Contributions of CYP2C9/CYP2C19 Genotypes and Drug Interaction to the Phenytoin Treatment in the Korean Epileptic Patients in the Clinical Setting

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We examined the contribution of CYP2C9 and CYP2C19 genotypes and drug interactions to the phenytoin metabolism among 97 Korean epileptic patients to determine if pharmacogenetic testing could be utilized in routine clinical practice. The CYP2C9 polymorphism is a well-known major genetic factor responsible for phenytoin metabolism. The CYP2C19 polymorphism, with a high incidence of variant alleles, has a minor influence on phenytoin treated Koran patients. Using a multiple regression model for evaluation of the CYP2C9 and CYP2C19 genotypes, together with other non-genetic variables, we explained 39.6% of the variance in serum phenytoin levels. Incorporation of genotyping for CYP2C9 and CYP2C19 into a clinical practice may be of some help in the determination of phenytoin dosage. However, because concurrent drug treatment is common in patients taking phenytoin and many environmental factors are likely to play a role in drug metabolism, these factors may overwhelm the relevance of CYP polymorphisms in the clinical setting. Further investigations with an approach to dose assessment that includes comprehensive interpretation of both pharmacogenetic and pharmacokinetic data along with understanding of the mechanism of drug interactions in dosage adjustment is warranted.

Keywords: CYP2C9, CYP2C19, Drug interaction, Korean, Phenytoin

Introduction

Phenytoin is metabolized predominantly by CYP2C9 with minor contributions from CYP2C19 (Horsmans et al., 1997). Genetic polymorphisms of the CYP2C subfamily are responsible for the great inter-individual variability found in phenytoin pharmacokinetics. In addition, the inhibition of CYP2C9 or CYP2C19 enzymes by other drugs can alter the metabolic clearance of phenytoin. Although there have been some studies evaluating the effect of the CYP2C genetic polymorphism on phenytoin metabolism in Asians, prior reports have not considered the alteration of metabolic clearance of phenytoin with concomitant medications. The use of more than one therapeutic agent is common in patients treated with phenytoin in the clinical setting. In order to implement pharmacogenetic testing in the clinical setting, the ultimate goal of pharmacogenomics, an understanding of the relative contribution of each CYP for different populations, as well as a realistic understanding of the clinical setting, is essential for the application of this new technology.

The purpose of this study was to examine the contribution of CYP2C9 and CYP2C19 genotypes and drug interactions in Korean epileptic patients treated with phenytoin, and to determine if pharmacogenetic testing could be utilized in routine clinical practice.

Materials and Methods

The ethics committee of Samsung Medical Center approved this study. Ninety-seven patients receiving oral phenytoin therapy were enrolled after providing informed consent. The study population consisted of 46 male and 51 female patients (49 brain tumors, 14 seizure disorders, 13 brain hemorrhage, 10 encephalitis or meningitis, 9 cerebrovascular diseases, and 2 with brain abscess) with an age range of 17 to 79 years. The mean (± SD) body weight was 68.5 (± 5.6) kg. Patients were excluded if they had hepatic or renal dysfunction as determined by biochemical profiles. Serum albumin
levels were within normal limits for all patients included. The pharmacokinetic parameters for each patient were estimated from at least two trough serum phenytoin concentrations by Bayesian analysis using the AbbottTruss Pharmacokinetic system (Abbott, USA). Serum phenytoin concentrations were measured by fluorescence polarization immunoassay (TDxFlx, Abbott, USA). Serum phenytoin concentrations were measured by fluorescence polarization immunoassay (TDxFlx, Abbott, USA). Serum phenytoin concentrations were measured by fluorescence polarization immunoassay (TDxFlx, Abbott, USA). Serum phenytoin concentrations were measured by fluorescence polarization immunoassay (TDxFlx, Abbott, USA).

All 97 patients were genotyped for CYP2C9 and CYP2C19 by PCR and sequencing (exon 3 and 7 for CYP2C9, exon 4 and 5 for CYP2C9). DNA was extracted from peripheral blood leukocytes. The PCR products were sequenced using the ABI PRISM BigDye terminator Cycle Sequencing Kit and an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, USA). Primers used and PCR conditions are available upon request.

Subjects were divided into five groups based on the CYP2C9 and CYP2C19 genotypes (Hung et al., 2004) (Table 1). For statistical analysis, we used SAS (ver. 9.13, SAS Inc., USA) and considered \( p < 0.05 \) as statistically significant. After evaluation for significance of differences in phenytoin metabolism among different groups using the Kruskal-Wallis test, the least significant difference test using ranks was implemented for multiple comparisons. Simple linear regression analysis and multiple linear regression analysis were applied to evaluate the effects of genetic (CYP2C9, CYP2C19) and nongenetic factors (age, gender, phenytoin dosage per body weight, additional drug administration) on phenytoin metabolism.

