2). Therefore the patients with CYP2C19 mutations, especially PMs of CYP2C19, should be treated carefully at higher daily doses.

Based on the development of biochemical technology, four members of the CYP2C subfamily, CYP2C8, CYP2C9, CYP2C18, and CYP2C19, have been identified in humans (10). Among the members of the CYP2C subfamily, CYP2C9, CYP2C18, and CYP2C19 were reported to contribute to the hydroxylation of PHT (2,3,35). We recently found genetic links between the CYP2C18 gene and the CYP2C19 gene in Japanese patients with epilepsy and speculated that the PMs of CYP2C19 might also be the PMs of CYP2C18 (36). These results suggest that CYP2C9 may be only one key enzyme for the hydroxylation of PHT in PMs of CYP2C19. Unfortunately, the heterozygote for Leu$^{359}$ with nonfunctional CYP2C19 was not observed in this study. The frequencies of amino acid substitutions are different among various ethnic groups; the frequencies of Leu$^{359}$ and Cys$^{144}$ variants in whites are higher than those in Asians (21,27,37). Therefore further study in a large population among various ethnic groups is required to elucidate the role of the CYP2C subfamily in PHT drug therapy.

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