ผลของภาวะพหุสัณฐานของยีน *CYP3A5* ต่อเภสัชจลนศาสตร์ของยาคาร์บามาซีพีนในผู้ป่วยไทย เมื่อใช้เป็นยาเดี่ยวหรือใช้ร่วมกับยาเฟนิทอยน์ ฟีโนบาร์บิทาล หรือวาลโพรอิกแอซิด

นางสาวธราธร ไตรยวงค์

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชกรรมคลินิก ภาควิชาเภสัชกรรมปฏิบัติ
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2553
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

EFFECT OF *CYP3A5* POLYMORPHISM ON CARBAMAZEPINE PHARMACOKINETICS IN THAI PATIENTS AS MONOTHERAPY OR COADMINISTRATION WITH PHENYTOIN, PHENOBARBITAL OR VALPROIC ACID

Miss Tharathorn Traiyawong

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Clinical Pharmacy
Department of Pharmacy Practice
Faculty of Pharmaceutical Sciences
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Thesis Title	EFFECT OF *CYP3A5* POLYMORPHISM ON CARBAMAZEPINE PHARMACOKINETICS IN THAI PATIENTS AS MONOTHERAPY OR COADMINISTRATION WITH PHENYTOIN, PHENOBARBITAL OR VALPROIC ACID
By	Miss Tharathorn Traiyawong
Field of Study	Clinical Pharmacy
Thesis Advisor	Associate Professor Duangchit Panomvana Na Ayudhya, Ph.D.
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ธราธร ไตรยวงค์ : ผลของภาวะพหุสัณฐานของยีน *CYP3A5* ต่อเภสัชจลนศาสตร์ของยาคาร์บามาซีพีนในผู้ป่วยไทย เมื่อใช้เป็นยาเดี่ยวหรือใช้ร่วมกับยาเฟนิทอยน์ ฟีโนบาร์บิทาล หรือวาลโพรอิกแอซิด (EFFECT OF *CYP3A5* POLYMORPHISM ON CARBAMAZEPINE PHARMACOKINETICS IN THAI PATIENTS AS MONOTHERAPY OR COADMINISTRATION WITH PHENYTOIN, PHENOBARBITAL OR VALPROIC ACID) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ภญ.ดร.ดวงจิตต์ พนมวัน ณ อยุธยา, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: นพ.สมชาย โตวณะบุตร, 130 หน้า.

งานวิจัยนี้มีสามวัตถุประสงค์หลัก วัตถุประสงค์ที่ 1 เปรียบเทียบอัตราการกำจัดยาและสัดส่วนระดับยาต่อขนาดยาของยาคาร์บามาซีพีนระหว่างผู้ป่วยที่มีอัลลีลแบบ *CYP3A5*1* และ *CYP3A5*3* เมื่อใช้เป็นยาเดี่ยวหรือใช้ร่วมกับยาเฟนิทอยน์ ฟีโนบาร์บิทาล หรือวาลโพรอิกแอซิด วัตถุประสงค์ที่ 2 สร้างสมการทำนายอัตราการกำจัดยาคาร์บามาซีพีนจากข้อมูลพื้นฐานของผู้ป่วยและภาวะพหุสัณฐานของยีน*CYP3A5* วัตถุประสงค์ที่ 3 ศึกษาความสัมพันธ์ระหว่างอัตราการกำจัดยาคาร์บามาซีพีนกับอัตราสูงสุดของการเมแทบอลิซึมของยาเฟนิทอยน์ อัตราการกำจัดยาฟีโนบาร์บิทาล และอัตราการกำจัดยาวาลโพรอิกแอซิด

การศึกษาแบบย้อนหลัง-ไปข้างหน้านี้เก็บข้อมูล ณ คลินิกผู้ป่วยนอกโรคลมชัก สถาบันประสาทวิทยา กรุงเทพมหานคร มีการตรวจยีน *CYP3A5* ในผู้ป่วย 70 ราย พบว่าร้อยละ 31 มีอัลลีลแบบ *CYP3A5*1* และร้อยละ 69 มีอัลลีลแบบ *CYP3A5*3* อัตราการกำจัดยาและสัดส่วนระดับยาต่อขนาดยาคาร์บามาซีพีนระหว่างผู้ป่วยที่มีอัลลีลแบบ *CYP3A5*1* และ *CYP3A5*3* ไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ อย่างไรก็ตามพบว่าในผู้ป่วยที่มีอัลลีลแบบ *CYP3A5*1* ที่ใช้ยาคาร์บามาซีพีนร่วมกับยากันชักที่เหนี่ยวนำเอนไซม์ (เฟนิทอยน์ หรือฟีโนบาร์บิทาล) มีแนวโน้มที่จะเกิดการเปลี่ยนแปลงอัตราการกำจัดยาคาร์บามาซีพีนได้มากกว่าและมีสัดส่วนระดับยาต่อขนาดยาลดลงมากกว่าผู้ป่วยที่มีอัลลีลแบบ *CYP3A5*3* จากการวิเคราะห์สมการถดถอยแบบหลายตัวแปรเพื่อทำนายอัตราการกำจัดยาคาร์บามาซีพีนจากข้อมูลพื้นฐานของผู้ป่วยและภาวะพหุสัณฐานของยีน *CYP3A5* (ผู้ป่วย 70 ราย) พบว่าสมการที่สร้างขึ้นไม่ได้เลือกปัจจัยอัลลีลที่ต่างกันของยีน *CYP3A5* เข้าในสมการ แต่ได้เลือกปัจจัยอื่น 4 ปัจจัยที่สัมพันธ์กับอัตราการกำจัดยาคาร์บามาซีพีน (ลิตรต่อกิโลกรัมต่อวัน) ได้แก่ ขนาดยาคาร์บามาซีพีน (มก./กก.) ขนาดยาเฟนิทอยน์ (มก./กก.) ขนาดยาฟีโนบาร์บิทาล (มก./กก.) และน้ำหนักตัว (กก.) สมการที่สร้างขึ้นสามารถอธิบายความแตกต่างของอัตราการกำจัดยาคาร์บามาซีพีนได้ 54.7% ($p<0.001$) เมื่อยากันชักสองตัวถูกใช้ควบคู่กันอัตราสูงสุดของการเมแทบอลิซึมของยาเฟนิทอยน์มีความสัมพันธ์ค่อนข้างสูงกับอัตราการกำจัดยาคาร์บามาซีพีน (ผู้ป่วย 14 ราย, R^2 = 78%, $p<0.001$) ขณะที่อัตราการกำจัดยาวาลโพรอิกแอซิดมีความสัมพันธ์ระดับปานกลางกับอัตราการกำจัดยาคาร์บามาซีพีน (ผู้ป่วย 16 ราย, R^2 = 41.2%, $p=0.007$) ส่วนความสัมพันธ์ระหว่างอัตราการกำจัดยาฟีโนบาร์บิทาลกับอัตราการกำจัดยาคาร์บามาซีพีนไม่ถึงระดับที่มีนัยสำคัญทางสถิติ (ผู้ป่วย 15 ราย, R^2 = 11%, $p=0.227$)

ภาควิชา....เภสัชกรรมปฏิบัติ.....ลายมือชื่อนิสิต.....ธราธร ไตรยวงค์.....

สาขาวิชา..เภสัชกรรมคลินิก.....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา.....2553.....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

5176566033 : MAJOR CLINICAL PHARMACY

KEYWORDS: CARBAMAZEPINE / PHARMACOKINETICS / *CYP3A5*/ POLYMORPHISM

THARATHORN TRAIYAWONG: EFFECT OF *CYP3A5* POLYMORPHISM ON CARBAMAZEPINE PHARMACOKINETICS IN THAI PATIENTS AS MONOTHERAPY OR COADMINISTRATION WITH PHENYTOIN, PHENOBARBITAL OR VALPROIC ACID. THESIS ADVISOR: ASSOC. PROF. DUANGCHIT PANOMVANA NA AYUDHYA, Ph.D., THESIS CO-ADVISOR : SOMCHAI TOWANABUT, M.D., 130 pp.

There were three main purposes in this present study; first, to compare clearance, level-to-dose ratio of carbamazepine (CBZ) between patients with *CYP3A5*1* and *CYP3A5*3* alleles either when CBZ was used as monotherapy or coadministration with phenytoin (PHT), phenobarbital (PB) or valproic acid (VPA), second, to provide regression equation to predict CBZ clearance from demographic data and polymorphism of *CYP3A5*, third, to determine relationship between CBZ clearance and the maximum rate of metabolism of PHT (V_{max}), PB clearance and VPA clearance.

A retro-prospective data were collected at the epilepsy outpatient clinic of Prasat Neurological Institute, Bangkok. Genotyping of *CYP3A5* was performed in 70 patients. The allele frequency of *CYP3A5*1* was 31% and *CYP3A5*3* was 69%. The CBZ clearance and level-to-dose ratio was not significantly different between patients with *CYP3A5*1* and *CYP3A5*3* alleles. However, in patients who used CBZ in combination with enzyme inducing antiepileptic drug (PHT or PB), individuals carrying *CYP3A5*1* allele yielded the trend toward more susceptible to changes in CBZ clearance and showed lower CBZ-level-to-dose ratio as compared to individuals carrying *CYP3A5*3* allele. Multiple regression analysis for prediction of CBZ clearance from demographic data and *CYP3A5* genotypes (N=70), which excluded *CYP3A5* genotypes while selected four other factors generated the model as being related to CBZ clearance (L/kg/day); CBZ dose (mg/kg), PHT dose (mg/kg), PB dose (mg/kg) and body weight (kg), this model could explain 54.7% of the variance in CBZ clearance ($p<0.001$). When two antiepileptic drugs were used concurrently, PHT V_{max} showed high correlation with CBZ clearance (N=14, R^2= 78%, $p<0.001$), VPA clearance showed moderate correlation with CBZ clearance (N=16, R^2 = 41.2%, $p=0.007$) while the correlation between PB clearance and CBZ clearance was not reach the statistically significant level (N=15, R^2 = 11%, $p=0.227$).

Department : Pharmacy Practice	Student's Signature Tharathorn Traiyawong
Field of Study : Clinical Pharmacy	Advisor's Signature Duangchit Panomvana
Academic Year : 2010	Co-Advisor's Signature Somchai Towanabut

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I would like to express my thanks and gratitude to all patients who participated in this study and all of those whose name have not been mentioned for helping me in any way for this study.

Finally, this study would not have been successful without endless love, care, and support from my parents and my brother.

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LIST OF ABBREVIATIONS

AED	=	Antiepileptic Drug
ANOVA	=	Analysis of Variance
CBZ	=	Carbamazepine
CL	=	Clearance
CYP1A2	=	Cytochrome P450, family 1, subfamily A, polypeptide 2
CYP2C8	=	Cytochrome P450, family 2, subfamily C, polypeptide 8
CYP3A4	=	Cytochrome P450, family 3, subfamily A, polypeptide 4
CYP3A5	=	Cytochrome P450, family 3, subfamily A, polypeptide 5
ddH_2O	=	Double distilled water
DNA	=	Deoxyribonucleic acid
EDTA	=	Ethylenediaminetetraacetic acid
HWE	=	Hardy-Weinberg Equilibrium
mcg	=	Microgram
mRNA	=	messenger Ribonucleic acid
OD	=	Optical Density
PCR	=	Polymerase Chain Reaction
PB	=	Phenobarbital
PHT	=	Phenytoin
SNP	=	Single Nucleotide Polymorphism
UDPGT	=	Uridine diphosphate glucuronosyltransferase
VPA	=	Valproic acid
V_d	=	Volume of distribution
V_{max}	=	Maximum rate of metabolism

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CHARPTER I
INTRODUCTION

Background and Rationale

Carbamazepine (CBZ) is a first-line antiepileptic drug for partial and generalized tonic–clonic seizures.[1-5] CBZ is used as monotherapy or coadministration with other antiepileptic drugs (AED) such as Phenytoin (PHT), Phenobarbital (PB), Valproic acid (VPA).[5-7] Additionally, it is commonly used for others neurological disease for instance pain relief in trigeminal neuralgia, bipolar disorder. [8] CBZ is metabolized 99% by the liver; *CYP3A4* and *CYP3A5* are the most importance enzymes. [8-11] The serum concentration of CBZ that reported to be the accepted therapeutic range is 4-12 mg/L when the drug is used for the treatment of seizures, however, the range for psychiatric disorders and trigeminal neuralgia is assumed to be the same. [9]

Studies about the clearance of CBZ are importance for therapeutic drug monitoring. Several studies reported that age, body weight, surface area, dose of CBZ, dose of PB, and co-medication with PHT, PB, or VPA are significant influence on CBZ clearance. [8, 9, 12-14] Recent pharmacogenomic studies found that *CYP3A5* polymorphism effects on CBZ clearance. Seo et al. [15] reported that CBZ clearance in patients with *CYP3A5*3/*3* was 8% higher than in patients with *CYP3A5*1/*1* and *CYP3A5*1/*3*. Park et al. [16] reported that the mean of level-to-dose-ratio of CBZ in patients with *CYP3A5*3/*3* was 31% significant higher than patients with *CYP3A5*1/*1* and *CYP3A5*1/*3* ($p = 0.032$), and the CBZ clearance was 29% significant lower ($p = 0.004$). Studies about the effect of *CYP3A5*3* on CBZ pharmacokinetics when comedication with other AEDs that reported to have pharmacokinetic interaction with CBZ have not been clearly defined. In Thailand there has never been study about the effect of *CYP3A5* polymorphism on CBZ clearance either in patients with CBZ monotherapy or coadministration with other AEDs which have drug interaction, such as, PHT, PB and VPA. Knowledge about the effect of *CYP3A5* polymorphism on CBZ pharmacokinetics may be useful in therapeutic plans to avoid serum drug concentration-related adverse

effects and reduce inappropriate dosage. A recent study reported that the frequency of *CYP3A5*3* allele in a Thai population was 66%. [17]

CBZ is mainly metabolized by the liver via CYP450, the same enzyme system as PHT and PB, at the same time, CBZ induces uridine diphosphate glucuronosyltransferase (UDPGT) which is the main metabolizing enzyme of VPA while VPA inhibits CBZ-10, 11-epoxide (active metabolite) metabolism via Epoxide hydrolase [6-9]. It is therefore highly possible that CBZ pharmacokinetic parameters could be related to pharmacokinetic parameters of PHT, PB and VPA. In Thailand the relationship between pharmacokinetic parameters of PHT and CBZ has been investigated and found that there was high correlation between clearance of CBZ and maximum rate of metabolism of PHT (PHT V_{max}) (correlation coefficient = 0.828), regression equations to predict CBZ clearance from PHT V_{max} or vice versa have also been provided [18], even though validation and application has never been performed. Additionally, the study of correlation between CBZ clearance and PB clearance and VPA clearance has never been investigated.

