EFFECT OF CYP2C POLYMORPHISMS ON THE PHARMACOKINETICS OF PHENYTOIN IN JAPANESE PATIENTS WITH EPILEPSY

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We examined the effect of CYP2C9/19 polymorphisms on the pharmacokinetics of phenytoin in 17 Japanese patients with epilepsy. The maximal elimination rate ($V_{\max}$) of phenytoin was slightly decreased (up to 14%) in patients with *CYP2C19* mutations for the defective allele. The $V_{\max}$ values in patients with a *CYP2C9* mutation for the heterozygous Ile/Leu³⁵⁹ allele were 40% lower than those in patients with wild-type *CYP2C9* for the homozygous Ile³⁵⁹ allele. These findings suggested that the genetic polymorphism of CYP2C isoenzymes plays an important role in the pharmacokinetic variability of phenytoin, and that the mutation in CYP2C9 proteins is a determinant of impaired metabolism of the drug.

KEY WORDS CYP2C19; CYP2C9; pharmacokinetics; phenytoin; polymorphism

Phenytoin is a commonly prescribed drug used in the treatment of epilepsy. However, the complicating factors of phenytoin treatment are non-linearity and large interindividual variability in the relationship between dosage and steady-state serum concentration.^{1,2)} Several genetic polymorphisms of drug metabolism have been documented in humans. One of the most characterized is that associated with 4'-hydroxylation of *S*-mephenytoin. Goldstein *et al.*³⁻⁵⁾ reported that CYP2C19 is the major *S*-mephenytoin 4'-hydroxylase in humans, and identified two kinds of genetic defects (m1 and m2) responsible for the polymorphism of *S*-mephenytoin metabolism in Japanese. However, phenytoin is not associated with the mephenytoin polymorphism in spite of the structural and metabolic similarities between phenytoin and mephenytoin.^{1,2)} On the other hand, Veronese *et al.*^{6,7)} showed that CYP2C9 catalyzes the metabolism of a wide range of therapeutic agents, including tolbutamide, warfarin, and phenytoin. In addition, Wang *et al.*⁸⁾ reported that the wild-type (wt) *CYP2C9* allele in Chinese subjects has Arg¹⁴⁴Tyr³⁵⁸Ile³⁵⁹Gly⁴¹⁷ in CYP2C9, and that there were 4 subjects with a *CYP2C9* mutation for the heterozygous Ile/Leu³⁵⁹ allele in 115 Chinese subjects. However, the contribution of the above mentioned CYP2C isoenzymes to the overall metabolism of phenytoin remains unclear. Here, we examined the effect of CYP2C9/19 polymorphisms on the pharmacokinetic variability of phenytoin in Japanese patients with epilepsy. To our knowledge, this is the first report describing the effect of a *CYP2C9* mutation on the *in vivo* metabolism of phenytoin.

METHODS

The subjects were seventeen patients between 2 and 29 years old, and no patients had hepatic or renal failure. Mean body weight of the patients was 42.1 kg. These patients had been routinely treated with oral administration of the conventional tablet and/or preformulated ground tablet of phenytoin (Aleviatin®, Dainippon Pharmaceutical Co., Osaka, Japan) at Kyoto University Hospital, and some of them had experienced the toxic levels of serum phenytoin concentration. Other antiepileptic drugs (*eg.* carbamazepine, valproic acid, and zonisamide) were also admini-

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stered concurrently with phenytoin to most of the patients. The mean daily dose of phenytoin was 5.30 mg/d/kg. The aim of the study was explained to the patients and/or to their parents, and informed consent was obtained. DNA was isolated from peripheral blood obtained from the selected patients, and the mutations in *CYP2C19* (m1 and m2) and *CYP2C9* (Leu³⁵⁹) were detected using the restricted fragment length polymorphism in conjunction with the polymerase chain reaction (PCR), as described previously.^{4,5,8)} The pharmacokinetic parameters of phenytoin in each patient were determined using routine therapeutic drug monitoring data. Seven serum phenytoin concentration values (on average) including the toxic levels were collected retrospectively for each patient, and the maximal elimination rate ($V_{\max}$), Michaelis-Menten constant (K_m), and volume of distribution (V) of phenytoin in individual patients were estimated by the bayesian analysis,⁹⁾ where the prior information is the population pharmacokinetic parameters described previously.¹⁰⁾ In addition, we normalized the $V_{\max}$ (in mg/d/kg) and V (in l/kg) values of phenytoin, assuming that the body weight is 42 kg in a typical patient, and that the bioavailability of the orally administered drug is 100%.¹⁰⁾

