Regular Article

Evaluation of Phenytoin Dosage Regimens Based on Genotyping of CYP2C Subfamily in Routinely Treated Japanese Patients†

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Summary: Two research groups have reported the effect of genetic polymorphisms of CYP2C9 and CYP2C19 on the pharmacokinetic parameters of phenytoin in Japanese epileptic patients. We measured the plasma phenytoin concentrations at steady-state in 20 routinely treated Japanese patients, and evaluated the usefulness of genotyping the CYP2C subfamily in predicting plasma concentrations and determining the dosage regimens of phenytoin. The plasma phenytoin concentrations predicted by genotypes of the CYP2C subfamily were well correlated with the observed concentrations in some patients, but not in some patients. The pharmacokinetic parameters (Vmax and Km) in individual patients, which were obtained from population estimates according to Bayes’ theorem, showed considerable interindividual variability even among patients with the same genotype. In addition, we assessed the effect of plasma protein binding on the residual interindividual variability in the clearance of phenytoin; however, there was no significant correlation between the unbound fraction and the intrinsic metabolic activity (Vmax/Km). These findings suggested that the mechanism responsible for the large variability in the clearance of phenytoin is not completely resolved, and that we should not overestimate the usefulness of genotyping the CYP2C subfamily in determining the dosage regimens of the drug.

Key words: CYP2C9; CYP2C19; genotyping; phenytoin; dosage regimen

Introduction

Although phenytoin is widely used in the management of a variety of seizure disorders, there are several complicating factors involved in the dose adjustment of the drug. The therapeutic range of phenytoin is narrow (10–20 µg/mL), and the drug exhibits nonlinear pharmacokinetics with large interindividual differences.1) Kutt et al. reported on the insufficient hydroxylation of phenytoin in a patient who showed intoxication.2) This insufficient metabolism of phenytoin was then suggested to be an inherited defect by pedigree studies.3) Recently, the genetically polymorphic enzymes, CYP2C9 and CYP2C19, have been shown to be involved in the principal metabolic pathway of phenytoin, namely the formation of 5-(4-hydroxyphenyl)-5-phenylhydantoin.4,5)

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of the CYP2C subfamily, and estimated the pharmacokinetic parameters in each group. The maximal elimination rate \((V_{max})\) of phenytoin is 33\% lower in patients heterozygous for \(CYP2C9*1/*3\) than that in patients homozygous for \(CYP2C9*1/*1\), and is slightly decreased (up to 14\%) in patients with the \(CYP2C19*2\) or \(CYP2C19*3\) allele compared with patients homozygous for \(CYP2C19*1/*1\). Mamiya et al. reported on the pharmacokinetic parameter set of phenytoin in 134 Japanese patients. A significant 42\% reduction in the \(V_{max}\) value was estimated in patients heterozygous for \(CYP2C9*1/*3\), compared with patients homozygous for \(CYP2C9*1/*1\) (Table 1).

In this study, we measured the plasma phenytoin concentrations in 20 routinely treated Japanese patients, and evaluated the usefulness of genotyping the CYP2C subfamily in predicting plasma concentrations and determining the dosage regimens of phenytoin.

### Methods

**Subjects and study design:** The subjects were 20 Japanese patients with epilepsy aged between 2 and 25 (mean ± SD: 11.9 ± 5.3) years old. Mean body weight (±SD) was 35.4 ± 13.8 kg, and no patients had severe hepatic or renal failure. These patients were routinely treated with oral administration of phenytoin (Aleviatin® 10\% powder, Dainippon Pharmaceutical Co., Osaka, Japan) at Toyama Medical and Pharmaceutical University Hospital. In this study, 4 patients were on monotherapy with phenytoin, whereas 16 patients received other antiepileptic drugs concurrently. Phenytoin was administered three times a day to most patients, and the mean daily dose was 5.19 mg/day/kg. We obtained a total of 121 blood samples at steady-state following repetitive dosing for more than two weeks. The plasma phenytoin concentration was quantified by a fluorescence polarization immunoassay (TDX™, Dainabot, Tokyo, Japan). In addition, the unbound plasma concentrations of phenytoin in 12 patients were measured by an ultrafiltration method.

The patients and/or their parents gave written informed consent to participate in this study, which was approved by the ethics committee of Toyama Medical and Pharmaceutical University.

**Genotyping procedures for CYP2C9 and CYP2C19:** Genomic DNA was isolated from peripheral blood with an extraction kit (Takara, Tokyo, Japan). \(CYP2C9*1\) and \(CYP2C9*3\) were determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method described by Nasu et al.\(^5\). \(CYP2C19*1\), \(CYP2C19*2\) and \(CYP2C19*3\) were determined by the PCR-RFLP method described by Lamba et al.\(^12\).