The purpose of this study was to determine the effect of *CYP3A5* polymorphism on CBZ clearance, provide the regression equation to predict CBZ clearance from demographic data and polymorphism of *CYP3A5* and investigate the correlation between CBZ clearance and PHT V_{max}, PB clearance or VPA clearance and develop regression equation to predict CBZ clearance from clearance of other AEDs or vice versa. The ultimate goal is to provide a more accurate and simplified method for predicting the appropriate dosage of CBZ and in turn, a higher efficiency and safety of drug used.

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Hypothesis

1. CBZ clearance was not different between patients with *CYP3A5*1* and *CYP3A5*3* alleles.
2. CBZ clearance was not correlated with PHT V_{max}, PB clearance or VPA clearance.

Objective

1. To compare clearance, level-to-dose-ratio of CBZ between patients with *CYP3A5*1* and *CYP3A5*3* either when CBZ was used as monotherapy or coadministration with PHT, PB or VPA.
2. To provide regression equation to predict CBZ clearance from demographic data and polymorphism of *CYP3A5*.
3. To determine relationship between CBZ clearance and PHT V_{max}, PB clearance and VPA clearance.

Significant of the study

1. Information about the difference between CBZ clearance in patients with *CYP3A5*1* VS *CYP3A5*3* may be useful for the dosage regimen plans.
2. Information about the factors that correlate with CBZ clearance may be used to therapeutic plans to avoid serum drug concentration-related adverse effects and add efficiency to drug used.
3. To provide equation to predict CBZ clearance, and in turn, to predict a more appropriate dosage regimen for the patient.

Scope of this study

1. Populations of this study are the outpatients at Prasat Neurological Institute who used CBZ as monotherapy or coadministration with PHT, PB or VPA.
2. Variables of this study: Dependent variables are CBZ clearance, CBZ level-to-dose-ratio. Independent variables are *CYP3A5* polymorphism, PHT V_{max}, PB clearance, VPA clearance and demographic data.

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Limitation of this study

Application of this study is limit to specific patients that have the same characteristics as the patients in this study.

Conceptual framework

Conceptual framework is shown in figure 1.

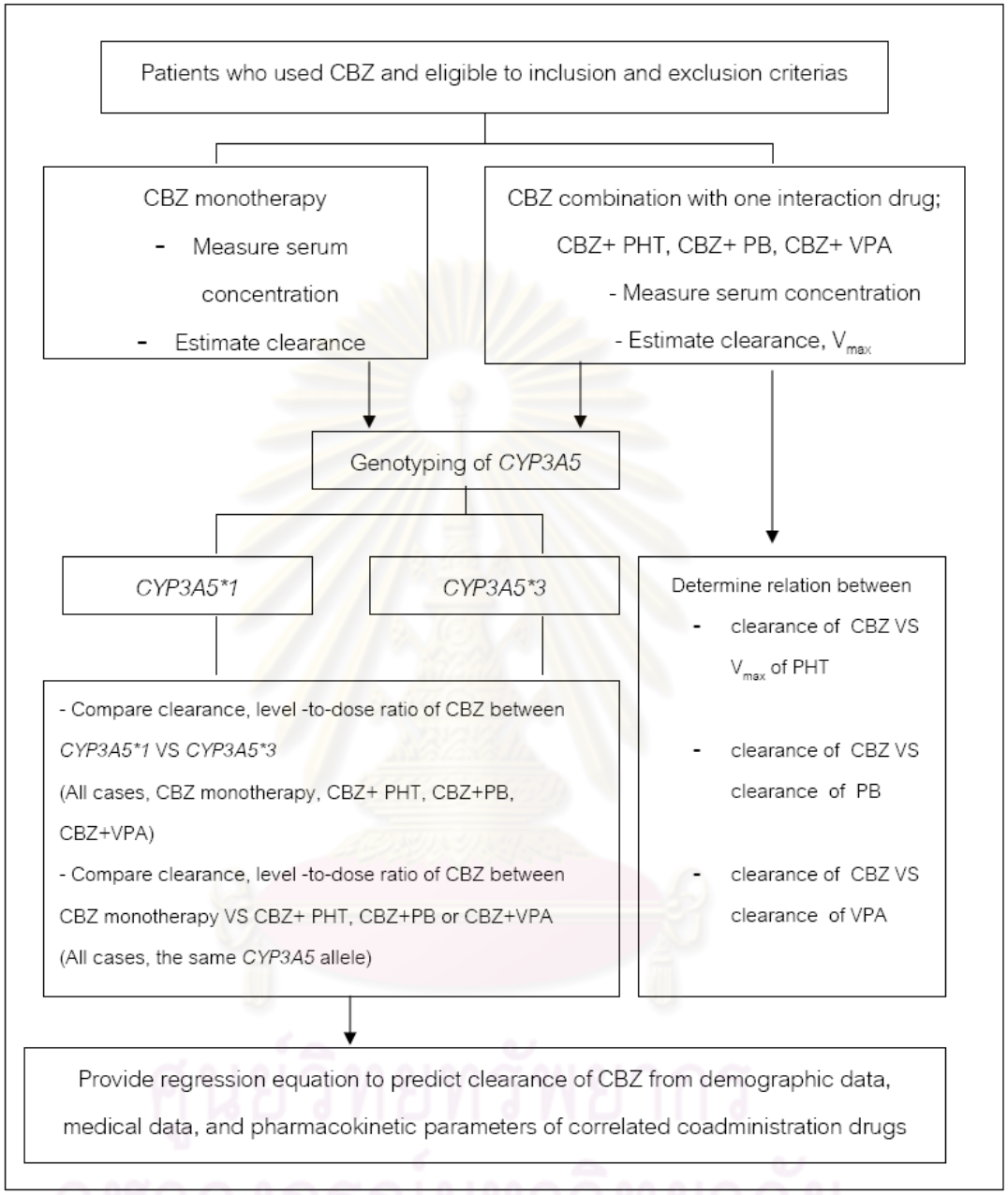

Figure1: Conceptual framework

Operational definition

1. *CYP3A5* polymorphism is genotype that control *CYP3A5* enzyme producing which has single-nucleotide polymorphism; *CYP3A5*3* allele is substitute amino acid at intron 3 (6986 A>G) when the reference allele is *CYP3A5*1*.
2. Antiepileptic drugs serum concentration measurement is a measurement of bound and unbound drug in serum (total drug) that the sampling time is not over one hour before the administration of the next dose in the morning (trough level).
3. Clearance is the ability of the body or organ (liver, kidney) to eliminate a drug. This pharmacokinetic parameter is calculated from serum concentration level at steady state.
4. Level-to-dose ratio is a ratio of antiepileptic drug serum level to dose per day of the drug.

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CHARPTER II
LITERATURE REVIEWS

Carbamazepine

CBZ is a first-line antiepileptic drug for partial and generalized tonic–clonic seizures. [1-5] Carbamazepine is used as monotherapy or coadministration with others antiepileptic drugs such as PHT, PB, VPA. [5-7] Additionally, it is commonly used for others neurological disease for instance pain relief in trigeminal neuralgia, bipolar disorder. [8] Molecular formula of CBZ is $C_{15}H_{12}N_2O$ (chemical name is 5H-dibenz [b, f] azepine-5-carboxamide). Chemical structure of CBZ is similar to tricyclic antidepressants (Figure 2), and it was synthesized in 1953 to complete with the newly introduced antipsychotic drug chlorpromazine. It was initially approved for the treatment of trigeminal neuralgia and for the treatment of seizures in 1974. [19, 20] Dosage forms of CBZ are available as immediate-release tablet, chewable tablet, oral suspension, controlled-release tablet and sustained-release capsule. [20, 21]

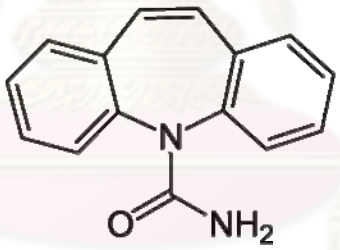

Figure 2: Chemical structure of CBZ.

Mechanism of action

CBZ acts by preventing repetitive firing of action potentials in depolarized neurons via use-and voltage-dependent sodium channels. [20] Voltage-gated sodium channels are the molecular pores that allow brain cells (neurons) to generate action potentials, the electrical events that allow neurons to communicate over long distances. After the sodium channels open to start the action potential, they inactivate, essentially closing the channel. CBZ stabilizes the inactivated state of sodium channels, meaning that fewer of these channels are available to subsequently open, making brain cells less excitable. [19, 20] CBZ has also been shown to potentiate GABA receptors made up of

alpha$_1$, beta$_2$, gamma $_2$ subunits subsequently open, making brain cells less excitable. [22]

Pharmacodynamic

CBZ has been considered the drug of choice for initial treatment of patients with simple, complex, or secondarily generalized partial seizures and for patients with primary generalized tonic-clonic seizures. It may exacerbate the rate of generalized absence and myoclonic seizures. [20]

The effectiveness of CBZ as an antiepileptic drug is associated with concentration of 4-12 mg/L and the range for psychiatric disorders and trigeminal neuralgia is assumed to be the same. This range is intended as a guide not an absolute, because of the variable amount of free drug, the contribution of the 10, 11-epoxide (active metabolite) and the interindividual variability in response. The target concentration for each patient should be determined by response and occurrence of side effects. [21]

Slow dosage titration allowed a patient time to develop tolerance to certain side effects associated with CBZ. The use of sustain-release or controlled-release dosage forms reduced the peak to trough fluctuations and may reduced associated side effects. The most common side effects of CBZ include dizziness, headache, diplopia, nausea, vomiting, sedation, and lethargy, and have been reported to be related to serum concentration. Other possible concentration- related side effects include hyponatremia, syndrome of inappropriate antidiuretic hormone and osteomalacia. An exact dose and concentration effect for these side effects has not been established, but they occur more frequently at higher doses or after prolonged exposure. [20, 21]

CBZ has been associated with atrioventricular block, especially in older women, and it is suggested that careful monitoring of the echocardiogram and drug concentration be done in elderly patients. Idiosyncratic reactions associated with CBZ include bone marrow suppression, aplastic anemia, agranulocytosis, toxic hepatitis, skin rash and rarely Steven-Johnson syndrome. [20, 21]

Pharmacokinetics

Absorption

CBZ is lipid-soluble compound that is slowly and variably absorbed from the gastrointestinal tract. Peak plasma concentration following immediate-release CBZ products occur approximately 6 hours (2-24 hrs) after oral ingestion. [9] Following chronic oral administration of CBZ tablets, extended-release tablets or extended-release capsules, peak plasma concentrations are reached in 4.5, 3-12, or 4.1-7.7 hours, respectively. [23] The time to peak increases with an increase in dose, suggesting that there is simultaneous first-order and zero order absorption. [20] Because of no intravenous form of CBZ is currently available for human trials, the oral bioavailability of CBZ has not been directly determined. [21] For clinical purpose the bioavailability (F) of CBZ is assumed to be approximately 80% for oral tablet, chewable tablet, or suspension. The bioavailability of extended-release CBZ products is assumed to be approximately 70%. [9] Concurrent administrations with food affect the rate but not the extent of absorption. Immediate-release tablets, extended-release tablets and suspension should be administered with meal, while the extended-release capsule can be taken without regard to food. [21]

Distribution

CBZ distributes rapidly and uniformly to various organs and tissues, achieving higher concentrations in organs of high blood flow for instance liver, kidney and brain. CBZ rapidly crosses the placenta and accumulates in fetal tissue with higher concentrations in the liver and kidney than the brain and lungs. CBZ has been detected in the cerebral spinal fluid, brain, duodenal fluids, bile and saliva. In breast milk CBZ concentration is about 25-60% of the concentration in mother's plasma. It was found that the correlations between saliva and plasma concentrations were strong and highly significant. [21]

On average, the volume of distribution (V_d) for CBZ is approximately 1.5 L/kg for neonates, 1.9 L/kg for children and 1.4 L/kg (0.8-1.9 L/kg) for adults based on total body weight. CBZ is primarily bound to albumin and alpha-1-acid glycoprotein. The percentage of protein binding of CBZ is 75-90% and the epoxide metabolite is 50-90%.

The free fraction of CBZ may vary with the presence of inflammation, trauma, concurrent AEDs therapy, and age. The free fraction of CBZ is approximately 0.2-0.3. In uremic patients, significant increases in free CBZ concentrations are seen. Although CBZ has significant binding to plasma proteins, there are very few clinical studies exploring alterations in plasma binding characteristics. This may be because CBZ is bound to multiple plasma proteins and with a free fraction of 0.2-0.3, fairly large changes in plasma binding to multiple plasma proteins would be required for the change in binding to become clinically significant. As a result of this, the use of free fraction CBZ serum concentrations are currently limited to those patients that have total concentrations within the therapeutic range but experience adverse effect usually seen at higher concentrations, or those patients that have total concentrations below the therapeutic range but have a therapeutic response usually observed at higher concentrations. However, there is no defined target concentration range for unbound CBZ and not routinely measured. [8, 9, 21]

Elimination

Metabolism

CBZ is about 99% metabolized by the epoxide-diol pathway, aromatic hydroxylation, and direct conjugation with glucuronic acid, and sulfur conjugation pathway. Epoxide diol and aromatic hydroxylation pathway are accounted for about 65% of it metabolism. The most important CBZ metabolite is 10, 11-epoxide, which appears to be active and contribute to efficacy and toxicity of CBZ. [20, 21] The epoxidation reaction is mediated by isoenzymes in the liver, *CYP3A4/5*, *CYP2C8* and *CYP1A2* with *CYP3A4/5* playing the most important role. [11, 20] The epoxide metabolite is further hydrolyzed to an inactive diol metabolite that is excreted in the urine. The aromatic hydroxylation is mediated by *CYP1A2*. UDPGT is also involved in the metabolism of CBZ. [20]

CBZ induces its own metabolism (autoinduction), which clearance increasing on continued dosing. Autoinduction begins 3-5 days after the initiation of therapy and take 3-5 weeks to complete. The autoinduction appears to be dose related, so each increase in dose will result in further autoinduction. The result of the autoinduction is that the

clearance of CBZ will increase and the half-life will become shorter with continued dosing. [20, 21]

Elimination parameters

Half-life

The half-life ($t_{1/2}$) of CBZ changes with continued dosing and is affected by other drugs that induce or inhibit enzymes. The time to steady state depends on the completion of autoinduction. Single dose studies predicted a CBZ half-life of approximately 25-65 hours, steady state data suggested a half-life of approximately 12-17 hours in adult patients receiving CBZ monotherapy, and approximately 5-14 hours in patients receiving other enzyme-inducing antiepileptic drugs (e.g. PHT, PB) concurrently. [21] Children metabolize CBZ more rapidly than adults with reported steady state half-life of 4-12 hours. [9] Table 1 summarizes the half-life and time to steady state.

