Effect of *CYP3A5* Genotypes on the Pharmacokinetics of Carbamazepine when used as Monotherapy or Co-Administered with Phenytoin, Phenobarbital or Valproic Acid in Thai Patients

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ABSTRACT - Purpose: To determine the effects of *CYP3A5* polymorphisms on carbamazepine (CBZ) pharmacokinetic parameters when CBZ is used either as monotherapy or co-administered with phenytoin (PHT), phenobarbital (PB) or valproic acid (VPA). **Methods:** Retrospective data were collected from an electronic database and medical records. Blood samples were obtained and drug concentrations analyzed as a part of routine therapeutic drug monitoring (TDM). Screening for wild-type *CYP3A5*1* and *CYP3A5*3* single nucleotide polymorphism (rs776746) by allelic discrimination assay using real-time polymerase chain reaction technique (real-time PCR) was performed. Pharmacokinetic parameters of CBZ; clearance and dose-adjusted CBZ levels in patients with different genotypes were calculated and compared. **Results:** Of the 70 patients assessed, 8 (11%) patients were homozygous *CYP3A5*1/*1*, 28 (40%) patients were heterozygous *CYP3A5*1/*3*, and 34 (49%) patients were homozygous *CYP3A5*3/*3*. The CBZ clearance and dose-adjusted CBZ levels did not significantly differ between patients with *CYP3A5*1* and *CYP3A5*3* alleles when CBZ was used as monotherapy. For patients who used CBZ in combination with an enzyme-inducing antiepileptic drug (AED: PHT or PB), individuals carrying the CYP3A5*1 allele (CYP3A5 expressers) showed a trend of having higher CBZ clearance and lower dose-adjusted CBZ level as compared to individuals carrying the CYP3A5*3 allele, even though no statistical significance was recorded. Nevertheless, it was observed that AEDs significantly increased CBZ clearance only in patients carrying the active CYP3A5*1 allele. **Conclusions:** When CBZ was used in combination with enzyme-inducing AED, *CYP3A5* expressers yielded a trend toward greater susceptibility to change in CBZ clearance and showed lower dose-adjusted CBZ levels compared to *CYP3A5* non-expressers. The dosage regimen should be adjusted accordingly to gain a better clinical outcome.

INTRODUCTION

Carbamazepine (CBZ) is a first-line antiepileptic drug for partial and generalized tonic–clonic seizures [1-5]. CBZ is commonly used as monotherapy or co-administered with other antiepileptic drugs (AED), such as phenytoin (PHT), phenobarbital (PB) and valproic acid (VPA). Additionally, it is commonly used for other neurological diseases, for instance, pain relief in trigeminal neuralgia or bipolar disorder [5-8]. The serum concentration of CBZ reported to be within the accepted therapeutic range is 4-12 mg/L when the drug is used for the treatment of seizures; accordingly, the range for psychiatric disorders and trigeminal neuralgia is assumed to be the same [9]. Ninety-nine percent of CBZ is metabolized by the liver; *CYP3A4* and *CYP3A5* are the most prominent enzymes that converted CBZ to CBZ-10, 11-epoxide (CBZ-E) in the major pathway of CBZ metabolism [8-11] (Figure 1).

Studies about the clearance of CBZ are important for therapeutic drug monitoring. Among several factors that have been reported as influencing the elimination of CBZ, age, body weight, surface area, dose of CBZ, and co-medication with PHT, PB, or VPA [8,9,12-14], *CYP3A5* polymorphism have more recently received greater focus. Seo et al. reported CBZ clearance in patients with *CYP3A5*3/*3* to be 8% significantly higher than that in patient with *CYP3A5*1/*1* or *CYP3A5*1/*3* [15].

