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Short communication

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 *CYP219* 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

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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 Abbottbase Pharmacokinetic system (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 PRISIM 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 *CYP2C* 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 appeared to have different pharmacokinetics compared to the other patients, in terms of the serum phenytoin level, Michaelis-Menten constant (K_m) and maximal elimination rate (V_{max}). There were significant differences among the five groups regarding serum phenytoin levels. In addition, the V_{max} differed significantly in comparisons between different groups; Group 1 vs. 3, Group 1 vs. 4, Group 2 vs. 3, and Group 2 vs. 4 ($p < 0.05$). Group 3, with two defective *CYP2C19* alleles, had a significantly lower V_{max} compared to Group 1 or Group 2 ($p < 0.05$).

We assume that, in patients with concurrent drug therapy in addition to phenytoin, the impact of the genetic polymorphism (especially for *CYP2C19*) on phenytoin metabolism may not be accurately evaluated. In patients receiving polytherapy, there was a substantial variation in pharmacokinetic parameters within each group and an overlap with other groups (Fig. 1A, Fig. 2). However, in patients receiving phenytoin monotherapy, we found a possible genetic effect of *CYP2C19* on phenytoin pharmacokinetics; their V_{max} values (mg/kg/day) tended to decrease in the following order by *CYP2C19* genotype (Fig. 1B): 7.64 ± 1.72 in Group 1, 6.88 ± 1.03 in Group 2, 5.27 ± 0.70, and in Group 3 ($p = 0.010$ by Jonckheere-Terpstra test). The difference reached statistical significance in the comparison between Groups 2 and 3 only in patients on monotherapy ($p = 0.010$), not in patients receiving polytherapy ($p = 0.609$). In the patients on phenytoin monotherapy, V_{max} values for patients in Group 3 were comparable to those in Group 4 (mean 5.27 vs. 5.54).

For the univariate regression model, the *CYP2C9* genotype and phenytoin dose showed significant effects on serum phenytoin ($p = 0.0003$ and $p = 0.001$) and V_{max} ($p = 0.0029$ and $p = 0.001$), while *CYP2C19* had some effect on serum phenytoin ($p = 0.0095$) and no effect on V_{max} ($p = 0.3687$). In a multiple regression analysis, phenytoin dose per body weight ($p = 0.0002$) and the *CYP2C9* ($p = 0.0006$) and *CYP2C19* ($p = 0.0001$) genotypes were independent variables predicting serum phenytoin levels. Total r^2 values for the multiple regression models for serum phenytoin and V_{max} were 0.396 and 0.284, respectively. In patients with phenytoin monotherapy, the r^2 values for the model were increased to; 0.520 for serum phenytoin and 0.390 for V_{max}.

Concurrent drug administration is common in epileptic patients receiving phenytoin therapy. Among the 97 patients in this study, 56 patients (57%) were taking additional medications that could have interfered with phenytoin pharmacokinetics, and 38 among them used more than two concomitant drugs.

Table 1. Genotypes, phenytoin dosages, serum phenytoin levels, and pharmacokinetic parameters in 97 patients

Group	*CYP2C9* genotype	*CYP2C19* genotype	No. of subjects (%)	Phenytoin dose mg/day/kg	Serum phenytoin (mg/L)/(mg/day/kg)	K_m mg/L	V_{max} mg/kg/day
1	**1/*1*	**1/*1*	36 (37.1)	5.62 ± 1.3 (5.1-6.1)	1.45 ± 0.6 (1.2-1.7)	4.24 ± 1.2 (3.8-4.6)	7.04 ± 1.6 (6.5-7.6)
2	**1/*1*	**1/*2*	32 (33.0)	5.54 ± 1.3 (5.1-5.9)	2.06 ± 0.9 (1.8-2.3)	4.44 ± 1.5 (4.0-4.9)	7.06 ± 1.4 (6.6-7.5)
		**1/*3*	12 (12.4)				
3	**1/*1*	**2/*2*	2 (2.1)	4.67 ± 1.1 (3.6-5.7)	2.92 ± 1.0 (2.0-3.9)	4.55 ± 1.8 (2.9-6.2)	6.12 ± 1.9 (4.4-7.8)
		**2/*3*	3 (3.1)				
		**3/*3*	2 (2.1)				
4	**1/*3*	**1/*1*	7 (7.2)	4.78 ± 1.3 (4.2-5.3)	3.36 ± 0.9 (2.7-4.1)	5.22 ± 2.5 (3.3-7.2)	5.43 ± 0.5 (5.0-5.9)
		**1/*2*	2 (2.1)				
5	**3/*3*	**1/*1*	1 (1.0)	4.47	5.27	9.73	3.06