Table 1: Half-life and Time to Steady State [21]

Dosing	Half-life (hr)	Time to Steady State [a]
Single dose	25-65	-
Chronic dose	12-17	60-85 hr
Concurrent antiepileptic drug	5-14	30-70 hr

[a] Time to steady state is not applicable to single doses and, due to autoinduction, is based on more realistically on the time for complete autoinduction.

Clearance

The Clearance (Cl) of CBZ increases with continued dosing and can be altered by enzyme-inducing or inhibiting drugs. The clearance appears to be age dependent, with higher clearances reported in younger children and lower clearances reported in older patients. CBZ is cleared more rapidly in the third trimester of pregnancy. Patients with significant liver disease may have a decreased clearance of CBZ. Renal disease and dialysis do not alter the clearance of CBZ. [20] The average clearance appears to be approximately 0.064 L/hr/kg in adult patients who received the chronic dosing while, in

patients who taking concurrent other enzyme-inducing antiepileptic drugs is approximately 0.1 L/hr/kg. In children with CBZ monotherapy, the clearance is approximately 0.11 L/hr/kg. [9]

Drug interaction

CBZ is an enzyme inducer and enhances the metabolism of many drugs that are metabolized by the *CYP450* system, including it self. CBZ induces and is metabolized extensively by the isoenzymes *CYP3A4/5*, and to a lesser extent *CYP1A2*, *CYP2B6*, *CYP2E1*, *CYP2C8*, *CYP2C9* and UDPGT. [11, 20, 21] Drugs that are inhibitors or inducers of the *CYP450* system, especially *CYP3A4/5* will decrease or increase the clearance of CBZ due to reduced or enhanced metabolism. Common drug interactions between CBZ and other drugs and the expected result were shown in Table 2 and Table 3.

Other types of interaction have been described. When lithium and CBZ or alcohol and CBZ are used together there are increased risks for neurological effects. Possible serotonin syndrome may result if CBZ is administered concurrently with an MOA inhibitor and combined therapy is contraindicated. CBZ and theophylline induce each other's metabolism resulting in change in the half-life and serum concentrations of both drugs. [21]

If administers CBZ undiluted suspension through polyvinyl chloride nasogastric feeding tubes, significant amounts of CBZ are lost. Dilution with an equal volume of diluent and flushing after administration can minimize the adsorption. Pharmacodynamic interactions have been reported between CBZ and lamotrigine and between CBZ and levetiracetam. When either lamotrigine or levetiracetam is added to regimen of patients taking CBZ there is an increase in incidence of central nervous system side effects. These effects are not associated with an increase in the concentration of either the CBZ or 10, 11-epoxide active metabolite. A dosage reduction of CBZ may be necessary when these drugs are added. [21]

Table 2: Drug interactions that CBZ change the concentrations [21, 24]

CBZ increases drug concentration	CBZ decreases drug concentration
Clomipramine	Acetaminophen
Primidone	Antidepressants (sertraline, citalopram, escitalopram,
Selegiline	duloxetine,
Phenytoin	bupropion, mirtazapine, trazodone, imipramine,
	amitriptyline, nortriptyline)
	Anticoagulants (warfarin, dicumarol)
	Antiepileptics(ethosuximide, lamotrigine, tiagabine,
	topiramate, valproate, zonisamide)
	Antifungal agents (fluconazole, itraconazole, ketoconazole)
	Antipsychotics (aripiprazole, clozapine, fluphenazine,
	haloperidol, olanzapine, risperidone, ziprasidone)
	Benzodiazepines (alprazolam, clonazepam, midazolam)
	Beta-blocker (propranolol)
	Corticosteroids (dexamethasone, prednisolone)
	Dihydropyridine calcium-channel blokers (felodipine,
	nifedipine)
	Immunosuppressants (cyclosporine, tracolimus)
	Protease inhibitors(indinavir)
	Statins (atorvastatin, lovastatin, simvastatin)
	Digoxin, Doxycycline
	Fentanyl , Methadone, Tramadol
	Hormonal contraceptives, Levothyroxine
	Methylphenidate, Pancuronium bromide, Vecuronium

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Table 3: Drug interactions that change the CBZ concentrations [21, 24]

Drug increases CBZ concentration	Drug decreases CBZ concentration
Acetazolamide	Antineoplastic agents (cisplatin,
Allopurinol (high-dose 600 mg/day)	doxorubicin)
Antifungal agents (fluconazole, itraconazole,	Rifampicin
ketoconazole)	Felbamate
Antihistamines (loratadine)	Phenobarbital
Antipsychotics (haloperidol, quetiapine, risperidone,	Primidone
loxapine, Chlopromazine)	Phenytoin
Macrolide antibiotics (clarithromycin, erythromycin)	Caffeine
Non-dihydropyridine calcium-channel blockers	
(diltiazem, verapamil)	
Protease inhibitors (ritonavir, saquinavir)	
Baclofen	
Cimetidine	
Danazol	
Felbamate (CBZ-E)	
Fluoxetine (CBZ, CBZ-E)	
Fluvoxamine	
Grapefruit juice	
Gemfibrozil	
Isoniazid	
Loxapine (CBZ-E)	
Nefazodone	
Niacinamide	
Omeprazole	
Pomegranate juice	
Propoxyphene, Dextropropoxyphene	
Valproic acid (CBZ-E)	

CBZ-E; 10, 11-epoxide

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Drug interaction between CBZ and PHT, PB or VPA

Up to 70% of patients diagnosed with epilepsy can be made seizure-free by currently available AEDs given as monotherapy. In patients who are unresponsive to monotherapy, however, a combination of two or more AEDs may be needed to optimize seizure control. However, combination therapy may have adverse effects. When two or more AEDs are used, the potential for drug interactions is substantial, and such interactions may have effect on patient's clinical responses. [25]

CBZ is used as monotherapy or coadministration with other antiepileptic drugs such as PHT, PB and VPA. Because of CBZ is a potent enzyme inducer, when used CBZ with PB the serum level of PB may decrease, while used CBZ with PHT, the serum level of PHT may decrease or increase. There is a complex interaction with VPA and the results are unpredictable. [21] The main enzymes that involved in drug interaction between CBZ and PHT, PB or VPA were shown in Table 4.

Table 4: Main enzymes that involved in drug interaction between CBZ and PHT, PB or VPA [7, 11]

	CYP3A4/5	*CYP2C9*	*CYP2C19*	UDPGT	Epoxide hydrolase
Substrate	CBZ	PHT PB VPA	PHT PB VPA	VPA	10,11-epoxide-CBZ
Enzyme-inducer	CBZ PHT PB	CBZ PHT PB	CBZ PHT PB	CBZ PHT PB	CBZ PHT PB
Enzyme-inhibitor	-	VPA	VPA	VPA	VPA

CBZ, carbamazepine; PHT, phenytoin; PB, phenobarbital; VPA, valproic acid; UDPGT, uridine diphosphate glucuronosyltransferase

CBZ is mainly metabolized by the liver via *CYP450* same as PHT, PB and CBZ is induces UDPGT which is mainly metabolizes VPA while VPA is inhibits CBZ metabolism via Epoxide hydrolase [6-9], it is highly possible that CBZ pharmacokinetics parameters

could be predict from pharmacokinetics parameters of PHT, PB and VPA, and vice versa, if so, it would be apply in CBZ and coadministration drugs therapeutic monitoring.

In 2007 Methaneethorn J. investigated the relationship between pharmacokinetics parameters of PHT and CBZ that found highly correlation between clearance of CBZ and maximum rate of metabolism of PHT (V_{max}) and provided a regression equation to predict CBZ clearance (Cl_{CBZ}) from V_{max} or vice versa: V_{max} *(mg/d/kg) = 1.421 x* Cl_{CBZ} *(L/d/kg) + 4.107* or Cl_{CBZ} *(L/d/kg) = 0.483 x* V_{max} *(mg/d/kg) – 1.340* (correlation coefficient = 0.828, p = 0.001) [18], even though validation and application has never been performed. Additionally the study of correlation between CBZ clearance and PB clearance or VPA clearance has never been investigated.

Usual dosage regimen and clinical applications

CBZ is induces its own metabolism (autoinduction) that takes approximately 3-5 weeks on fixed dosing regimen. Generally doses are started at one-fourth to one-third of the expected maintenance dose and gradually increased to allow for development of tolerance to side effects, especially central nervous system related side effects. The dose is titrated based on the patient's clinical response and tolerability of side effects. [21] The initial and maximum maintenance dosing of CBZ for the treatment of trigeminal neuralgia and bipolar disorder is shown in Table 5 and for the treatment of epilepsy is shown in Table 6.

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Table 5: The initial and maximum maintenance dosing of CBZ for trigeminal neuralgia and bipolar disorder [23]

Indication	Initial dose	Subsequence dose	Maintenance dose
Trigeminal neuralgia	100 mg twice daily	Increase up to 200 mg/day at weekly interval, bid	1,200 mg/day
Bipolar disorder	200-600 mg daily, tid or qid	Tritate upward according to patient response and tolerability	1,600 mg/day

Table 6: The initial and maximum maintenance dosing and dosage forms of CBZ for epilepsy [1, 21]

Dosage form and age groups	Initial dose	Subsequent dose	Maintenance dose
Oral (tablets and suspension): Elderly	100 mg once or twice daily	Increase in weekly interval by 100 mg daily	1,000 mg/day
Over 12 yr	200 mg twice daily	Increase up to 200 mg/day at weekly interval, bid or tid	800-1,000 mg/day (12-15 yr) 1,200 mg/day (>15 yr) 1,600 mg/day (adult in rare instances)
6-12 yr	100 mg twice daily	Increase up to 100 mg/day at weekly interval, bid or tid	1,000 mg/day
Under 6 yr	10-20 mg/kg/day tid or qid	Increase 5 mg/kg/week to achieve optimal clinical response tid or qid	35 mg/kg/day
Oral (tablets or suspension): Rapid loading for critically ill patients			
Children (≤12 yr)	10	-	-
Adult (>12 yr)	8	-	-

Clinical practice in CBZ and other drugs therapy for epilepsy was considered from type of epilepsy that classified by ILAE 1981 [3] (Table 7). In generally the first line drugs was choose before considered the second line drugs or add on therapy. Pragmatically, the choice of AED among first line agents needs to be individualized mainly on the basis of the patient profile, including the efficacy for the seizure or the

epilepsy syndrome, tolerability, safety, ease of use, pharmacokinetics (in consideration of the current or likely future need for concomitant medication for comorbidity), and finally cost. Patients with more than one type of seizures should received AED with broad spectrum or more than one mechanism of action. [3-5] AEDs provide satisfactory control of seizures for most patients with epilepsy.

Table 7: Thai guideline of selection of AEDs.[1]

Type of seizure	Drug selection			
	First line drug			Second line drug (add on drug)
	Drug list A	Drug list D	Not in National List of Essential Medicines 2008	
Absence	Sodium valproate	Lamotrigine		Clonazepam [B]
Myoclonic, atonic, tonic	Sodium valproate			Topiramate * [D] Lamotrigine * [D] Clonazepam [B] Nitrazepam
Generalized tonic clonic	Phenobarbital Sodium valproate Phenytoin Carbamazepine	Lamotrigine Topiramate	Oxcarbazepine	Levetiracetam Clonazepam [B] Clobazam
Partial	Carbamazepine Phenytoin Sodium valproate Phenobarbital	Lamotrigine Topiramate	Levetiracetam Oxcarbamazepine	Gabapentin [D] Clonazepam [B] Clobazam
Infantile spasm		Vigabatrin		Sodium valproate Nitrazepam [D] Clonazepam [B] Clobazam Topiramate [D]

* For treat Lennox-Gastaut syndrome in children

Sub list A, B, C, D and E of National List of Essential Medicines 2008.

Therapeutic and toxic plasma concentration

The accepted therapeutic range for CBZ is 4-12 mg/L. [8, 9, 21] The therapeutic range for a given patient must be individually determined with the goal of therapy as cessation of seizure while minimizing side effects. Little prospective work has been done to establish the therapeutic range for unbound CBZ serum concentration or clinical situations where unbound CBZ serum concentration measurement is useful. As an initial guide, 25% of the total CBZ therapeutic range has been used to establish a preliminary desirable range for unbound CBZ serum concentration of 1-3 mg/L. [8]

The 10, 11-epoxide metabolite of CBZ is active and contributes to efficacy and toxicity. Drug interactions may increase the concentration of the metabolite with out changing the CBZ concentration. Ideally, the clinician should measure both the parent drug and metabolite, but an assay for 10, 11-epoxide is not commercially available. Currently, the therapeutic range of 10, 11-epoxide is not known although a suggested range of 0.4-4 mg/L is used by several research centers. [8, 21]

In the upper end of the therapeutic range (> 8 mg/L) some patients will begin to experience the concentration-related adverse effects of CBZ treatment; neusea, vomiting, lethargy, dizziness, drowsiness, headache, blurred vision, diplopia, unsteadiness, ataxia, incoordination. Because of CBZ induces its own hepatic metabolism, these adverse effects can also be seen early during dosage titration periods soon after dosage increases are made. [8, 9]

CBZ serum concentration should be measured in most of patients. Because epilepsy is an episode disease state, patients do not experience seizures on a continuous basis. Thus, during dosage titration it is difficult to tell if the patient is responding to drug therapy or simply is not experiencing any abnormal central nervous system discharges at that time. CBZ serum concentrations are also valuable tools to avoid adverse drug effects. [8] As a general rule, samples should be obtained at steady state and before the morning dose (trough concentration) to decrease the variation owing to daily fluctuation and avoid multiple peak concentration phenomena. [9]

Factors associated with CBZ pharmacokinetics

The studies about clearance of CBZ are importance for therapeutic drug monitoring. Several studies were found that age, body weight, surface area, dose of CBZ, dose of PB, and co-medication with PHT, PB, or VPA are significant influence on CBZ clearance. [8, 9, 12-14]

Reith DM. et al. examined the influence of weight, height, surface area, autoinduction, age, gender, and comedication upon clearance of CBZ using NONMEM V for population pharmacokinetic analysis. A total of 946 CBZ plasma concentrations from 91 subjects, ages 0.7-37 years, were collected and analyzed using a one compartment, first-order absorption and elimination model. They concluded that surface area and dose were important explanatory variables in the modeling of CBZ population pharmacokinetics in children and adults. CBZ clearance increased with increased surface area and dose. The model was: *CL (L/hr) = (2.24 x Surface area (m^2)) + (0.047 x Dose (mg/kg))*. A bootstrap analysis was used to assess the accuracy and robustness of population model. The estimates for those parameters contributing to clearance and residual error were all within 15% of the bootstrapped means.