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However, Park et al. reported the mean dose-adjusted CBZ level in patients with *CYP3A5*3/*3* to be 31% significantly higher than that in patients with *CYP3A5*1/*1* or *CYP3A5*1/*3* (p = 0.032). Consequently, CBZ clearance was reported to be 29% significantly lower in patients with *CYP3A5*3/*3* compared to the rate in patients with *CYP3A5*1/*1* or *CYP3A5*1/*3* (p = 0.004) [16]. The effects of *CYP3A5* 3* on CBZ pharmacokinetics (PK) when co-medicated with other AEDs reported to have a pharmacokinetic interaction with CBZ have never been clearly defined. Knowledge about the effect of *CYP3A5* polymorphism on CBZ pharmacokinetics either when used alone or used in combination with other AEDs may be useful in therapeutic plans to avoid serum drug concentration-related adverse effects and inefficacy.

The purpose of this study was to determine the effects of *CYP3A5* polymorphism on CBZ pharmacokinetic parameters when CBZ was used either as monotherapy or co-administered with PHT, PB or VPA.

METHODS

Study Design

A retrospective study design was used. The protocol was approved by The Institutional Review Board/Independent Ethics Committee, Prasat Neurological Institute (Bangkok, Thailand).

Patients

During February 2010 - September 2010, patients at the epilepsy outpatient clinic of Prasat Neurological Institute, aged over 13 years old who used CBZ as monotherapy or co-administered with PHT, PB or VPA and whose therapeutic drug monitoring data (TDM) had been recorded and were available were included in this study. CBZ and VPA took the form of controlled-release tablets (Tegretol CR or Zeptol CR and Depakine Chrono, respectively) while the rest took prompt release dosage forms. Patients were on a stable dosage regimen for at least 6 weeks; hence, a steady state was assumed and the pharmacokinetic interaction, if any, was assumed to be complete. We excluded pregnant women, patients with hepatic disease or renal disease. Additionally, patients who were concurrently using drugs which may interact with CBZ pharmacokinetics (except PHT, PB and VPA) were excluded, namely verapamil, diltiazem, haloperidol, theophylline, ticlopidine, cimetidine, omeprazole, trazodone, fluoxetine, risperidone, clarithromycin, erythromycin, rifampicin, isoniazid, isotretrinion, gemfibrozil and metronidazole [14-16]. Grapefruit consumers, if any, were excluded. All subjects gave written informed consent.

Blood Sampling and Assay

Blood samples were obtained as a part of routine TDM. All blood samples were drawn in the morning before drug intake (trough level). Total CBZ, PHT, PB and VPA concentrations in serum were determined using an immuno-turbidimetry assay method with an automated analyzer (Synchron LX® Systems, Beckman Coulter Inc., Fullerton, California, USA). The assay method has been routinely validated according to company guidance. The analytical range for CBZ level was 2.0-20.0 mg/L, while the precision specification was 0.6 mg/L or 5.0%. The analytical range for PHT level was 2.5-40.0 mg/L, while the precision specification was 0.5 mg/L or 4.0%. The analytical range for PB level was 5.0-80.0 mg/L, while the precision specification was 1.0 mg/L or 4.0%. The analytical range for VPA level was 10.0-150.0 mg/L, while the precision specification was 3.6 mg/L or 6.0%.

Genotyping

Genomic DNA was extracted from peripheral whole blood using QIAamp® DNA Blood Mini kit (Qiagen, Valencia, California, USA).

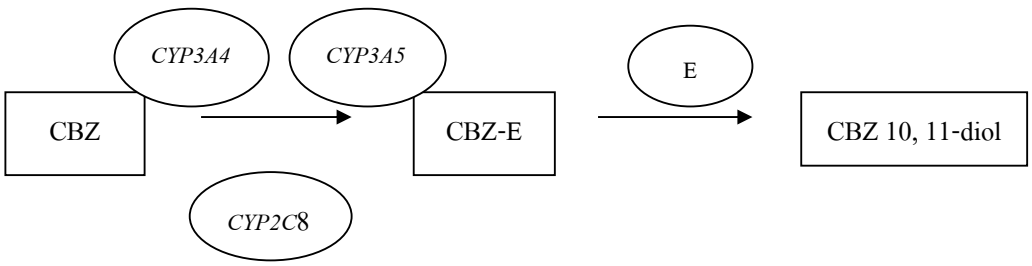

Figure 1: The epoxide-diol pathway of CBZ metabolism E-epoxide hydrolase

CYP3A5 genotyping was determined by allelic discrimination assay using real-time polymerase chain reaction technique (real-time PCR) with specific probe and primer (TaqMan® MGB probes, FAM™ and VIC® dye-labeled; Assay ID: C_26201809_30, rs: 776746)