**Predictive performance for plasma phenytoin concentration:** The plasma phenytoin concentrations in individual patients were predicted according to the pharmacokinetic parameters depicted in Table 1. In addition, Odani et al. reported that the parameter of power function of weight to adjust the maximal elimination rate \((V_{max})\) is 0.463, and the Michaelis-Menten constant \((K_m)\) of phenytoin is increased 16\% by concurrently administered zonisamide. Therefore, we used not only the genotype information of the CYP2C subfamily but also the patient’s body weight and the coadministered drug to predict plasma phenytoin concentrations.\(^9,10\) The predicted drug concentration was compared with the observed (measured) concentrations.

**Estimation of pharmacokinetic parameters in individual patients:** Mean pharmacokinetic parameters and their interindividual variations in 20 patients were estimated from 121 measured plasma phenytoin concentration data at steady-state \((\text{Css})\) using the nonlinear mixed effects model (NONMEM) program.\(^14\) The pharmacokinetic parameters in individual patients were then obtained from the population estimates according to Bayes’ theorem using the NONMEM POSTHOC option.\(^15\) In brief, the plasma phenytoin concentration at steady-state \((\text{Css})\) was modeled using the following equation: \(^{16}\)

### Table 1. Mean pharmacokinetic parameters of phenytoin in Japanese patients

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<tbody>
<tr>
<td></td>
<td>(V_{max}) (mg/day/kg)</td>
<td>(K_m) (µg/mL)</td>
<td>(V_{max}) (mg/day/kg)</td>
<td>(K_m) (µg/mL)</td>
</tr>
<tr>
<td>I</td>
<td>(*1/*1)</td>
<td>(*1/*1)</td>
<td>10.74</td>
<td>8.52</td>
</tr>
<tr>
<td>II</td>
<td>(*1/*1)</td>
<td>(*1/*2)</td>
<td>9.75</td>
<td>9.38</td>
</tr>
<tr>
<td>III</td>
<td>(*1/*1)</td>
<td>(*2/*2)</td>
<td>9.18</td>
<td>9.16</td>
</tr>
<tr>
<td>IV</td>
<td>(*1/*3)</td>
<td>(*1/*1)</td>
<td>7.49</td>
<td>10.0</td>
</tr>
<tr>
<td>V</td>
<td>(*1/*3)</td>
<td>(*1/*2)</td>
<td>6.37</td>
<td>10.3</td>
</tr>
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</table>

\[ CSS_{ij} = \frac{D_{ij}/\tau_{ij} \cdot K_{mi}}{V_{max ij} - D_{ij}/\tau_{ij}} (1 + \varepsilon_{ij}) \]  

where \( D_{ij} \) and \( \tau_{ij} \) are the dose and the dosing interval, respectively, for the \( ij \)th plasma concentration of the \( i \)th patient (\( CSS_{ij} \)). \( \varepsilon_{ij} \) is a random variable describing intraindividual variability with mean zero. \( V_{max} \) is the maximal elimination rate, and \( K_{mi} \) is the Michaelis-Menten constant in the \( i \)th patient. \( V_{max} \) and \( K_{mi} \) were modeled using the following equations:

\[ V_{max i} = \bar{V}_{max} \cdot (1 + \eta_{V_{max} i}) \]  
\[ K_{mi} = \bar{K}_{m} \cdot (1 + \eta_{K_{mi}}) \]

where \( \bar{V}_{max} \) and \( \bar{K}_{m} \) are the population mean values to be estimated, and \( \eta_{V_{max} i} \) and \( \eta_{K_{mi}} \) are random variables describing interindividual variability of \( V_{max} \) and \( K_{mi} \) with mean zero, respectively.

**Statistical analysis:** Multiple comparison was performed using Scheffé-type test following Kruskal-Wallis analysis. \( P \) values of less than 0.05 were considered to be significantly different.

**Results and Discussion**

Our object in this study was to evaluate the usefulness of genotyping of the CYP2C subfamily in predicting plasma phenytoin concentrations and determining the phenytoin dosage regimens. We recruited 20 epileptic patients, and determined their genotypes of the CYP2C subfamily. These patients were divided into five groups according to the genotype of the CYP2C subfamily as reported previously (Table 1). In the present study, Group I consisted of five patients who were homozygous for the wild-type alleles of CYP2C9 and CYP2C19. Seven patients belonged to Group II: three patients were homozygous for the \( CYP2C19^{*1}/*2 \) alleles, and four patients were heterozygous for the \( CYP2C19^{*1}/*3 \) alleles. Six patients belonged to Group III: one patient was homozygous for the \( CYP2C19^{*2} \) allele, three patients were heterozygous for the \( CYP2C19^{*2}/*3 \) alleles, and two patients were homozygous for the \( CYP2C19^{*3} \) allele. No patient belonged to Group IV, which expressed the \( CYP2C9^{*1}/*3 \) and \( CYP2C19^{*1}/*1 \) genotypes. Two patients were heterozygous for the \( CYP2C9^{*1}/*3 \) alleles (Group V): one patient was heterozygous for the \( CYP2C19^{*1}/*2 \) alleles, and the other was heterozygous for the \( CYP2C19^{*1}/*3 \) alleles.