Jiao Z. et al. investigated the pharmacokinetic profile of CBZ in Chinese epilepsy patients to facilitate the dosing schedule by NONMEM analysis with a one compartment, first-order absorption and elimination. 687 of serum samples through concentrations at steady state were collected prospectively from 585 patients, ages 1.2-85.1 years. They were found that the important determinants of clearance were total body weight (TBW), dose, patient age over 65 years (E), and comedication with PHT, PB, or VPA when VPA daily dose was greater than 18 mg/kg. The final model was: $CL\ (L/hr) = 0.0722 \times Dose\ (mg/kg/day)^{0.403} \times TBW\ (kg)^{0.697} \times 1.45^{PHT} \times 1.17^{PB} \times 1.21^{VPA} \times 0.851^{E}$. The value of the coefficient of variation for interpatient variability in CL was 15.9% and the residual error standard deviation was 0.987 mg/L.

Vucicevic K. et al. developed a population pharmacokinetic model for CBZ using NONMEM analysis with a one compartment, first-order absorption and elimination. 423 Steady state CBZ plasma concentrations were collected from 265 patients. The influence of weight, age, gender, smoking, allergy, CBZ daily dose, and cotherapy on clearance was evaluated. They were found that patients' gender, age, smoking, allergy,

cotherapy with lamotrigine and benzodiazepines had no effect on CBZ clearance, but patient's weight (WT), daily CBZ dose (DCBZ), daily dose of PB (DPB) and VPA, when its daily dose exceeded 750 mg significantly influenced CBZ clearance and were included in the final model: $CL\ (L/hr) = 5.35[\text{DCBZ (mg/kg/day)}/15]^{0.591} \times [1 + 0.414(\text{DPB(mg/kg/day)}/2)] \times [\text{WT(kg)}/70]^{0.564} \times 1.18^{\text{VPA}}$. The interindividual coefficient of variability for clearance was 36.5%, whereas the residual variability was 1.18 mcg/mL.

Prediction of the suitable dosage regimens for patients treated with CBZ is difficult because of its erratic absorption, autoinductive metabolism, active metabolite, diurnal fluctuations, and narrow therapeutic range (4–12 mg/L). In addition, anticonvulsant therapy can be further complicated by concomitant use of other AEDs with induction and inhibition properties. All these variations in its pharmacokinetic characteristics necessitate individualized dosing regimens. A better understanding of the intraindividual and interindividual variability in pharmacokinetic behavior can lead to more efficacious and safer drug use.[8, 9, 20, 21, 23]

Nowadays pharmacogenomics which are the studies of the complex effects of genome-wide composition on drug disposition and effects during there route from administration to the target site, the drugs can interact with hundreds of proteins like receptors, transporters, and metabolizing enzymes. Polymorphic genes affect the quantity or activity of these protein products and may be explain interindividual variability in pharmacokinetics and pharmacodynamics of many drugs. Several studies investigated the influence of *CYP3A5* polymorphism on CBZ pharmacokinetics. They were found that *CYP3A5* polymorphism affects CBZ clearance.[15, 16]

Cytochrome P450 3A5 *(CYP3A5)* Polymorphism

Cytochrome P450, family 3, subfamily A, polypeptide 5 named *CYP3A5* is a protein that in humans is encoded by the *CYP3A5* gene. The *CYP3A* enzymes in human consist of *CYP3A4*, *CYP3A5*, *CYP3A7* and *CYP3A43*. *CYP3A4* and *CYP3A5* are regarded as predominant functional form of human *CYP3A* in the liver and intestine. They are involved in the phase I metabolism of more than 50% of currently prescribed drugs and endogenous compounds.[26-30]

This gene, *CYP3A5*, encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This protein localizes to the endoplasmic reticulum and its expression is induced by glucocorticoids and some pharmacological agents. The enzyme metabolizes drugs such as nifedipine and cyclosporine as well as the steroid hormones testosterone, progesterone and androstenedione. This gene is part of a cluster of cytochrome P450 genes that locus of 231 kb located on chromosome 7q21.1.[31]

CYP3A5 is polymorphically expressed in liver, small intestine and kidney. The allele nomenclature of the *CYP3A5* was shown in Table 8. The most frequent and functionally important Single-nucleotide polymorphism (SNP) in the *CYP3A5* gene is a mutation of adenosine (*CYP3A5*1* wild-type allele) to guanosine (*CYP3A5*3* mutated allele) at the position 6986 within intron 3 (Figure 3). This mutation creates an alternative splice site in the pre-messenger ribonucleic acid (mRNA) and production of aberrant mRNA (SV1-mRNA) that contains 131 bp of intron 3 sequence (exon 3B) inserted between exon 3 and exon 4 (Figure 4). The exon-3B insertion results in a frameshift and encoded a protein that is truncated at amino acid 102 and is inactive. [30, 32, 33]

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Table 8: *CYP3A5* allele [30]

Allele	Location	Nucleotide changes	Amino Acid substitution	Expression
CYP3A5*1A				
CYP3A5*1B	5'UTR	G-86A		
CYP3A5*1C	5'UTR	C-74T		
CYP3A5*1D	3' UTR	C31611T		
CYP3A5*2	Exon 11	C27289A	T398N	
CYP3A5*3A	Intron 3	A6986G, C31611T	Splicing defect	None
CYP3A5*3B	Intron 3	C3705T, 3709 ins G, A6986G, C31611T	H30Y, splicing defect splicing defect	None
CYP3A5*3C	Intron 3	A6986G		None
CYP3A5*4	Exon 7	A14665G	Q200R	
CYP3A5*5	Intron 5	T12952C	splicing defect	Alternatively spliced mRNA
CYP3A5*6	Exon 7	G14690A	splicing defect	None (skip Exon 7)
CYP3A5*7	Exon 11	27131 ins T	stop codon at 348	None

UTR= untranslated region

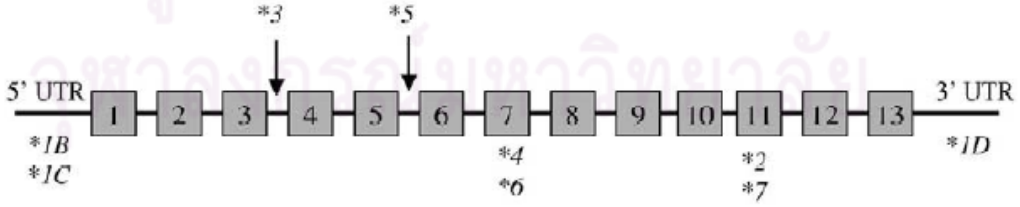

Figure 3: Distribution of mutation in the *CYP3A5* gene [30]

The absence of *CYP3A5* expression was recently correlated to a genetic polymorphism (*CYP3A5*3*). Because CYP3A5 may represent up to 50% of total *CYP3A*

protein in individuals polymorphically expressing *CYP3A5*, it may have a major role in variation of CYP3A-mediated drug metabolism. [30]

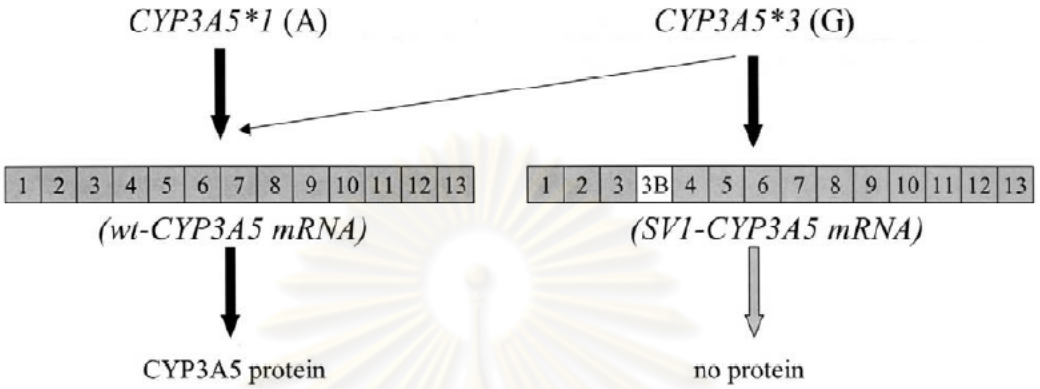

Figure 4: SNP in *CYP3A5* gene within intron 3 (A6986G) [30]

Prevalence of *CYP3A5* polymorphism

Several polymorphic of *CYP3A5* have been recently reported in difference populations. In Thai population the allele frequency of *CYP3A5*3* was 66% and *CYP3A5*1* was 34%, that is similar to other Asian population but significant difference from Caucasian and African American. The frequency of *CYP3A5*3* allele in Thai population was lower and higher than Caucasian and African American respectively. Other *CYP3A5* coding variants have been described, but occur at relatively low allele frequencies. [17, 34-38] The comparison of allele frequency between Thai population and other ethnic populations was shown in Table 9

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Table 9: Allele frequencies of the *CYP3A5* in Thai population and other ethnic populations

Ethnicity	Number of subject	% Allele frequency		p-value
		**1*	**3*	
Thai [17]	150	34	66	-
Chinese [34]	302	22	78	0.059
Indian [35]	90	41	59	0.307
Malaysian [35]	98	39	61	0.463
Japanese [36]	200	23	77	0.085
Dutch Caucasian [37]	500	8	92	<0.001
African American [38]	20	45	48	0.042

Effects of *CYP3A5* polymorphism on CBZ clearance

The human *CYP3A* subfamily plays a most important role in the metabolic elimination of recently prescribed drugs, includes CBZ. *CYP3A4* was the first discovered gene, which plays a most dominant role in *CYP3A* subfamily. There is no evidence of null allele for *CYP3A4*. More than 30 SNPs have been identified in the *CYP3A4* gene. Generally, variant in the coding regions of *CYP3A4* occur at allele frequencies less than 5% and appear as heterozygous with wild-type allele. These coding variants may contribute to but are not likely to be the major cause of interindividual differences in *CYP3A*-dependent clearance, because of the low allele frequencies and limited alterations in enzyme expression or catalytic function. Recent reports indicated that *CYP3A5* plays a crucial role in the metabolism of *CYP3A* substrates. Therefore on the basis of the in vitro evidence, *CYP3A5* is functionally and quantitatively important in relation to total *CYP3A,* especially exhibited comparable metabolic activity as *CYP3A4* (90-110%) toward CBZ, and may play an important role in the disposition of CBZ in vivo. Several genetic variants have been described for *CYP3A5* and the most common, the *CYP3A5*3* allele, causes loss of *CYP3A5* activity. Thus, only people with at least one *CYP3A5*1* allele can express large amounts of *CYP3A5*. [11, 23, 30]

Several studies reported the effects of *CYP3A5* polymorphism on pharmacokinetics of *CYP3A* substrates. The causes of interindividual variability of clearance of amlodipine, tracolimus, cyclosporine, saquinavir, simvastatin and alprazolam are likely from *CYP3A5* polymorphism. [39-44] Recent years, there are 2 studies of the effect of *CYP3A5* polymorphism on pharmacokinetics of CBZ. [15, 16]

Seo T. et al. investigated the effect of *CYP3A5* polymorphism on pharmacokinetics of CBZ in Japanese patients with epilepsy using nonlinear mixed effect regression program and 1-compartment model. They evaluated *CYP3A5* genotype and other covariates: age, body weight, gender, CBZ daily dose, and coadministration of PHT, PB, or VPA. Over all 144 patients, the frequency of homozygous *CYP3A5*3/*3* was 52% and the remaining 48% were *CYP3A5*1/*1* and heterozygous *CYP3A5*1/*3*. Factors influence the clearance of CBZ were body weight, CBZ daily dose, coadministration of PHT or PB, and *CYP3A5*3/*3* genotype which results of 8% significant higher in CBZ clearance than other genotypes ($p < 0.01$). They incorporated *CYP3A5*3* in the final model for the prediction of CBZ clearance: $Cl/F = 0.17 \times (BW/40)^{0.11} \times Dose^{0.45} \times 1.40^{PHT} \times 1.21^{PB} \times 1.08^{*3/*3}$. Although the data modeling showed that the CBZ doses influenced its pharmacokinetic parameters, particularly, the autoinducibility of CBZ was not considered.

Park PW. et al. investigated the effect of *CYP3A5* polymorphism on pharmacokinetics of CBZ at steady state serum concentrations in Korean patients with epilepsy. The selected patients were treated with CBZ monotherapy and were not using co-medication drugs with CBZ pharmacokinetics drug interaction. Plasma concentrations were prospectively collected and analyzed using Baysian estimation program and a one compartment, first-order absorption and elimination model. Over all 35 patients, the frequency of homozygous *CYP3A5*3/*3* was 60% and the remaining 40% were *CYP3A5*1/*1* and heterozygous *CYP3A5*1/*3*. The comparison of CBZ serum concentration between difference genotypes found that patient with *CYP3A5*3/*3* genotype has significant higher level-to-dose ratio than patient with *CYP3A5*1/*1* and *CYP3A5*1/*3* genotypes (13.07 ± 4.46 ng/mL/mg vs 9.94 ± 3.38 ng/mL/mg, $p = 0.032$) or 31% higher. The CBZ clearance in patient with *CYP3A5*3/*3* genotype was significant

lower than patient with *CYP3A5*1/*1* and *CYP3A5*1/*3* genotypes (0.040 ± 0.014 L/h/kg vs 0.056 ± 0.017 L/h/kg, p = 0.004) or 29% lower.