Pharmacokinetic Parameter Calculation

CBZ clearance (CL) was calculated using the following equation:

$$CL/F = (S)\,(Dose) / (\tau)\,(C_{ss\ ave})$$

F is the bioavailability, S is the salt fraction for CBZ = 1, τ is the dosing interval (hour or day), and $C_{ss\ ave}$ is the measured serum drug concentration at steady state (mg/L). Because CBZ absolute bioavailability is unknown, apparent clearance (CL/F) was estimated [8, 9].

DATA ANALYSIS

PK parameters were calculated using Microsoft Excel and statistical analyses were determined using SPSS version 17.0 (SPSS Co., Ltd., Bangkok Thailand). The level of significance was set at $p \leq 0.05$. Continuous variables were determined for normality of the distribution using the Kolmogorov–Smirnov test and determined for homogeneity of variance using Levene's test. Statistical comparisons of CBZ clearance and dose-adjusted CBZ levels between patients with *CYP3A5*1* and *CYP3A5*3* were performed using independent *t*-test or Mann-Whitney U test, and between patients with CBZ monotherapy or co-administered with PHT, PB or VPA were performed using one-way ANOVA or Kruskal-Wallis test. All p-values were corrected for multiple testing.

RESULTS

Seventy patients who used CBZ as monotherapy or co-administered with PHT, PB or VPA and met the inclusion-exclusion criteria were included in this study. Their demographic data and plasma levels of related drugs were collected from the electronic database and medical records at the epilepsy outpatient clinic of Prasat Neurological Institute.

Frequencies of *CYP3A5*

The allele frequency of *CYP3A5*1* was 31% and *CYP3A5*3* was 69%. Allelic frequencies of *CYP3A5* genotypes did not significantly differ from those predicted by Hardy-Weinberg Equilibrium.

Demographic Data

Of the 70 patients, 67 were diagnosed as suffering epilepsy and 3 neuropathic pain. Among these, 36 patients used CBZ as monotherapy, 7 patients used CBZ in combination with PHT, 11 patients used CBZ in combination with PB and 16 patients used CBZ in combination with VPA. The patient's gender, age, body weight and BMI did not significantly differ among the three different *CYP3A5* genotype groups, as shown in Table 1.

Effect of *CYP3A5* Polymorphism on CBZ Pharmacokinetics

CBZ Monotherapy

Thirty six patients who used CBZ as monotherapy were categorized into a *CYP3A5* expressor group and a *CYP3A5* non-expressor group. The *CYP3A5* expressor group comprised the genotypes *CYP3A5*1/*1* and *CYP3A5**1/*3. The *CYP3A5* non-expressor group consisted of the *CYP3A5*3/*3* genotype. There were no statistically significant differences in patient's demographic characteristics (i.e., age, body weight, BMI, and male gender) and pharmacokinetic parameters of CBZ between the two groups. The details are shown in Table 2.

Table 1. Demographic characteristics of patients when categorized into three groups based on *CYP3A5* genotypes (N=70)

Demographic data	*CYP3A5*1/*1*	*CYP3A5*1/*3*	*CYP3A5*3/*3*	p-value
No. of patients	8 (11%)	28 (40%)	34 (49%)	
Gender (male)	3/8 (38%)	12/28 (43%)	16/34 (47%)	0.602 [a]
Age (yrs) mean±SD	50.96±20.61	38.97±11.47	43.68±13.16	0.078 [b]
(range)	(16.53-82.05)	(18.35-64.90)	(17.81-69.77)	
Body weight (kg) mean±SD	66.48±12.51	58.56±9.04	64.95±12.89	0.061 [b]
(range)	(52.00-88.00)	(40.10-77.00)	(43.30-104.00)	
BMI (kg/m^2) mean±SD	24.01±2.26	22.73±2.85	24.93±4.79	0.093 [b]
(range)	(21.37-27.85)	(17.26-29.34)	(16.50-37.53)	

[a] Chi-square test, [b] One-way ANOVA.