The plasma concentrations predicted according to the reports of Odani et al.\(^9\) and Mamiya et al.\(^10\) were compared with the observed concentrations at steady-state in individual patients (Fig. 1). In this study, each patient was identified by the group and number. For example, the second patient in Group III was designated as III-2. As shown in Fig. 1, the correlation between the predicted and observed plasma concentrations was good in some patients (e.g. II-1, III-3, and V-2). However, the observed plasma concentrations in some patients (e.g. II-6, II-7, and III-6) were significantly higher than the predicted concentrations. Patient II-6 was a 17-year-old male weighing 49.1 kg who was treated with phenytoin, valproic acid, and carbamazepine. Patient II-7 was a 5-year-old male weighing 16.6 kg who was treated with phenytoin, valproic acid, clobazam, and zonisamide. Patient III-6 was a 14-year-old male weighing 43.9 kg who was treated with phenytoin, valproic acid, and zonisamide. Although Mamiya et al.\(^10\) estimated the pharmacokinetic parameters of phenytoin in 134 Japanese adult patients aged between 18 and 76 years old, Odani et al.\(^9\) estimated the pharmacokinetic parameters of phenytoin in 44 pediatric and adult patients between 1 and 33 years old. In addition, no patients had hepatic or renal failure. In the present study, we used the information about the patient’s body weight and coadministered zonisamide to predict plasma phenytoin concentration as described in the Method section, and none of the three patients (II-6, II-7, and III-6) had received any other potent inhibitors of CYP2C9 and CYP2C19 (e.g. omeprazole, ticlopidine). Accordingly, we could not explain the reduced clearance of phenytoin by the patient’s age, physical diagnosis, and coadministered drug in these three patients.

We estimated the pharmacokinetic parameters of phenytoin in individual patients according to Bayes’ theorem in order to evaluate the effect of genotypes of the CYP2C subfamily on the pharmacokinetics and to estimate its interindividual variability. As shown in Fig. 2, the influences of genotype of the CYP2C subfamily on \( V_{max} \) and \( V_{max}/K_{m} \) were observed, though this was not statistically significant (0.05 < \( P < 0.1 \), Kruskal-Wallis analysis). However, it was clear that there is a large interindividual variation in the pharmacokinetic parameters (\( V_{max} \), \( K_{m} \), and \( V_{max}/K_{m} \)) even among patients with the same genotype (Fig. 2). The coefficient of variation in the intrinsic clearance (\( V_{max}/K_{m} \)) for Group I, II, and III was estimated to be 19.0%, 23.8%, and 21.2%, respectively (Fig. 2C).

The unbound fraction of phenytoin is known to show large interindividual differences among patients, and an increased unbound fraction would result in a proportional increase in metabolic drug clearance.\(^11\)\(^17\) In this study, therefore, we evaluated the plasma unbound fraction of phenytoin in 12 patients. Figure 3 shows the relationship between the intrinsic metabolic activity (\( V_{max}/K_{m} \)) and the plasma unbound fraction of phenytoin in 12 patients who belonged to Group 1, Group 2, or Group 3. The unbound fraction of phenytoin showed considerable interindividual variability in each genotype group; however, there was no positive correlation between the unbound fraction and...
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Fig. 1. Steady-state plasma concentrations vs. weight (WT)-corrected daily dose of phenytoin in 20 Japanese patients. Open circles indicate the observed plasma phenytoin concentrations. Solid and dotted lines indicate the predicted curve according to the pharmacokinetic parameters reported by Odani et al. (1997) and Mamiya et al. (1998), respectively.

$V_{max}/K_m$ (Fig. 3). This suggested that the unbound fraction of phenytoin was unlikely to be responsible for the large interindividual variability of the pharmacokinetics of phenytoin. This observation may be consistent with that of Yacobi et al., who reported that protein binding is not a cause of the pronounced interindividual differences in phenytoin clearance of subjects with normal renal function.

The findings in this study indicated that the genetic polymorphism of the CYP2C subfamily is the major
factor affecting the pharmacokinetics of phenytoin, but that the mechanism responsible for the large variability in the clearance of phenytoin is not completely resolved. Therefore, we should not overestimate the usefulness of genotyping the CYP2C subfamily in determining the phenytoin dosage regimens, and should monitor the plasma (unbound) concentrations of phenytoin when treating epileptic patients.

References