There are conflicting results of two studies above and the studies of effect of *CYP3A5*3* on CBZ pharmacokinetics when combination with others drugs that have drug interaction were not clearly define in other countries and in Thailand has never been study the effect of *CYP3A5* polymorphism on CBZ clearance either in patients with CBZ monotherapy or coadministration with others drugs which have drug interaction such as PHT, PB and VPA. Knowledge of effect of *CYP3A5* polymorphism on pharmacokinetics may be useful in therapeutic plans to avoid serum drug concentration-related adverse effects and reduce inappropriate dosage.

Table 10: Comparison the effect of *CYP3A5* polymorphism on CBZ clearance

	Seo T. et. al. (2006)	**Park PW. et. al. (2009)**
Population	Japanese	Korean
Number of subject	144	35
Average age (yr)	15	35
Co-administration with other AEDs	Monotherapy or used with PHT, PB, or VPA	None
Result	*CYP3A5* polymorphism affected CBZ clearance: *CYP3A5*3/*3* has 8% higher than *CYP3A5*1/*1* and *CYP3A5*1/*3* ($p < 0.01$)	*CYP3A5* polymorphism affected CBZ clearance: *CYP3A5*3/*3* has 29% lower than *CYP3A5*1/*1* and *CYP3A5*1/*3* ($p = 0.004$)

***CYP3A5* genotyping**

Published methods for genotyping *CYP3A5* have relied on gene sequencing or the use of mismatched primers to generate restriction sites to enable restriction fragment length polymorphism (RFLP) analysis. Sequencing is expensive and requires specialized equipment. RFLP may be an option, but can be time-consuming. In the case

of *CYP3A5* analysis, the amplification, digestion and visualization methods are technically more involved than standard RFLP protocols. This is due to the absence of naturally occurring splice site for known restriction endonucleases. Allelic discrimination assay is an alternative method which is rapid and reliable for genotyping *CYP3A5* polymorphism. In allele specific polymerase chain reaction amplification, oligonucleotides specific for hybridizing with the common or variant alleles are used for parallel amplification reaction and then identify for the presence or absence of the appropriate amplified DNA products by real-time fluorescence-based analysis, melt curve analysis or gel electrophoresis. [42-45]

Antiepileptic drug analytical methods

The AEDs have been measured by a wide variety of analytical methods in serum, plasma, blood, saliva, tissue, and urine. For the older AEDs (CBZ, PHT, PB, VPA) and some of the newer AEDs (felbamate, topiramate, zonisamide), automated enzyme multiplied immunoassay (EMIT) and Fluorescence polarization immunoassay (FPIA) are available and allow rapid and accurate determination of concentrations in biological fluids, usually serum or plasma. For the other AEDs, laboratories rely on chromatrographic methods; gas-liquid chromatography (GC) and high-performance liquid chromatography (HPLC) with a variety of detection methods, which are more labor-intensive and relatively more expensive. There are also new technological advances in the use of capillary electrophoresis (CE) for therapeutic drug monitoring. Like other chromatographic methods, CE allows simultaneous measurement of several AEDs and can provide automation of procedures, low cost, and rapid speed with high specificity. As shown in Table 11, there are effective methods of analysis for AEDs. [20]

Table 11: Antiepileptic drug analytical methods

Method of detection	GC			HPLC						
	FID	NPD	MS	UV	ECD	FD	MS	CE	EMIT	FPIA
CBZ	-	-	-	√	-	-	√	√	√	√
CBZ-epoxide	-	-	-	√	-	-	√	√	-	-
Felbamate	√	√	-	√	-	-	-	√	√	√
Gabapentin	√	-	-	√	-	√	-	√	-	-
Lamotrigine	-	√	√	√	-	-	-	√	-	-
Levetiracetam	-	√	-	√	-	-	-	-	-	-
Oxcarbazepine	-	-	√	√	-	-	-	-	-	-
PB	√	-	-	√	-	-	-	√	√	√
PHT	-	-	√	√	-	-	√	√	√	√
Tiagabine	-	-	√	-	√	-	√	-	-	-
Topiramate	√	√	-	√	-	-	√	√	-	√
VPA	√	-	√	√	-	-	-	√	√	√
Zonisamide	-	-	-		-	-	-		-	√

GC: gas chromatography, FID: flame ionization detection, NPD: nitrogen-phosphorus detections, MS: mass spectrometry, HPLC: high-performance liquid chromatography, UV: ultraviolet detection, ECD: electrochemical detection, FD: fluorometric detection, CE: capillary electrophoresis, EMIT: enzyme-multiplied immunoassay technique, FPIA: fluorescence polarization immunoassay.

Pharmacokinetic parameters calculation of CBZ, PHT, PB and VPA [8, 9]

1. Maximum rate of metabolism (V_{max}) of PHT calculated from formula

   $V_{max} = (SFD/\tau)(Km + Css_{ave}) / C_{ss\ ave}$

2. Clearance of CBZ, PB and VPA calculated from formula

   $Cl = SFD / (\tau)(C_{ss\ ave})$

   S is the salt fraction (CBZ = 1, PHT= 0.92 for capsule and = 1 for chewable tablet, PB= 0.9, VPA= 1)

   F is the bioavailability factor (CBZ = 0.7, PHT= 1, PB= 1, VPA= 1)

D is the dose (mg)

τ is the dosing interval (hr or day)

K_m is the population Michaelis constant = 4 mg/L

$C_{ss\ ave}$ is the average plasma concentration at steady state (mg/L)

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CHARPTER III
PATIENTS AND METHOD

This study was conducted from February to September 2010 at Prasat Neurological Institute, Bangkok, Thailand.

1. Study design

A retro-prospective descriptive method was used. Demographic data and measured drugs serum concentrations from patients were collected, *CYP3A5* genes were genotyped, and the data were then analyzed.

2. Patients

2.1 Population and samples

2.1.1 Population is patients with epilepsy or neurological disease who used CBZ as monotherapy or coadministration with PHT, PB or VPA.

2.1.2 Samples are patients with epilepsy or neurological disease who were outpatients at Prasat Neurological Institute during February to September 2010 and met the inclusion criteria.

2.2 Inclusion criteria

2.2.1 Age not less than 13 years old.

2.2.2 Patients who were diagnosed to have epilepsy or neurological disease.

2.2.3 Patients who were treated with CBZ monotherapy or comedication with one of the other classical AEDs, PHT, PB or VPA.

2.2.4 Patients who received stable dose of CBZ in control released dosage form not less than 1 month before blood sampling.

Patients in comedication groups should used the coadministration drug not less than 1 month before blood sampling.

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Patients who co administered with VPA should receive controlled released dosage form only.

2.2.5 All patients consented to enroll in this study.

2.3 Exclusion criteria

2.3.1 Patients with acute or chronic hepatic disease.

2.3.2 Patients with acute or chronic kidney disease.

2.3.3 Patients with drug non-compliance detected from interviewing by the investigator.

2.3.4 Patients who treated chronic diseases with drugs that reported to have some effects on pharmacokinetics of CBZ, such as, verapamil, diltiazem, gemfibrozil, isotretrinion, isoniazid, haloperidol, theophylline, ticlopidine, cimetidine, omeprazole, trazodone, fluoxetine, risperidone, clarithromycin, erythromycin, rifampicin.

2.3.5 Patients whose medical records were not complete or whose required data could not be revealed or were missing.

2.4 Sample size determination

2.4.1 CBZ monotherapy [46]

The purpose of this study was to determine whether patients with difference allele of *CYP3A5*, *CYP3A5*1* and *CYP3A5*3*, would show difference in their CBZ clearance which was a hypothesis testing about the difference of the means of two independent groups of population.

A study in Thailand found that the frequency of *CYP3A5*1* allele in Thai population was 34% and *CYP3A5*3* allele was 66%, that is, the ratio of *CYP3A5*1*: *CYP3A5*3* was 1:2. [17]

To assign:

N was the sample size of CBZ monotherapy patients

N_1 was the sample size of CBZ monotherapy patients with *CYP3A5*1*

N_2 was the sample size of CBZ monotherapy patients with *CYP3A5*3*

$N = N_1 + N_2, N_2 = 2N_1$

$$\frac{N_1 N_2}{N_1 + N_2} = \frac{(Z_\alpha + Z_\beta)^2 S_p^2}{D^2}$$

$$\frac{2N_1^2}{3N_1} = \frac{(Z_\alpha + Z_\beta)^2 S_p^2}{D^2}$$

$$N_1 = \frac{3 (Z_\alpha + Z_\beta)^2 S_p^2}{2D^2}$$

$$S_p^2 \text{ (pooled variance)} = \frac{S_1^2 + S_2^2}{2}$$

Previous study by Park PW. et al. reported that the polymorphism of *CYP3A5* effects on CBZ clearance. Patients with *CYP3A5*1* have higher CBZ clearance than *CYP3A5*3* (0.056 ± 0.017 L/hr/kg VS 0.040 ± 0.014 L/hr/kg, $p<0.05$).

To assign:

$\alpha = 0.05$, $Z_\alpha = 1.64$

$\beta = 0.20$, $Z_\beta = 0.84$

$$S_p^2 \text{ (pooled variance)} = \frac{(0.017)^2 + (0.014)^2}{2}$$

$$= 0.0002425$$

Park PW. et al. found that the difference of CBZ clearance between patients with *CYP3A5*1* VS *CYP3A5*3* was 29%, so, this study set the difference of CBZ clearance to detect to be 25%.

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D (mean difference) = 0.01379

$$N_1 = 3 \frac{(1.64+0.84)^2 (0.0002425)}{2(0.01379)^2}$$

$$= 11.76 \approx 12$$

$N_2 = 24$, $N = 36$

The sample size of CBZ monotherapy patients was 36.

2.4.2 CBZ coadministration with PHT, PB or VPA. [47]

The study of the correlations between CBZ clearance and V_{max}, PB clearance and VPA clearance estimated sample size from this formula

$$N = \frac{(Z_\alpha + Z_\beta)^2}{Z_0^2} + 3$$

$Z_0 = (0.5) \ln (1+r/1-r)$

$\alpha = 0.05$, $Z_\alpha = 1.64$

$\beta = 0.20$, $Z_\beta = 0.84$

r = correlation coefficient

To assign correlation coefficient = 0.60

$Z_0 = (0.5) \ln [(1+0.6) / (1-0.6)] = 0.693$

$$N = \frac{(1.645+0.84)^2}{0.693^2} + 3 = 15.86 \approx 16$$

The sample size of each combination therapy groups (CBZ+PHT, CBZ+PB and CBZ+VAP) was at least 16.

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3. Study protocol

3.1 Study protocol was approved by the ethical committee of Prasat Neurological Institute.

3.2 Patients were selected following inclusion and exclusion criterias.

3.3 The investigator explained the objective and study protocol to the selected patients or their legal representatives. Patients or their legal representatives signed in the informed consent form.

3.4 Demographic data were collected from medical records.

3.5 Made an appointment for patient to have his/her blood sample collected at the next visited time. [Before a visit date the investigator called to remind the patient to bring his/her morning antiepileptic drug(s) along on the visit date and had his/her blood sample drawn before taking antiepileptic drug(s), blood samples for CBZ, PHT, PB and VPA levels monitoring were drawn at steady state, at trough level that was, before the administration of the next dose in the morning.]

3.6 Coordinated the doctor to order blood samples drawing for antiepileptic drug levels measurement and *CYP3A5* genotyping.

3.7 Coordinated the medical technologist for blood sample drawing to measure antiepileptic drug levels and *CYP3A5* genotyping.

3.8 Measured antiepileptic drug levels and *CYP3A5* genotyping.

3.9 Collected all the required data and analyzed.

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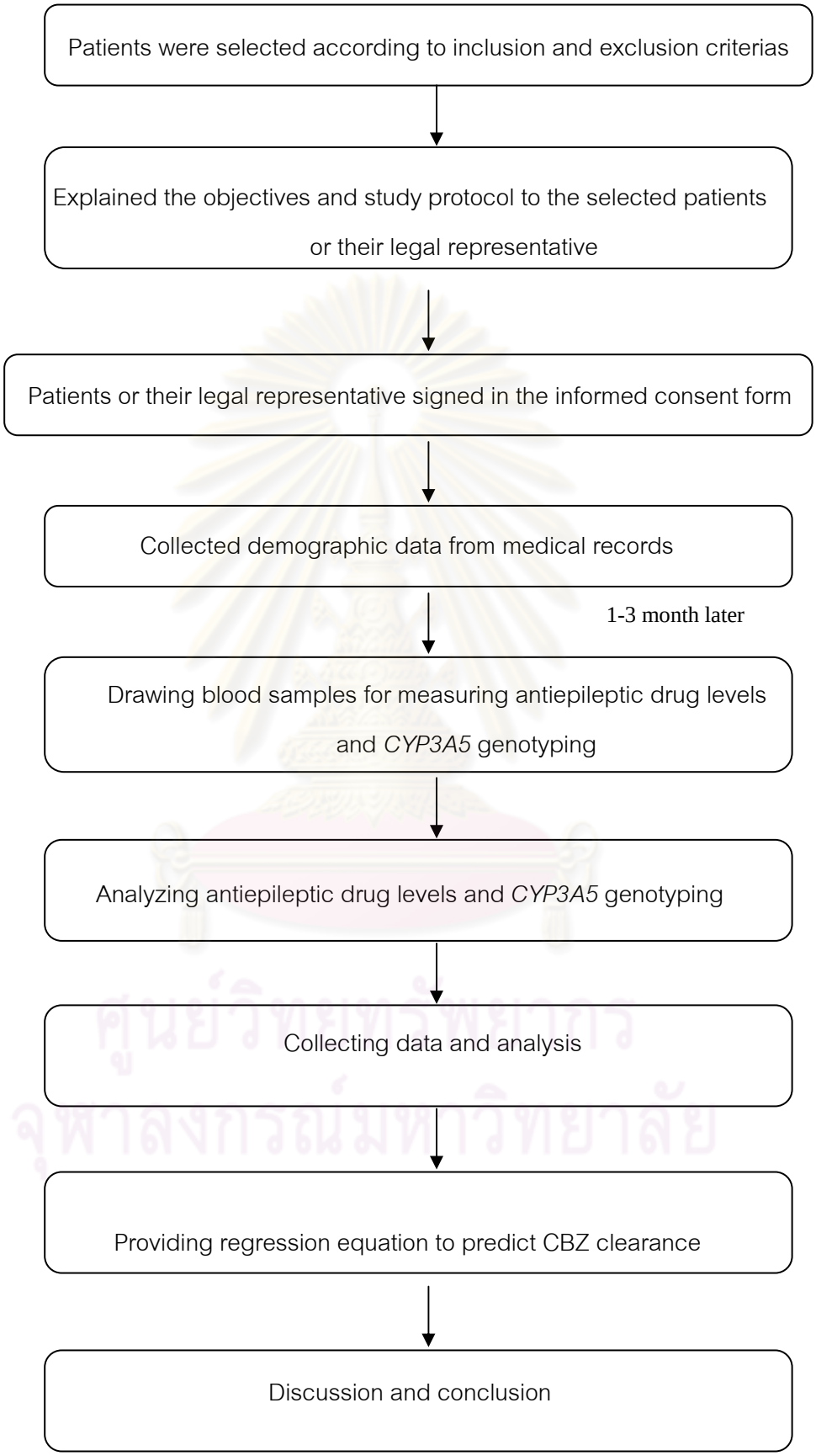
Figure 5: Study protocol



4. Sampling

Eighty five patients who met the inclusion criteria were participated in this study. Blood sampling for CBZ, PHT, PB and VPA concentrations were obtained at steady state. Whole blood was drawn from patients before the administration of the next dose of antiepileptic drugs in the morning. Volume of blood sample was 10 mL for the patients who received CBZ monotherapy and 15 mL for the patients who received CBZ with PHT, PB or VPA. Blood samples were collected in 2 tubes, 5 or 10 mL of clot blood tube (red-stopper) for measured antiepileptic drugs level measurement and 5 ml of Vacutainer® tube (purple-stopper) containing EDTA for *CYP3A5* genotyping.