CBZ used in combination with other AEDs

CBZ in combination with enzyme-inducing AED group (CBZ+PHT or CBZ+PB)

Comparisons of CBZ PK parameters between *CYP3A5* expressor group and *CYP3A5* non-expressor group among 18 patients who used CBZ in combination with enzyme-inducing AED revealed that the CBZ CL/F (L/kg/hr) in the *CYP3A5* expressor group showed a higher trend than that observed in the *CYP3A5* non-expressor group, but did not reach a statistically significantly different level. The details are shown in Table 3.

CBZ+VPA

Comparisons of CBZ PK parameters between *CYP3A5* expressor group and *CYP3A5* non-expressor group among the 16 patients who used CBZ in combination with VPA showed no statistically significantly difference in either CBZ PK parameters as demonstrated in Table 3.

The Induction Effect of Other AEDs upon CBZ Pharmacokinetics in Patients Carrying Different *CYP3A5* Genotypes

Tables 4A and 4B show comparisons of PK parameters of CBZ within the same genotype group between patients who used CBZ as monotherapy with those who used CBZ in combined treatment with a different second AED, i.e. PHT, PB and VPA.

***CYP3A5* Expressor Group**

CBZ dose (mg/day, mg/kg/day) and CBZ level (mg/L) did not significantly differ among the three groups categorized based on different second AED used in combination with CBZ; however, when the level was standardized with the dose, dose-adjusted CBZ level (mcg/L/mg) and CBZ CL/F (L/hr, and L/kg/hr) significantly differed between the CBZ monotherapy group and CBZ combined with second AED group. The details are shown in Table 4A.

Post hoc comparisons of the CBZ PK parameters between CBZ monotherapy, CBZ + enzyme-inducing AEDs and CBZ+VPA within the *CYP3A5* expressor group revealed that the mean CBZ CL/F (L/kg/hr) in patients who used CBZ + enzyme-inducing AEDs (PHT, PB) was significantly higher than the mean CBZ CL/F in patients who used CBZ as monotherapy (43.28%, p=0.013). Accordingly, the mean dose-adjusted CBZ level (mcg/L/mg) in patients who used CBZ + enzyme-inducing AEDs was significantly lower than that obtained in patients who used CBZ as monotherapy (78.10%, p=0.010), while the mean CBZ CL/F and the mean dose-adjusted CBZ level (mcg/L/mg) in patients who used CBZ + VPA did not significantly differ statistically from patients who used CBZ as monotherapy.

Table 2: Comparisons of pharmacokinetic parameters of CBZ between *CYP3A5* expressor and *CYP3A5* non-expressor patients using CBZ monotherapy

Demographic data /Parameter	*CYP3A5* expressor group (N=21)	*CYP3A5* non-expressor group (N=15)	p-value
Gender (male)	8/21 (38%)	7/15 (47%)	0.607 [a]
Age (yrs) mean±SD	43.47±15.62	43.30±14.25	0.974 [b]
(range)	(16.53-82.05)	(17.81-69.77)	
Body weight (kg) mean±SD	57.12±9.36	61.37±11.33	0.227 [b]
(range)	(40.10-80.50)	(45.00-89.00)	
BMI (kg/m^2) mean±SD	22.17±2.57	23.33±3.44	0.253 [b]
(range)	(17.26-27.85)	(16.73-30.80)	
CBZ dose (mg/day) median	800	800	0.300 [c]
(range)	(400-1,600)	(200-1,400)	
(mg/kg/day) mean±SD	13.98±5.72	14.29±5.46	0.871 [b]
(range)	(6.67-29.09)	(3.33-23.53)	
CBZ level (mg/L) mean±SD	8.02±2.29	8.39±2.51	0.645 [b]
(range)	(3.70-11.80)	(4.40-11.90)	
(mcg/L/mg) mean±SD	11.06±3.92	10.61±3.65	0.727 [b]
(range)	(5.40-21.50)	(6.75-22.00)	
CBZ CL/F (L/hr) mean±SD	4.23±1.52	4.25±1.08	0.972 [b]
(range)	(1.94-7.71)	(1.90-6.17)	
(L/kg/hr) mean±SD	0.076±0.033	0.071±0.019	0.552 [b]
(range)	(0.032-0.184)	(0.032-0.101)	