Abbreviations. K_m, Michaelis-Menten constant; V_{max}, maximal elimination rate

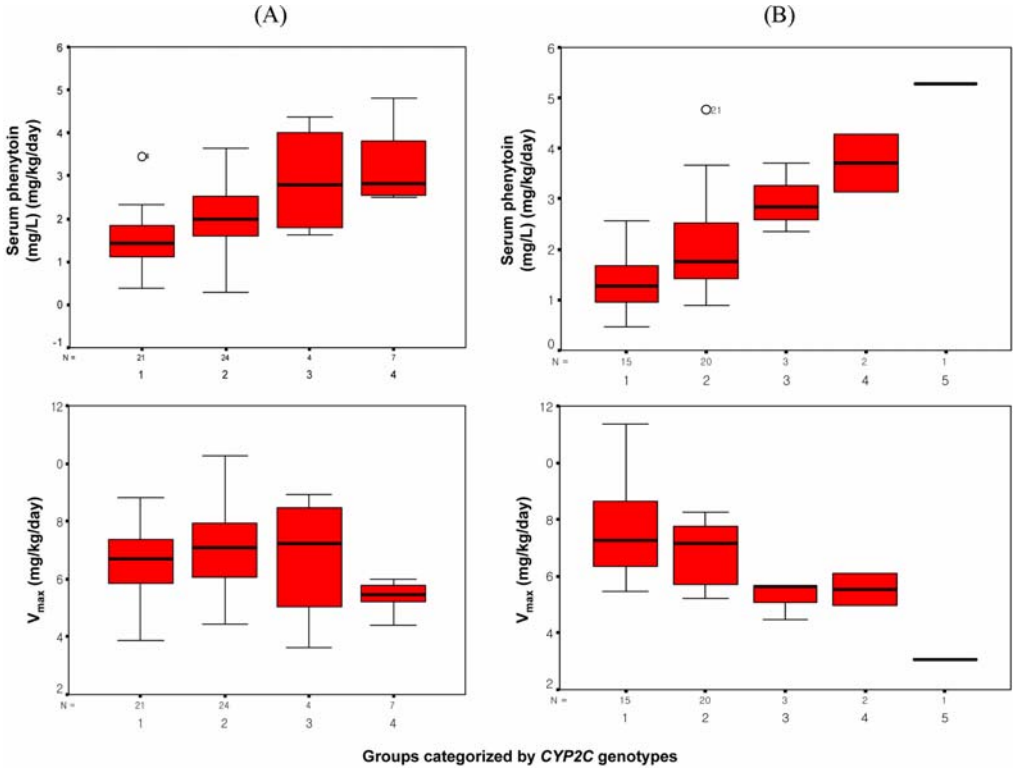

Fig. 1. Relationship between *CYP2C* genotypes and phenytoin metabolism (serum phenytoin level, Vmax) in 97 epileptic patients with (A) or without (B) additional medications.

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.

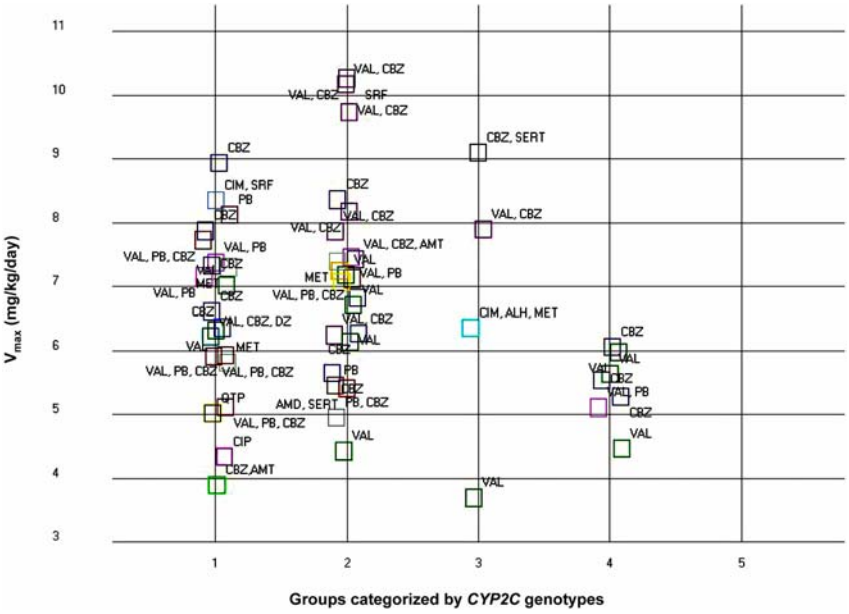

Fig. 2. Relationship between *CYP2C* genotypes and Vmax for phenytoin in 56 epileptic patients with additional medications. Abbreviations. CBZ, carbamazepine; PB, phenobarbital; VAL, valproate; MET, metronidazole; DZ, diazepam; AMT, amitriptyline; CIM, cimetidine; SERT, sertraline; AMD, amiodarone; QTP, quetiapine; SRF, sucralfate; CIP, ciprofloxacin; ALH, aluminum hydroxide.

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_{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_{max} values, wide inter-individual variation in phenytoin metabolism within given genotype groups cannot be explained by *CYP2C* 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_{max} compared to those with wild-type genotypes; the PMs of CYP2C19 revealed similar V_{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 *CYP219* 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

data along with understanding of the mechanism of drug interactions in dosage adjustment is warranted.

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