Whole blood in the EDTA tube was prepared as buffy coat by centrifuge at 2,500 x g for 10 minutes at room temperature. After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes. Pipette 200 mcL of buffy coat into microcentrifuge tube size 1.5 mL and stored in a freezer at -20 °C until extracted for DNA.

5. Bioanalysis

5.1 DNA extraction

Buffy coat were used for DNA extraction by QIAamp® DNA Blood Mini kit.

5.1.1 Materials

Chemical and reagents

1.	Absolute etanol	Carlo erba	Italy
2.	Buffer AL	Qiagen	Germany
3.	Buffer AW1	Qiagen	Germany
4.	Buffer AW2	Qiagen	Germany
5.	Buffer AE	Qiagen	Germany
6.	QIAGEN®protease	Qiagen	Germany
7.	Protease solvent	Qiagen	Germany

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Apparatus

1.	Centrifuge (Universal 320)	Hettick	Germany
2.	Vortex mixer (S0100-220)	Labnet	USA
3.	Heating block (Dri-block DB-2D)	Techne	UK
4.	Microcentrifuge (5415R)	Eppendorf	Germany
5.	Spectrophotometer (Smart spec 3000)	Bio-rad™	USA
6.	Freezer	Sanyo	Japan
7.	Real-Time PCR system (Applied Biosystems 7500)		USA

Supplies

1.	Microcentrifuge tube (1.5 ml)	Treff AG.	Switzerland
2.	Pipette tip (Blue and Yellow)	Scientific Plastics	USA
3.	Micropipette 1,000 mcL	Eppendorf	Germany
4.	Micropipette 200 mcL	Eppendorf	Germany
5.	Micropipette 20 mcL	Eppendorf	Germany
6.	QIAamp Mini spin Column	Qiagen	Germany
7.	Collection tube 2 mL	Qiagen	Germany
8.	Disposable gloves		

5.1.2 DNA Extraction method

1. Equilibrate samples and reagents to room temperature.
2. Heat a heating block to 56°C.
3. Pipette 20 mcL QIAGEN Protease into a 1.5 mL microcentrifuge tube containing buffy coat 200 mcL.
4. Mix by vortex mixer for 15 seconds.
5. Add 200 mcL buffer AL to the sample. Mix by vortex mixer for 15 seconds.
6. Incubate at 56°C for 10 minutes.
7. Briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.

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8. Add absolute ethanol (96–100%) 200 mcL to the sample, and mix again by vortex mixer for 15 seconds. After mixing, briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.
9. Carefully apply the mixture to the QIAamp Mini spin column (in a 2 mL collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 minute. Place the QIAamp Mini spin column in a clean 2 mL collection tube, and discard the tube containing the filtrate.
10. Carefully open the QIAamp Mini spin column and add 500 mcL Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 minute. Place the QIAamp Mini spin column in a clean 2 mL collection tube, and discard the collection tube containing the filtrate.
11. Carefully open the QIAamp Mini spin column and add 500 mcL Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 minutes.
12. Place the QIAamp Mini spin column in a new 2 mL collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 minute.
13. Place the QIAamp Mini spin column in a clean 1.5 mL microcentrifuge tube, and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 200 mcL Buffer AE or distilled water. Incubate at room temperature (15 – 25°C) for 1 minute, and then centrifuge at 6000 x g (8000 rpm) for 1 minute.
14. For long-term storage of DNA, eluting in Buffer AE and storing at –20°C.

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5.1.2 Optical Density measurement

After DNA isolation should bring a sample to measure the amount and quality of DNA by OD measurement. These steps should be done with spectrophotometer as following.

1. Dilute a sample of DNA isolation in 1:5 concentrations, by using DNA 20 mcL add ddH_2O 80 mcL.
2. Prepare dH_2O 100 mcL for control.
3. Set spectrophotometer measure OD at 260 and 280 nm.
4. Calculate OD 260/280 ratio to observe purity and estimate concentration of DNA following this formula.

DNA concentration in mcg/mL or ng/mcL = OD260 x 50 x dilution factor

5.2 *CYP3A5* genotyping

CYP3A5 genotyping was determined by Allelic discrimination assay using real-time polymerase chain reaction (real-time PCR) technique with specific probe and primer (TaqMan® MGB probes, FAM™ and VIC® dye-labeled). See methods at Appendix D.

5.3 Drugs concentration measurement

CBZ, PHT, PB and VPA concentrations in serum were determined by the biochemistry laboratory of Prasat Neurological Institute using an immuno-turbidimetry assay method with an automate analyzer (Synchron LX® Systems, Beckman Coulter Inc., Fullerton, California). The analytical range of CBZ level was 2.0-20.0 mg/L, while the precision specification was 0.6 mg/L or 5.0%. The analytical range of PHT level was 2.5-40.0 mg/L, while the precision specification was 0.5 mg/L or 4.0%. The analytical range of PB level was 5.0-80.0 mg/L, while the precision specification was 1.0 mg/L or 4.0%. The analytical range of VPA

level was 10.0-150.0 mg/L, while the precision specification was 3.6 mg/L or 6.0%.

6. Statistical analysis

Statistical analyses were determined using the Statistical Package for Social Sciences (SPSS Co., Ltd., Bangkok Thailand) software version 17.0. Both descriptive and inferential statistics were determined. The level of significance was set at an α = 0.05.

Continuous variables was determined for normality of the distribution using Kolmogorov–Smirnov test and determined for homogeneity of variance using Levene's test.

Demographic data were determined and presented as mean ± SD, median, percentage or frequency where appropriate for qualitative or quantitative variables.

Statistical comparisons of CBZ clearance and level-to-dose-ratio between patients with *CYP3A5*1* and *CYP3A5*3* were performed using independent t-test or Mann-Whitney U test. Statistical comparisons of CBZ clearance and level-to-dose-ratio between patients with CBZ monotherapy or coadministration with PHT, PB or VPA were performed using one-way ANOVA, median test or Kruskal-Wallis H test.

The correlation between CBZ clearance and demographic data such as weight, gender, age, CBZ dose, coadministration drugs, *CYP3A5* allele were determined by multiple regression analysis.

The correlation between CBZ clearance and PHT V_{max}, PB clearance and VPA clearance were determined using simple linear regression. The assumptions of linear regression were tested; linearity of the relationship between dependent and independent variables, independence of the errors (no serial correlation), homoscedasticity (constant variance) of the errors versus the predictions (or versus any independent variable) and the normality of the error distribution

Regression equation to predict CBZ clearance from demographic data and polymorphism of *CYP3A5* was provided using regression analysis or multiple regression analysis with forward-inclusion method.

CHARPTER IV
RESULTS

Part 1 Clinical pharmacokinetics of carbamazepine as monotherapy and in combination with classical antiepileptic drugs

Eighty five patients who used CBZ as monotherapy or coadministration with PHT, PB or VPA and their therapeutic drug monitoring data (TDM) had been recorded and available and met the inclusion criteria were included into this study. Four years retro-prospective data, August 2006 - August 2010, were collected from electronic database and medical record at the epilepsy outpatient clinic of Prasat Neurological Institute.

Demographic data

Of the 85 patients recruited, 3 patients were excluded; one patient had the PHT level lower than the analytical range, 2 patients used CBZ once daily at bedtime which CBZ levels obtained in the morning were not the trough levels. Data used for analysis included from the total of 82 patients, 79 were diagnosed to be epilepsy and 3 were neuropathic pain. Of the 79 epileptic patients, 13 had a generalized seizure and 66 had a localized seizure. Among these, 36 patients used CBZ as monotherapy, 15 patients used CBZ combination with PHT, 15 patients used CBZ combination with PB and 16 patients used CBZ combination with VPA; the details are shown in Table 12.

Table 13 presents CBZ pharmacokinetic parameters from the total patients included into the study.

Table 14 shows the comparisons of patient's characteristics and PK parameters of CBZ when categorized patients into 4 groups based on other AEDs used in combination with CBZ; CBZ monotherapy, CBZ combination with PHT (CBZ+PHT), CBZ combination with PB (CBZ+PB), and CBZ combination with VPA (CBZ+VPA). Patient's age, body weight, CBZ daily dose per body weight were not significantly different among these 4 groups, but the CBZ daily dose, CBZ level, CBZ level-to-dose ratio and CBZ clearance were significantly different among the 4 groups.

Table 12: Demographic data of patients (N=82)

Characteristic	Frequency, (mean ± SD or median)	% (range)
Number of patients	82	100
Gender		
Male	34	41.5
Female	48	58.5
Age (years)	(39.70±15.02)	(13.87–82.05)
Weight (kgs)	(61.60±12.21)	(37-104)
Indication of CBZ used		
Epilepsy	79	96
Neuropathic pain	3	4
Type of epilepsy		
Generalized seizure	13	16
Localized seizure	66	84
Combination therapy		
CBZ monotherapy	36	44
CBZ+PHT	15	18
CBZ+PB	15	18
CBZ+VPA	16	20

Table13: Pharmacokinetic parameters of CBZ from total patients included (N=82)

PK parameters (N=82)	Minimum	Maximum	Mean ± SD or Median
CBZ dose (mg/day)	200	2,000	800
(mg/kg/day)	3.33	32.33	15.45±6.53
CBZ level (mg/L)	2.10	11.90	7.50±2.43
(mcg/L/mg)	1.61	22.00	9.03±3.71
CBZ clearance (L/hr)	1.33	18.10	3.31
(L/day)	31.82	434.48	79.44
(L/kg/hr)	0.022	0.259	0.057
(L/kg/day)	0.53	6.21	1.37

Multiple comparisons of the pharmacokinetic parameters of CBZ among the 4 groups of different drug treatment in order to identify which group was different from other group were shown in details in Table 15. The result indicated that the CBZ level-to-dose ratio in CBZ monotherapy group was significantly higher than all of the other groups, and this parameter in the CBZ+PHT group was significantly lower than that observed in all of the other groups. Comparisons of the median of CBZ clearance (L/kg/hr or L/kg/day) among the 4 groups indicated that the CBZ monotherapy group had significantly lower CBZ clearance as compared to the CBZ+PHT and CBZ+PB groups, but this CBZ clearance was not significantly different from the CBZ clearance obtained from the CBZ+VPA group. At the same time, the median CBZ clearance of the CBZ+PHT group was significantly higher than that of the CBZ+VPA group.

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Table 14: Comparisons of some patient's characteristics and pharmacokinetic parameters of CBZ among CBZ monotherapy and difference combination therapy groups

Parameter	Mean ± SD or Median				P-value
	CBZ (N=36)	CBZ+PHT (N=15)	CBZ+PB (N=15)	CBZ+VPA (N=16)	
Age (years) [a]	43.38 ± 14.84	34.25 ± 16.32	39.16 ± 13.37	37.02 ± 14.80	0.199
(range)	(16.53 – 82.05)	(14.13 – 64.90)	(13.87 – 61.69)	(18.35 – 65.51)	
Body weight (kgs) [b]	58.70	60.00	64.20	64.35	0.113
(range)	(40.10 – 89.00)	(37.00 – 82.00)	(47.30 – 82.00)	(43.30 – 104.00)	
CBZ dose (mg/day) [b]	800	900	1,000	1,000	0.039 *
(range)	(200 – 1,600)	(300 – 2,000)	(400 – 1,600)	(400 – 1,600)	
(mg/kg/day)	13.33	19.15	17.39	15.27	0.288
(range)	(3.33 – 29.09)	(5.19 – 27.91)	(6.23 – 30.77)	(7.08 – 32.33)	
CBZ level (mg/L) [a]	8.18 ± 2.36	5.16 ± 2.24	7.41 ± 2.16	8.24 ± 1.64	<0.001 *
(range)	(3.70 – 11.90)	(2.10 – 9.20)	(3.80 – 10.80)	(3.70 – 10.90)	
(mcg/L/mg) [b]	10.50	5.58	6.75	8.88	<0.001 *
(range)	(5.40 – 22.00)	(1.61 – 13.14)	(3.80 – 13.50)	(5.36 – 13.83)	
CBZ clearance (L/hr) [b]	2.78	5.22	4.32	3.42	<0.001 *
(range)	(1.33 – 5.40)	(2.22 – 18.10)	(2.16 – 7.68)	(2.11 – 5.44)	
(L/day)	66.67	125.37	103.70	81.86	<0.001 *
(range)	(31.82 – 129.63)	(53.26 – 434.48)	(51.85 – 184.21)	(50.60 – 130.67)	
(L/kg/hr)	0.049	0.097	0.064	0.056	0.003 *
(range)	(0.022 – 0.129)	(0.036 – 0.259)	(0.035 – 0.139)	(0.027 – 0.111)	
(L/kg/day)	1.17	2.34	1.52	1.35	0.003 *
(range)	(0.53 – 3.09)	(0.87 – 6.21)	(0.83 – 3.34)	(0.66 – 2.66)	

* Statistical significant difference ($p < 0.05$), [a] one way ANOVA test, [b] Median Test.