RESULTS

The seventeen patients were divided into 4 groups, based on the results of genotyping of *CYP2C9* and *CYP2C19*. Table 1 summarizes the genotypes of *CYP2C9/19* and the $V_{\max}$ and K_m values of phenytoin in individual groups. The $V_{\max}$ values of phenytoin in patients with *CYP2C19* mutations for the defective alleles (Group 2 and 3) were slightly lower (up to 14%) than those in patients with wt *CYP2C19* (Group 1). On the other hand, the $V_{\max}$ values in patients with a *CYP2C9* mutation for the heterozygous Leu³⁵⁹ allele (Group 4) were 40% lower than those in patients with wt *CYP2C9* (Group 1); ($p < 0.1$ by a Scheffé-type test). In addition, no significant change in the K_m and V values of phenytoin was observed among the groups. The mean K_m and V values in 17 patients were 9.45 $\mu\text{g/ml}$ and 1.24 l/kg, respectively.

Table 1. *CYP2C9/19* Genotypes and $V_{\max}$ Values of Phenytoin in 17 Patients with Epilepsy

Group	<i>n</i>	<i>CYP2C9</i>	<i>CYP2C19</i>	$V_{\max}$ (mg/d/kg) ^a	K_m ($\mu\text{g/ml}$) ^a
1	4	wt/wt	wt/wt	10.4 (9.5 - 11.3)	8.9 (6.8 - 10.2)
2	8	wt/wt	wt/m1 or wt/m2	9.6 (8.5 - 11.6)	9.7 (7.2 - 10.6)
3	3	wt/wt	m1/m1 or m1/m2	8.9 (8.1 - 9.9)	8.9 (7.7 - 10.0)
4	2	wt/Leu ³⁵⁹	wt/wt	6.2 (6.1 - 6.3)	10.4 (10.0 - 10.8)

^a Mean values for *n* patients are presented with ranges.

DISCUSSION

Previously, we estimated the population pharmacokinetic parameters of phenytoin in Japanese patients with epilepsy.¹⁰⁾ The bayesian analysis with the prior information allows us to estimate the pharmacokinetic parameters of phenytoin in individuals from the limited number of serum concentration values.⁹⁾ Herein, we examined the effect of *CYP2C9* and *CYP2C19* polymorphisms on the pharmacokinetics of phenytoin in Japanese patients whose drug concentration had been monitored routinely, and estimated the contribution of the *CYP2C* isoenzymes to the overall metabolism of phenytoin and its interindividual variability.

The impaired metabolism of *S*-mephenytoin in a poor metabolizer results from the defective hepatic expression of *CYP2C19* in human liver,¹¹⁾ and the *CYP2C19* mutations (m1/m1, m1/m2, and m2/m2) lead to the complete defect of *CYP2C19*.^{4,5)} *CYP2C19* is also important in the *in*

vivo metabolism of a number of related hydantoin and barbiturates, as well as in that of structurally dissimilar drugs such as omeprazole, proguanil, and citalopram.⁵⁾ However, CYP2C19 seems to be only partly responsible for the overall metabolism of phenytoin. Fritz *et al.*¹⁾ analyzed the association between mephenytoin polymorphism and enantioselective 4'-hydroxylation of phenytoin. The minor pathway, production of *R*-form of 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (HPPH) from non-chiral phenytoin, was absent in a poor metabolizer of mephenytoin. However, the major pathway leading to *S*-HPPH was not altered in a poor metabolizer of mephenytoin.¹⁾ In the present study, the $V_{\max}$ values of phenytoin in patients with CYP2C19 mutations for the defective alleles were decreased slightly, as compared with those in patients with wt CYP2C19 (Table 1).

In the present study, there was no patient with a CYP2C9 mutation for the homozygous Leu³⁵⁹ allele in CYP2C9 proteins. However, the $V_{\max}$ values in patients with a CYP2C9 mutation for the heterozygous Ile/Leu³⁵⁹ allele were lower than those in patients with CYP2C19 mutations for the defective alleles (Table 1). This suggested that CYP2C9 is involved in the *in vivo* metabolism of phenytoin, and that the mutation in CYP2C9 proteins is a determinant of impaired metabolism of the drug. Previous studies using site-directed mutagenesis and cDNA expression in COS cells have shown that the amino acid Ile³⁵⁹ has an important role in CYP2C9-substrate binding.^{7,12)} The highly conservative mutation (Ile³⁵⁹→Leu) in CYP2C9 proteins profoundly decreased the phenytoin and tolbutamide hydroxylation rate.⁷⁾ In addition, the same amino acid change was shown to alter the regio- and stereoselectivity of warfarin metabolism.¹²⁾

In conclusion, the present findings indicated that the genetic polymorphism of CYP2C isoenzymes plays an important role in the pharmacokinetic variability of phenytoin. The simple PCR-based genetic test for the CYP2C isoenzymes may be useful in designing the dosage regimen of phenytoin and/or in detecting patients at risk for the drug intoxication.

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