Results and Discussion

The dosage of phenytoin, serum phenytoin levels, and estimated pharmacokinetic parameters in the five groups categorized by CYP2C9 genotypes are shown in Table 1. Group 5 was not included in the statistical analyses because there was only one patient in this group. However, the patient in Group 5 included in the statistical analyses because there was only one genotype (Hung, 2004) (Table 1). For statistical analysis, we used SAS (ver. 9.13, SAS Inc., USA) and considered \( p < 0.05 \) as statistically significant. After evaluation for significance of differences in phenytoin metabolism among different groups using the Kruskal-Wallis test, the least significant difference test using ranks was implemented for multiple comparisons. Simple linear regression analysis and multiple linear regression analysis were applied to evaluate the effects of genetic (CYP2C9, CYP2C19) and nongenetic factors (age, gender, phenytoin dosage per body weight, additional drug administration) on phenytoin metabolism.

<table>
<thead>
<tr>
<th>Group</th>
<th>CYP2C9 genotype</th>
<th>CYP2C19 genotype</th>
<th>No. of subjects (%)</th>
<th>Phenytoin dose mg/day/kg</th>
<th>Serum phenytoin mg/L (mg/day/kg)</th>
<th>( K_m ) mg/L</th>
<th>( V_{max} ) mg/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>*1/*1</td>
<td>*1/*1</td>
<td>36 (37.1)</td>
<td>5.62 ± 1.3 (5.1-6.1)</td>
<td>1.45 ± 0.6 (1.2-1.7)</td>
<td>4.24 ± 1.2 (3.8-4.6)</td>
<td>7.04 ± 1.6 (6.5-7.6)</td>
</tr>
<tr>
<td>2</td>
<td>*1/*1</td>
<td>*1/*2</td>
<td>32 (33.0)</td>
<td>5.54 ± 1.3 (5.1-5.9)</td>
<td>2.06 ± 0.9 (1.8-2.3)</td>
<td>4.44 ± 1.5 (4.0-4.9)</td>
<td>7.06 ± 1.4 (6.6-7.5)</td>
</tr>
<tr>
<td>3</td>
<td>*1/*1</td>
<td>*2/*2</td>
<td>12 (12.4)</td>
<td>4.67 ± 1.1 (3.6-5.7)</td>
<td>2.92 ± 1.0 (2.0-3.9)</td>
<td>4.55 ± 1.8 (2.9-6.2)</td>
<td>6.12 ± 1.9 (4.4-7.8)</td>
</tr>
<tr>
<td>4</td>
<td>*1/*3</td>
<td>*1/*1</td>
<td>2 (2.1)</td>
<td>4.78 ± 1.3 (4.2-5.3)</td>
<td>3.36 ± 0.9 (2.7-4.1)</td>
<td>5.22 ± 2.5 (3.3-7.2)</td>
<td>5.43 ± 0.5 (5.0-5.9)</td>
</tr>
<tr>
<td>5</td>
<td>*3/*3</td>
<td>*1/*1</td>
<td>1 (1.0)</td>
<td>4.47</td>
<td>5.27</td>
<td>9.73</td>
<td>3.06</td>
</tr>
</tbody>
</table>

Abbreviations. \( K_m \), Michaelis-Menten constant; \( V_{max} \), maximal elimination rate.
Hepatic enzyme induction or inhibition is the common cause of pharmacokinetic drug interactions with phenytoin treatment. The known CYP2C9 inhibitors are amiodarone, fluconazole, metronidazole, ketoconazole, cimetidine, and valproate (Riva et al., 1996; Anderson, 1998). The CYP2C19 inhibitors are felbamate, omeprazole, cimetidine, fluoxetine, diazepam, and ticlopidine (Riva et al., 1996). In addition, phenytoin may compete with drugs metabolized by the same CYP isoenzymes (Anderson, 1998). In our patients, valproate (19 patients), carbamazepine (30 patients) and phenobarbital (13 patients) were commonly used in patients taking phenytoin. Valproate was administered concomitantly with phenytoin in 10 patients; three of these patients had the lowest $V_{max}$ in their group categorized by CYP genotypes; Group 2, 3 and 4 each (Fig. 2). These findings led us to infer that valproate might change the CYP2C enzyme activity. In addition, the $V_{max}$ values for patients taking carbamazepine along with valproate were higher than the values in patients taking valproate alone. Carbamazepine can increase phenytoin metabolism through the induction of CYP2C enzymes; however, it can lower phenytoin bioavailability (Spina et al., 1996). The reported effects of phenobarbital on the pharmacokinetics of phenytoin have been inconsistent (Riva et al., 1996). Phenobarbital is an inducer of CYP2C9 but may compete with phenytoin as a substrate of CYP2C9 (Riva et al., 1996; Anderson, 1998). Inhibition of CYP enzyme activity by selective serotonin reuptake inhibitors has been reported (Schmider et al., 1997; Mamiya et al., 2001). Interaction between sertraline and phenytoin has been shown with substantial elevations of phenytoin concentrations (Haselberger et al., 1997). Unfortunately, we could not discern the primary effect of sertraline on phenytoin pharmacokinetics in our study, because sertraline was prescribed in only two patients, one also taking carbamazepine and the other amiodarone. One patient taking quetiapine had a low $V_{max}$ value even though this patient had wild type genotypes for both CYP2C9 and CYP2C19. Amitriptyline, which is a CYP2C9 substrate (Nasu et al., 1997), was used in two patients along with other medications. Two patients with cimetidine also used aluminum hydroxide or sucralfate concomitantly. Cimetidine inhibits the clearance of phenytoin (Frigo et al., 1983) but aluminum hydroxide or sucralfate can alter phenytoin absorption (Carter et al., 1981).