Table 15: Multiple comparisons of the pharmacokinetic parameters of CBZ between CBZ monotherapy and combination therapy

CBZ level (mg/L) [a]	Group	CBZ	CBZ+PHT	CBZ+PB	CBZ+VPA
	CBZ				
	CBZ+PHT	0.000*			
	CBZ+PB	0.667	0.029*		
	CBZ+VPA	1.00	0.001*	0.714	
	Mean±SD	8.18±2.36	5.16±2.24	7.41±2.16	8.24±1.64
CBZ level-to-dose ratio (mcg/L/mg) [b]	Group	CBZ	CBZ+PHT	CBZ+PB	CBZ+VP
	CBZ				
	CBZ+PHT	0.000*			
	CBZ+PB	0.008*	0.040*		
	CBZ+VPA	0.043*	0.005*	0.333	
	Median	10.50	5.58	6.75	8.88
CBZ Clearance [b]	Group	CBZ	CBZ+PHT	CBZ+PB	CBZ+VPA
	CBZ				
	CBZ+PHT	0.000*			
	CBZ+PB	0.008*	0.040*		
	CBZ+VPA	0.029*	0.009*	0.514	
(L/hr)	Median	2.78	5.22	4.32	3.42
(L/day)	Median	66.67	125.37	103.70	81.86
CBZ Clearance [b]	Group	CBZ	CBZ+PHT	CBZ+PB	CBZ+VP
	CBZ				
	CBZ+PHT	0.000*			
	CBZ+PB	0.036*	0.054		
	CBZ+VPA	0.341	0.002*	0.252	
(L/kg/hr)	Median	0.049	0.097	0.064	0.056
(L/kg/day)	Median	1.17	2.34	1.52	1.35

* Statistical significant differences, [a] Post Hoc test (Tukey HDS), [b] Mann-Whitney-U test.

The details about the other classical AEDs which used in combination with CBZ are shown in Table 16.

Table 16: Pharmacokinetic parameters of other AEDs used in combination with CBZ

PK parameters of other AEDs	Minimum	Maximum	Mean±SD or Median
CBZ+PHT (N=15)			
PHT dose (mg/day)	200.00	400.00	298.33 ± 69.09
PHT dose/BW (mg/kg/day)	3.33	6.67	5.01 ± 1.07
PHT level (mg/L)	4.50	32.20	15.32 ± 8.61
CBZ+PB (N=15)			
PB dose (mg/day)	30	180	120
PB dose/BW (mg/kg/day)	0.54	2.68	1.53 ± 0.73
PB level (mg/L)	7.00	32.80	13.60
CBZ+VPA (N=16)			
VPA dose (mg/day)	500	1,750	1,100
VPA dose/BW (mg/kg/day)	8.85	39.26	19.25 ± 7.68
VPA level (mg/L)	12.70	95.20	62.56 ± 20.93

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Therapeutic outcome

Therapeutic outcomes were organized from the evaluations of physicians which put in the medical records. Among the 36 patients of CBZ monotherapy group, 3 patients used CBZ for neuropathic pain while 33 patients used for epilepsy. Within these 33 epileptic patients, 6 patients (18%) had uncontrolled seizure even though their CBZ levels were within the therapeutic range. A second drug had been added to 4 patients; topiramate to 3 patients and the remainder received VPA, their seizures were improved later. Because of the precipitating factors (fever, sleep late), two patients still received the same dosage of CBZ. None of the patients in CBZ monotherapy group showed sign of noticeable adverse effect (Table 17).

Among the 15 patients of CBZ+PHT combination therapy group, 4 patients (27%) still had seizure; the dosages of CBZ were increased in 2 patients and the dosages of PHT were increased in one patient, their seizures were improved later, one patient still received the same dosages of CBZ+PHT since seizure was due to precipitating factor (sleep late). There were 5 patients who had their PHT levels above the therapeutic range, 2 of them had adverse effects; nystagmus and ataxia, and their PHT dosages had been decreased (Table 17).

Among the 15 patients of CBZ+PB combination therapy group, 2 patients (13%) still had seizure; the dosage of CBZ was increased in one patient, while the rest one patient still received the same dosages of CBZ+PB since her seizure was due to precipitating factor (perimenstruation period). One patient noticed mild dizziness (Table 17).

Among the 16 patients of CBZ+VPA combination therapy group, 7 patients (44%) still had seizure; the dosage of VPA was increased in one patient and the third drug (topiramate or lamotrigine) were added in 2 patients, their seizures were improved later, the remainder 4 patients still received the same dosages of CBZ+VPA since their seizures were due to precipitating factors (sleep late, stress, perimenstruation period). One patient had mild tremor (Table 17).

Table 17: Therapeutic outcome of patients

Therapeutic levels	Efficacy		Adverse effect
	Controlled seizure	Uncontrolled seizure	
CBZ monotherapy (N=33)			
Subtherapeutic range (CBZ level < 4mg/L)	2	-	-
Therapeutic range (CBZ level 4-12 mg/L)	25	6	-
Above therapeutic range (CBZ level > 12 mg/L)	-	-	-
CBZ+PHT (N=15)			
	CBZ PHT	CBZ PHT	
Subtherapeutic range (CBZ level < 4mg/L and/or PHT <10 mg/L)	1 3	2 3	-
Therapeutic range (CBZ level 4-12 mg/L and PHT 10-20 mg/L)	3 3	1 1	-
Above therapeutic range (CBZ level > 12 mg/L and/or PHT > 20 mg/L)	- 5	- -	2
CBZ+PB (N=15)			
	CBZ PB	CBZ PB	
Subtherapeutic range (CBZ level < 4mg/L and/or PB <10 mg/L)	1 3	- -	-
Therapeutic range (CBZ level 4-12 mg/L and PB10-40 mg/L)	9 9	2 2	1
Above therapeutic range (CBZ level > 12 mg/L and/or PB > 40 mg/L)	- -	- -	-
CBZ+VPA (N=16)			
	CBZ VPA	CBZ VPA	
Subtherapeutic range (CBZ level < 4mg/L and/or VPA <50 mg/L)	1 3	- 1	1
Therapeutic range (CBZ level 4-12 mg/L and VPA 50-100 mg/L)	6 6	6 6	-
Above therapeutic range (CBZ level > 12 mg/L and/or VPA > 100 mg/L)	- -	- -	-

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Part 2 Correlation between pharmacokinetic parameters of carbamazepine and other classical antiepileptic drugs when used in combination

Data from 46 patients of the 82 patients from previous part (part 1) were recruited into part 2 of this study.

Demographic data

Data included for analysis were from 46 epileptic patients, 8 had a generalized seizure and 38 had a localized seizure. There were 15 patients who used CBZ in combination with PHT, 15 patients who used CBZ in combination with PB and 16 patients who used CBZ in combination with VPA. Neither patient had serum albumin which was lower than the normal range. Demographic data of each combination therapy group is shown in table 18.

Table 18: Demographic data

Parameter	Mean ± SD or Median		
	CBZ+PHT (N=15)	CBZ+PB (N=15)	CBZ+VPA (N=16)
Age (years)	34.25 ± 16.32	39.16 ± 13.37	37.02 ± 14.80
(range)	(14.13 – 64.90)	(13.87 – 61.69)	(18.35 – 65.51)
Body weight (kgs)	61.05 ± 14.78	62.77 ± 9.98	67.09 ± 14.48
(range)	(37.00 – 82.00)	(47.30 – 82.00)	(43.30 – 104.00)
CBZ dose (mg/day)	900	1,000	1,000
(range)	(300 – 2,000)	(400 – 1,600)	(400 – 1,600)
CBZ dose/BW (mg/kg/day)	19.15	17.39	15.27
(range)	(5.19 – 27.91)	(6.23 – 30.77)	(7.08 – 32.33)
CBZ level (mg/L)	5.16 ± 2.24	7.41 ± 2.16	8.24 ± 1.64
(range)	(2.10 – 9.20)	(3.80 – 10.80)	(3.70 – 10.90)
CBZ level/dose (mcg/L/mg)	5.58	6.75	8.88
(range)	(1.61 – 13.14)	(3.80 – 13.50)	(5.36 – 13.83)
CBZ level/dose/BW (mcg/L/mg/kg)	0.12 ± 0.06	0.13 ± 0.05	0.14 ± 0.04
(range)	(0.05 – 0.23)	(0.07 – 0.24)	(0.07 – 0.22)

The details of the combination drugs which were used concurrently with CBZ are shown in Table 19. The mean daily dose per body weight of PHT from 15 patients was 5.01 ± 1.07 mg/kg/day while the mean serum level of PHT was 15.32 ± 8.61 mg/L. The mean daily dose per body weight of PB from 15 patients was 1.53 ± 0.73 mg/kg/day while the median serum level of PB was 13.60 mg/L. The mean daily dose per body weight of VPA from 16 patients was 19.25 ± 7.68 mg/kg/day and the mean serum level of VPA was 62.56 ± 20.93 mg/L.

Table 19: Pharmacokinetic parameters of AEDs used in combination with CBZ

PK parameters of other AEDs	Minimum	Maximum	Mean±SD or Median
CBZ+PHT (N=15)			
PHT dose (mg/day)	200.00	400.00	298.33 ± 69.09
PHT dose/BW (mg/kg/day)	3.33	6.67	5.01 ± 1.07
PHT level (mg/L)	4.50	32.20	15.32 ± 8.61
PHT level/dose (mg/L/mg)	0.011	0.083	0.520 ± 0.025
CBZ+PB (N=15)			
PB dose (mg/day)	30	180	120
PB dose/BW (mg/kg/day)	0.54	2.68	1.53 ± 0.73
PB level (mg/L)	7.00	32.80	13.60
PB level/dose (mg/L/mg)	0.10	0.40	0.19 ± 0.07
CBZ+VPA (N=16)			
VPA dose (mg/day)	500	1,750	1,100
VPA dose/BW (mg/kg/day)	8.85	39.26	19.25 ± 7.68
VPA level (mg/L)	12.70	95.20	62.56 ± 20.93
VPA level/dose (mg/L/mg)	0.025	0.095	0.053± 0.022

Table 20 shows pharmacokinetic parameters of each patient in CBZ+PHT combination therapy group. CBZ clearance ranged from 0.87 – 6.21 L/kg/day (mean 2.45 ± 1.28 L/kg/day). PHT Vmax ranged from 4.32 – 9.93 mg/kg/day (mean 6.29 ± 1.50 mg/kg/day). The correlation between CBZ clearance and PHT Vmax was determined using regression analysis. The scatter plot of CBZ clearance versus PHT Vmax is shown in figure 6, which likely to be a simple linear correlation. The correlation between CBZ clearance and PHT Vmax was highly significant ($r = 0.817$, $p < 0.001$). There was an outlier data which was the data from patient number 2, when we excluded this data, slightly increasing in the correlation coefficient was found ($r = 0.883$, $p < 0.001$) .The regression equations between CBZ clearance and PHT Vmax are shown in Table 21.

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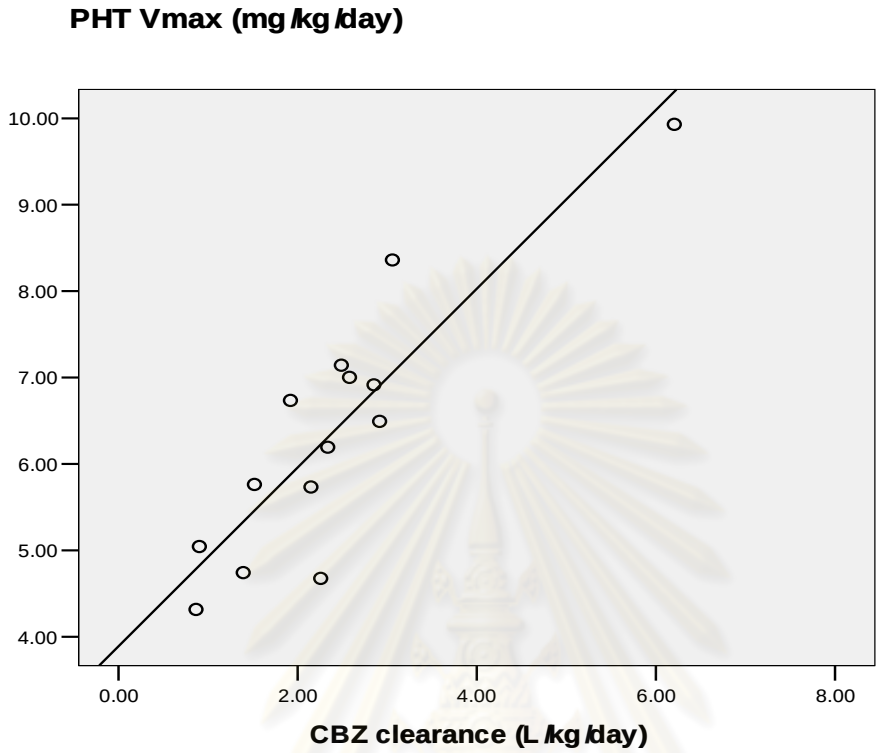
Table 20: Pharmacokinetic parameters of individual patient in CBZ+PHT combination therapy group

Patient No	CBZ dose (mg/kg)	CBZ CL (L/day)	CBZ CL (L/kg/day)	CBZ CL (L/kg/hr)	PHT dose (mg/kg)	PHT Vmax (mg/day)	PHT Vmax (mg/kg/day)	PHT Vmax (mg/kg/hr)
1	20.90	128.61	1.92	0.080	4.48	451.24	6.73	0.28
2	10.00	200.00	3.33	0.139	3.33	317.82	5.30	0.22
3	20.00	112.90	2.26	0.094	4.00	233.73	4.67	0.19
4	19.15	134.04	2.85	0.119	6.38	325.07	6.92	0.29
5	8.11	95.45	2.58	0.107	5.41	259.10	7.00	0.29
6	26.67	112.00	2.49	0.104	6.67	321.43	7.14	0.30
7	27.91	125.37	2.92	0.121	5.81	279.22	6.49	0.27
8	25.71	434.48	6.21	0.259	5.71	695.11	9.93	0.41
9	11.14	108.95	1.52	0.063	5.57	413.71	5.76	0.24
10	6.76	159.09	2.15	0.090	5.41	424.29	5.73	0.24
11	5.19	66.67	0.87	0.036	3.90	332.33	4.32	0.18
12	11.86	53.26	0.90	0.038	4.24	297.67	5.05	0.21
13	19.23	159.09	3.06	0.127	6.25	434.78	8.36	0.35
14	9.76	114.29	1.39	0.058	3.96	388.88	4.74	0.20
15	24.69	189.19	2.34	0.097	4.01	501.67	6.19	0.26
Mean ± SD	16.47 ±7.85	146.23 ±89.16	2.45 ± 1.28	0.102 ± 0.053	5.00 ±1.07	378.40±116.76	6.29 ± 1.50	0.26 ± 0.06
Range	5.19 -27.91	53.26 -434.48	0.87 -6.21	0.036 -0.259	3.33 -6.67	233.73 -695.11	4.32 -9.93	0.18 -0.41



r = 0.883, p <0.001

Figure 6: Scatter plot of CBZ clearance (L/kg/day) versus PHT maximum rate of metabolism (mg/kg/day) (N=14).