[a] Chi-square test, [b] Independent *t*-test, [c] Mann-Whitney *U* Test

***CYP3A5* Non-expressor Group**

CBZ dose (mg/day, mg/kg/day), CBZ level (mg/L, mcg/L/mg), CBZ CL/F (L/hr) and CBZ CL/F standardized with body weight (L/kg/hr) did not significantly differ among the three groups categorized based on different second AED used in combination with CBZ. The details are shown in Table 4B. The mean dose-adjusted CBZ level (mcg/L/mg) in CBZ+ enzyme-inducing AEDs group was 20.83% lower than the mean in the monotherapy group; however, this figure did not reach a statistically significant level. The mean of CBZ CL/F (L/kg/hr) in patients who used CBZ+ enzyme-inducing AEDs was 16.47% higher than the mean of CBZ CL/F in patients who used CBZ as monotherapy – but this also did not statistically differ significantly. At the same time, the mean of CBZ CL/F in patients who used CBZ+VPA was 18.39% higher than the mean of CBZ CL/F in patients who used CBZ monotherapy, and showed no statistically significant difference.

DISCUSSION

The observed allelic frequency of *CYP3A5*3* among the 70 patients participating in this study was 69%. This frequency was similar to previous studies in the Thai population (66%) and in most other Asian populations, including Chinese, Indian, Malaysian and Japanese (78%, 59%, 61% and 77% respectively). However, this frequency of *CYP3A5*3* allele observed in our study was lower than that reported for Caucasians (92%) and higher than that recorded for African-Americans (48%). Other *CYP3A5* coding variants have been described, but occur at relatively low allele frequencies [17-22].

In addition to *CYP3A5*, *CYP3A4*, as the most abundant isoform of the *CYP3A* family may play an important role in the metabolism of CBZ. The *CYP3A4* gene is also very polymorphic; therefore, its genetic polymorphisms may influence the levels of CBZ obtained. However, unlike the *CYP3A5* gene, there is no evidence of a 'null' allele for *CYP3A4* [23]. Nonetheless, the over 30 SNPs identified in the *CYP3A4* gene [24] may contribute to the marked inter-individual variation in *CYP3A4*-catalysed metabolism. However, since the frequencies of different *CYP3A4* alleles are very low, the impact may not be distinct. In vitro data show that *CYP3A5* exhibited metabolic activity comparable to that of *CYP3A4* toward CBZ epoxidation [11]. The *CYP3A5*3* causes alternative splicing and protein truncation, resulting in an absence of protein activity, whereas individuals with at least one *CYP3A5*1* allele express the *CYP3A5* isoenzyme. Because *CYP3A5* may represent up to 50% of the total *CYP3A* protein in individuals polymorphically expressing *CYP3A5*, it may have a major role in the variation of *CYP3A*-mediated drug metabolism [22-23].