Previous studies have demonstrated that the frequency of mutations in the CYP2C9 gene is low in Asian populations. In two Japanese studies, CYP2C9*3 was found in 7% and 10.6% of epileptic patients (Hung et al., 2004; Soga et al., 2004). In our study, CYP2C 9*3 was found in 10% of patients (1 homozygote and 9 heterozygotes), while the proportions of intermediate metabolizers (IMs) and poor metabolizers (PMs) of CYP2C19 were high (42% and 7%, respectively). Our results indicated that CYP2C9 has a dominant role in phenytoin metabolism regardless of concurrent therapy; this is based on the findings of Groups 4 and 5 (the carriers of CYP2C9*3), which had lower $V_{max}$ values compared to the other genotype groups.
While only 2-5% of White or Black racial groups are PMs, 13-23% of Asians are PMs for CYP2C19 (Goldstein et al., 1997; Yoon et al., 2001). As the frequency of PMs for CYP2C19 in the Asian population is considerably higher than in Caucasians, the role of CYP2C19 may be more important for dose adjustment of phenytoin in Asians. Odani et al. suggested that the \( V_{\text{max}} \) values of phenytoin had decreased (up to 14%) among Japanese patients with CYP2C19 mutations compared with patients with wild type CYP2C19 (Wedlund, 2000). Mamiya et al. reported that the mean \( K_m \) value in PMs of CYP2C19 was 54% higher than that in extensive metabolizers (EMs), whereas the IM was intermediate between these two values, suggesting a gene dosage effect (Odani et al., 1997). Our study demonstrated different metabolic activities of CYP2C19 variant alleles, which was consistent with the suggestion of Mamiya et al. (1998).

Although there is a general trend toward higher serum phenytoin and lower \( V_{\text{max}} \) values, wide inter-individual variation in phenytoin metabolism within given genotype groups cannot be explained by CYP2C19 genotypes alone. In a multiple regression model, the CYP2C9 and CYP2C19 genotypes and the phenytoin dosage were independent and statistically significant factors contributing to the total variability in serum phenytoin levels. However, they only accounted for 39.6% of the total variability in serum phenytoin levels. Although, genetic polymorphisms of CYP2C9 and CYP2C19 are important variables that affect phenytoin metabolism, their relative contributions can be modified by many environmental factors including concurrent drug therapy in the clinical setting.

In previous studies analyzing the effect of genetic polymorphisms on phenytoin therapy (Odani et al., 1997; Mamiya et al., 1998; Watanabe et al., 1998; van der Weide et al., 2001), investigators have ignored the influence of drug interactions, even though most of their patients studied were taking additional medications. We examined CYP2C polymorphisms, in conjunction with the effect of other drugs, on phenytoin metabolism. Patients with the CYP2C19 polymorphism appeared to have a lower \( V_{\text{max}} \) compared to those with wild-type genotypes; the PMs of CYP2C19 revealed similar \( V_{\text{max}} \) values compared to heterozygotes for CYP2C9. However, the extent of the effect on metabolism by enzyme induction or inhibition by other drugs as well as genetic predisposition remains to be defined. Phenytoin and other drugs interact through multiple mechanisms and polytherapy is not uncommon in epileptic patients. Therefore, the final outcome may range from no change to significant alterations in phenytoin pharmacokinetics for each patient. Further studies are needed to quantitatively predict the relationships between CYP2C genotypes or alleles, drug interactions, metabolic activities, and clinical outcome with phenytoin therapy.

The CYP2C9 polymorphism is a major genetic factor responsible for phenytoin metabolism. The CYP2C19 polymorphism, with its high incidence of variant alleles, has a minor influence on phenytoin treatment in Korean patients. Incorporation of genotyping for CYP2C9 and CYP2C19 into a clinical practice may be of some help in the determination of phenytoin dosage. However, as concurrent drug treatment is common in patients on phenytoin therapy and many environmental factors may overwhelm the relevance of CYP polymorphisms in the clinical setting, comprehensive interpretation of both pharmacogenetic and pharmacokinetic

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**Fig. 2.** Relationship between CYP2C genotypes and \( V_{\text{max}} \) for phenytoin in 56 epileptic patients with additional medications. Abbreviations. CBZ, carbamazepine; PB, phenobarbital; VAL, valproate; MET, metronidazole; DZ, diazepam; AMT, amitriptyline; CIM, cimetidine; SERT, sertraline; AMD, amiodarone; QTP, quetiapine; SRF, sucralfate; CIP, ciprofloxacin; ALH, aluminum hydroxide.
data along with understanding of the mechanism of drug interactions in dosage adjustment is warranted.

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References