Table 3: Comparisons of pharmacokinetic parameters of CBZ between *CYP3A5* expressor and *CYP3A5* non-expressor patients using CBZ in combination with other AEDs

CBZ with other AEDs	PK parameters of CBZ		*CYP3A5* expressor group	*CYP3A5* non-expressor group	p-value
CBZ+enzyme-inducing AED	CBZ dose (mg/day)	mean±SD	971.43±335.23	909.09±478.44	0.768 [a]
	(mg/kg/day)	mean±SD	15.64±5.12	13.28±5.95	0.400 [a]
	CBZ level (mg/L)	mean±SD	5.76±2.78	6.85±2.58	0.406 [a]
		(range)	(2.10-10.50)	(2.22-9.90)	
	(mcg/L/mg)	mean±SD	6.21±2.74	8.40±3.25	0.161 [a]
		(range)	(2.63-10.50)	(3.70-13.14)	
	CBZ CL/F (L/hr)	mean±SD	8.12±4.11	5.82±2.62	0.164 [a]
		(range)	(3.97-15.87)	(3.17-11.26)	
	(L/kg/hr)	mean±SD	0.134±0.074	0.085±0.031	0.139 [a]
		(range)	(0.052-0.264)	(0.050-0.139)	
CBZ + VPA	CBZ dose (mg/day)	mean±SD	1,000±427.62	1,100±385.45	0.631 [a]
	(mg/kg/day)	mean±SD	15.50±7.63	16.72±6.96	0.745 [a]
	CBZ level (mg/L)	mean±SD	8.52±2.17	7.96±0.93	0.510 [a]
		(range)	(3.70-10.90)	(6.60-9.30)	
	(mcg/L/mg)	mean±SD	9.34±2.87	7.96±2.63	0.335 [a]
		(range)	(5.81-13.83)	(5.36-13.17)	
	CBZ CL/F (L/hr)	mean±SD	4.87±1.57	5.69±1.61	0.325 [a]
		(range)	(3.01-7.17)	(3.17-7.77)	
	(L/kg/hr)	mean±SD	0.076±0.031	0.087±0.032	0.510 [a]
		(range)	(0.039-0.135)	(0.049-0.158)	

[a] Independent *t*-test.

A previous study by Seo et al. of 144 Japanese epileptic patients reported that patients with *CYP3A5*3/*3* exhibited CBZ clearance 8% higher than patients not carrying *CYP3A5*3/*3* [15]. This result conflicted with the result from the study by Park et al. of 35 Korean epileptic patients, who reported that the CBZ clearance in patients with homozygous *CYP3A5*3/*3* was 29% lower than that observed in patients with at least one *CYP3A5*1* allele [16]. Seo et al. [15] recruited patients who used CBZ as either monotherapy or concurrently with the potent inducer of *CYP3A*, i.e. PHT and PB, into their study which may confound the effect of *CYP3A5* genotypes on CBZ pharmacokinetics. The reason that CBZ clearance was found to be higher in the *CYP3A5*3/*3* group might be due to the number of patients who used CBZ concurrently with the potent inducer being also higher in that group. Park et al. included only patients who used CBZ as monotherapy with their results indicating that *CYP3A5*3/*3* would result in lower CBZ clearance.

Table 4: The induction effect of other AEDs upon CBZ pharmacokinetics in patients carrying different *CYP3A5* genotypes: A. Among patients carrying *CYP3A5*1/*1* or *CYP3A5*1/*3* genotype

Parameter	CBZ monotherapy (N=21)	CBZ+ enzyme-inducing AEDs (N=7)	CBZ+VPA (N=8)	p-value
CBZ dose (mg/day) median	800 (400-1,600)	1,000 (400-1,400)	1,000 (400-1,600)	0.149 [b]
(mg/kg/day) median	13.33 (6.67-29.09)	17.39 (5.19-20.90)	15.26 (7.08-30.19)	0.541 [b]
CBZ level (mg/L) mean±SD	8.02±2.29 (3.70-11.80)	5.76±2.78 (2.10-10.50)	8.52±2.17 (3.70-10.90)	0.062 [a]
(mcg/L/mg) mean±SD	11.06±3.92 [c] (5.40-21.50)	6.21±2.74 [c] (2.63-10.50)	9.34±2.87 (5.81-13.83)	0.012 [a*]
CBZ CL/F (L/hr) mean±SD	4.23±1.52 [c, d] (1.94-7.71)	8.12±4.11 [c] (3.97-15.87)	4.87±1.57 [d] (3.01-7.17)	0.001 [a*]
(L/kg/hr) mean±SD	0.076±0.033 [c, d] (0.032-0.184)	0.134±0.074 [c] (0.052-0.264)	0.076±0.031 [d] (0.039-0.135)	0.012 [a*]

* Statistically significant difference, [a] One-way ANOVA, [b] Kruskal- Wallis test

[c] Post hoc comparisons (bonferroni) between CBZ monotherapy group and CBZ+ enzyme-inducing AEDs group; p-value = 0.010 for dose adjusted CBZ level (mcg/L/mg), p-value = 0.001 for CBZ CL/F (L/hr) and p-value = 0.013 for CBZ CL/F standardized with body weight (L/kg/hr)

[d] Post hoc comparisons (bonferroni) between CBZ monotherapy group and CBZ+VPA group; p-value = 0.741 for dose-adjusted CBZ level (mcg/L/mg), p-value = 1.000 for CBZ CL/F (L/hr) and p-value = 1.000 for CBZ CL/F standardized with body weight (L/kg/hr)

B. Among patients carrying *CYP3A5*3/*3* genotype

Parameter	CBZ monotherapy (N=15)	CBZ+ enzyme inducing AEDs (N=11)	CBZ+VPA (N=8)	p-value [a]
CBZ dose (mg/day) mean±SD	866.67±335.23 (200-1,400)	909.09±478.44 (400-2,000)	1,100±385.45 (600-1,600)	0.404
(mg/kg/day) mean±SD	14.29±5.46 (3.33-23.53)	13.28±5.95 (6.23-24.69)	16.72±6.96 (9.30-32.33)	0.465
CBZ level (mg/L) mean±SD	8.39±2.51 (4.40-11.90)	6.85±2.58 (2.20-9.90)	7.96±0.93 (6.60-9.30)	0.244
(mcg/L/mg) mean±SD	10.61±3.65 (6.75-22.00)	8.40±3.25 (3.70-13.14)	7.96±2.63 (5.36-13.17)	0.125
CBZ CL/F (L/hr) mean±SD	4.25±1.08 (1.90-6.17)	5.82±2.62 (3.17-11.26)	5.69±1.61 (3.17-7.77)	0.069
(L/kg/hr) mean±SD	0.071±0.019 (0.032-0.101)	0.085±0.031 (0.050-0.139)	0.087±0.032 (0.049-0.158)	0.259

[a] One-way ANOVA

In this study, in order to avoid the confounding effect of the enzyme-inducing factor, the effects of *CYP3A5* polymorphism on CBZ pharmacokinetic parameters were determined by grouping the patients based on the AEDs used, i.e., CBZ monotherapy, CBZ+PHT or CBZ+PB, CBZ+VPA. Since a previous study on population pharmacokinetics by Vucicevic et al [14] reported that smoking had no effect on CBZ clearance, smokers were not excluded from this study.

Comparisons of CBZ PK parameters between groups of different genotypes among the 36 patients who used CBZ as monotherapy, either categorized patients into three groups as *CYP3A5*1/*1*, *CYP3A5*1/*3* and *CYP3A5*3/*3* or categorized patients into two groups as *CYP3A5* expressors and *CYP3A5* non-expressors. CBZ level and CBZ CL/F showed no significant difference between groups of different genotypes (comparisons of the categorized patients into three groups are not shown). These results conflict with the results reported by Park et al., who reported that the mean of dose-adjusted CBZ level in *CYP3A5* expressors (9.94±3.38 mcg/L/mg) was significantly lower (p=0.032) than the mean of dose-adjusted CBZ level in *CYP3A5* non-expressors (13.07±4.46 mcg/L/mg), while the mean of CBZ CL/F in *CYP3A5* expressors (0.056±0.017 L/kg/hr) was significantly higher (p=0.004) than the mean of CBZ CL/F in *CYP3A5* non-expressors (0.040±0.014 L/kg/hr) [16]. In our study, the mean of dose-adjusted CBZ level in *CYP3A5* expressors was 11.06±3.92 mcg/L/mg, while the mean of CBZ level-to-dose ratio in *CYP3A5* non-expressors was 10.61±3.65 mcg/L/mg, meaning they were nearly equal and were not statistically significantly different (p=0.727). At the same time, the mean of CBZ CL/F in *CYP3A5* expressors was 0.076±0.033 L/kg/hr while the mean of CBZ CL/F in *CYP3A5* non-expressors was 0.071±0.019 L/kg/hr – 6.58% lower but not statistically significantly different (p=0.552).

Significantly higher CBZ CL/F (L/kg/hr) and in turn significantly lower dose-adjusted CBZ levels (mcg/L/mg) between CBZ+ enzyme-inducing AEDs as compared to CBZ monotherapy were more commonly recorded within patients carrying *CYP3A5*1/*1*, or *CYP3A5*1/*3* than within patients carrying *CYP3A5*3/*3*. This might indicate that the effects of enzyme induction from the second AEDs were more prominent on the *CYP3A5*1/*1* and *CYP3A5*1/*3* genotypes than on the *CYP3A5*3/*3* genotype.

The results also demonstrated that the enzyme-inducing effect of PB and PHT on CBZ clearance was stronger than the effect caused by polymorphism of the *CYP3A5* genotype.

Even though the *CYP3A5* is not the only enzyme for CBZ metabolism, the drug-drug interaction that inhibits or induces this enzyme may cause significant alteration of CBZ clearance because it is a prominent enzyme in the major pathway of CBZ metabolism. PB and PHT are the common potent inducer of the *CYP*450 enzymes including *CYP3A5*. These AEDs will increase the clearance of CBZ due to enhanced metabolism [8-11, 25-26]. Previous studies reported the increment of CBZ clearance to be within the range of 16-44% when concurrently used with PB, while the increment was 42-45 % in patients who used CBZ in combination with PHT as compared to patients who used CBZ monotherapy [13-14, 27-28]. Consistent with this study, especially in *CYP3A5* expressors who used CBZ+ enzyme-inducing AEDs, whose CBZ clearance was 43.28% higher when compared to that obtained in patients using CBZ monotherapy, while in *CYP3A5* non-expressors CBZ clearance increased less (by 16.47%). However, the effect of AEDs on the induction of *CYP3A5* has not been directly investigated. There are conflicting results on the effect of VPA on CBZ clearance: increase, decrease, or no change [7, 13-14, 25-28]. In this study, the CBZ clearance in *CYP3A5* expressors who used CBZ + VPA was quite similar to patients who used CBZ monotherapy, while in *CYP3A5* non-expressors the CBZ clearance in patients who used CBZ + VPA was 18.39% higher than patients who used CBZ monotherapy. VPA which has a higher protein-binding capacity than CBZ could increase CBZ clearance by replacing protein-bound CBZ and changing CBZ to a free form that would be eliminated faster [13, 29]. The inability to measure CBZ-epoxide and CBZ-diol which are the major metabolites of CBZ and the too low sample size are the limitations of this study.

CONCLUSIONS

The *CYP3A5* genotype did not substantially affect the pharmacokinetics of CBZ especially when the drug was used as monotherapy. However, in patients who used CBZ in combination with enzyme-inducing AED (PHT or PB), individuals carrying *CYP3A5*1* allele yielded a trend toward being more susceptible to increments in CBZ clearance and showed lower dose-adjusted CBZ level as compared to individuals carrying *CYP3A5*3*.

Genotype identifying might be an advantage for predicting individual dosage requirements for

targeted therapeutic levels, especially for those who have used CBZ in combination with enzyme-inducing AED. Thus, better clinical outcome can be achieved.

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Conflict of Interest

The authors have no conflicts of interest.

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