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Table 21: Regression equations show correlation between PHT maximum rate of metabolism and CBZ clearance

Regression equation	R	R Square	P- value
(N=15)			
PHT Vmax (mg/day) = 1.064 x CBZ CL (L/day) + 222.802	0.813	0.660	< 0.001
CBZ CL (L/day) = 0.621 x PHT Vmax (mg/day) – 88.595			
PHT Vmax (mg/kg/day) = 0.956 x CBZ CL (L/kg/day) + 3.945	0.817	0.668	< 0.001
CBZ CL (L/kg/day) = 0.699 x PHT Vmax (mg/kg/day) – 1.942			
(N=14)[§]			
PHT Vmax (mg/day) = 1.127 x CBZ CL (L/day) + 222.285	0.857	0.735	< 0.001
CBZ CL (L/day) = 0.652 x PHT Vmax (mg/day) – 107.266			
PHT Vmax (mg/kg/day) = 1.034 x CBZ CL (L/kg/day) + 3.889	0.883	0.780	< 0.001
CBZ CL (L/kg/day) = 0.754 x PHT Vmax (mg/kg/day) – 2.405			

[§]: excluded 1 patient (No.2 out lier data).

Table 22 shows pharmacokinetic parameters of each patient in CBZ+PB combination therapy group. CBZ clearance ranged from 0.83 – 3.34 L/kg/day (mean= 1.68 ± 0.77 L/kg/day). PB clearance ranged from 0.033 – 0.183 L/kg/day (mean= 0.084 ± 0.034 L/kg/day). The correlation between CBZ clearance and PB clearance was determined using regression analysis. There were no significant correlation between CBZ clearance versus PB clearance ($r = 0.332$, $p = 0.227$).The regression equations between CBZ clearance and PB clearance were performed and are shown in Table 23.

Table 22: Pharmacokinetic parameters of individual patient in CBZ+PB combination therapy group

Patient No	CBZ dose (mg/kg)	CBZ CL (L/day)	CBZ CL (L/kg/day)	CBZ CL (L/kg/hr)	PB dose (mg/kg)	PB CL (L/day)	PB CL (L/kg/day)	PB CL (L/kg/hr)
1	14.55	69.14	1.26	0.052	0.82	4.82	0.088	0.0037
2	16.00	84.85	1.13	0.047	0.80	5.19	0.069	0.0029
3	11.27	58.95	.83	0.035	1.69	5.10	0.072	0.0030
4	30.19	103.70	1.96	0.082	1.13	3.97	0.075	0.0031
5	30.77	169.70	3.26	0.136	1.15	5.51	0.106	0.0044
6	17.39	80.00	1.16	0.048	0.87	2.28	0.033	0.0014
7	7.14	51.85	0.93	0.039	2.68	4.12	0.074	0.0031
8	17.86	104.48	1.87	0.078	0.54	3.86	0.069	0.0029
9	6.23	56.00	0.87	0.036	1.87	4.58	0.071	0.0030
10	16.91	101.82	2.15	0.090	2.54	8.64	0.183	0.0076
11	20.00	111.36	1.59	0.066	0.86	4.03	0.058	0.0024
12	12.20	118.64	1.45	0.060	1.46	7.71	0.094	0.0039
13	17.65	103.70	1.53	0.064	1.76	7.94	0.117	0.0049
14	18.12	184.21	3.34	0.139	2.17	4.25	0.077	0.0032
15	17.67	127.27	1.87	0.078	2.65	5.51	0.081	0.0034
Mean ± SD	16.93±6.82	101.71 ± 38.45	1.68 ± 0.77	0.070 ±0.032	1.53±0.73	5.17 ± 1.73	0.084 ±0.034	0.0035 ±0.0014
Range	6.23-30.77	51.85 – 184.21	0.83 –3.34	0.035 – 0.139	0.54-2.68	2.28 – 8.64	0.033 – 0.183	0.0014 –0.0076

Table 23: Regression equations show correlation between PB clearance and CBZ clearance

Regression equation	R	R Square	P- value
PB CL (L/day) = 0.007 x CBZ CL (L/day) + 4.458 CBZ CL (L/day) = 3.465 x PB CL (L/day) + 83.807	0.155	0.024	0.580
PB CL(L/kg/day) = 0.014 x CBZ CL (L/kg/day) + 0.06 CBZ CL (L/kg/day) = 7.673 x PB CL (L/kg/day) + 1.032	0.332	0.110	0.227

Table 24 shows pharmacokinetic parameters of each patient in CBZ+VPA combination therapy group. CBZ clearance ranged from 0.66 – 2.66 L/kg/day (mean= 1.37 ± 0.52 L/kg/day). VPA clearance ranged from 0.149 – 0.697 L/kg/day (mean= 0.357 ± 0.193 L/kg/day). The correlation between CBZ clearance and VPA clearance was determined using regression analysis. The assumption of the linear regression was tested when we conducted the correlation equation between CBZ clearance (L/kg/day) and VPA clearance (L/kg/day). It was found that when generated the equation to predict VPA clearance from CBZ clearance, the error (observed value – predicted value) was not normally distributed, then, the CBZ clearance was transformed using log transformation (ln CBZ clearance) and the error was normally distributed. In contrary, when we generated the equation to predict CBZ clearance from VPA clearance, the error showed normal distribution. The scatter plot of ln CBZ clearance versus VPA clearance is shown in figure 7 and the scatter plot of VPA clearance versus CBZ clearance is shown in figure 8. The correlation between ln CBZ clearance and VPA clearance was moderately significant ($r = 0.661$, $p = 0.005$). The correlation between VPA clearance and CBZ clearance was moderately significant ($r = 0.642$, $p = 0.007$). The regression equations showed correlation between CBZ clearance and VPA clearance were generated and are shown in Table 25.

Table 24: Pharmacokinetic parameters of individual patient in CBZ+VPA combination therapy group

Patient No	CBZ dose (mg/kg)	CBZ CL (L/day)	CBZ CL (L/kg/day)	Ln CBZ CL (L/kg/day)	CBZ CL (L/kg/hr)	VPA dose (mg/kg)	VPA CL (L/day)	VPA CL (L/kg/day)	VPA CL (L/kg/hr)
1	18.92	108.89	1.47	0.39	0.061	23.65	20.00	0.270	0.0113
2	32.33	115.29	2.66	0.98	0.111	39.26	27.64	0.638	0.0266
3	14.06	78.87	1.39	0.33	0.058	17.57	12.06	0.212	0.0088
4	15.15	76.09	1.15	0.14	0.048	15.15	15.38	0.233	0.0097
5	9.30	53.16	0.82	-0.19	0.034	15.50	13.26	0.206	0.0086
6	15.38	64.22	0.99	-0.01	0.041	15.38	14.79	0.228	0.0095
7	9.09	57.73	0.66	-0.42	0.027	11.36	14.51	0.165	0.0069
8	21.92	119.15	1.63	0.49	0.068	20.55	37.78	0.518	0.0216
9	12.90	84.85	1.37	0.31	0.057	24.19	38.66	0.624	0.0260
10	13.33	71.79	1.20	0.18	0.050	16.67	18.02	0.300	0.0125
11	30.19	120.43	2.27	0.82	0.095	30.19	26.36	0.497	0.0207
12	15.87	90.91	1.44	0.37	0.060	23.81	34.25	0.544	0.0227
13	9.35	50.60	0.79	-0.24	0.033	15.58	10.50	0.164	0.0068
14	7.08	75.68	1.34	0.29	0.056	8.85	39.37	0.697	0.0290
15	15.38	120.43	1.16	0.15	0.048	11.54	15.52	0.149	0.0062
16	17.50	130.67	1.63	0.49	0.068	18.75	21.93	0.274	0.0114
Mean ± SD	16.11±7.08	88.67±26.86	1.37±0.52	0.25±0.37	0.057±0.022	19.25±7.68	22.50±10.15	0.357±0.193	0.0149±0.0080
Range	7.08-32.33	50.60–130.67	0.66 –2.66	-0.42-0.98	0.027–0.111	8.85-39.26	10.50–39.37	0.149–0.697	0.0062 – 0.0290

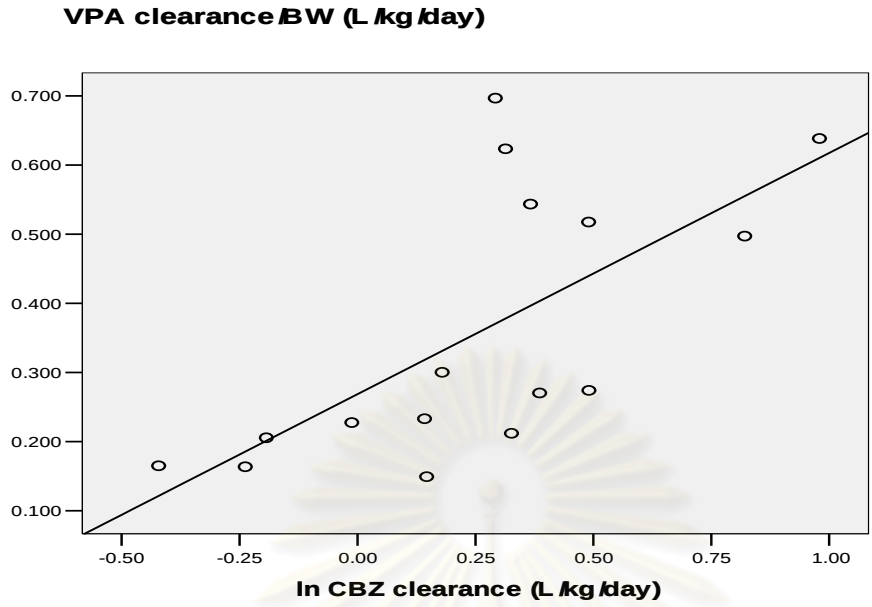

Figure 7: Scatter plot of ln CBZ clearance (L/kg/day) versus VPA clearance (L/kg/day).

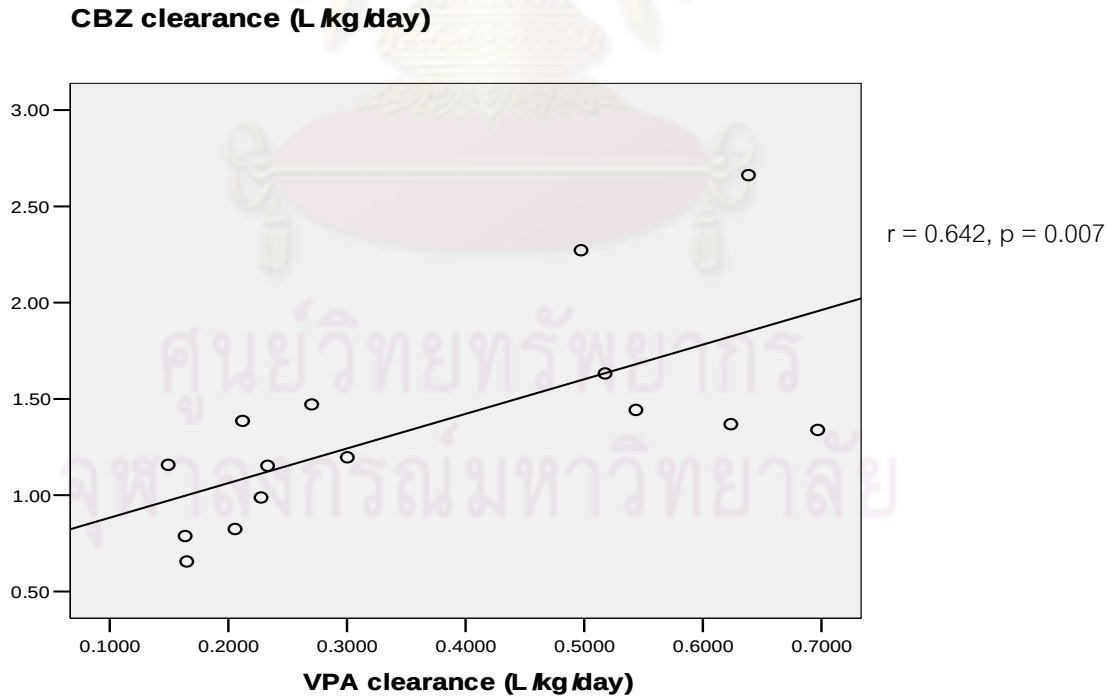

Figure 8: Scatter plot of VPA clearance (L/kg/day) versus CBZ clearance (L/kg/day).

Table 25: Regression equations show correlation between VPA clearance and CBZ clearance

Regression equation	R	R Square	P- value
VPA CL (L/day) = 0.154 x CBZ CL (L/day) + 8.882 CBZ CL (L/day) = 1.075 x VPA CL (L/day) + 64.477	0.406	0.165	0.118
VPA CL(L/kg/day) = 0.349x ln CBZ CL (L/kg/day) + 0.269	0.661	0.437	0.005
CBZ CL (L/kg/day) = 1.732 x VPA CL (L/kg/day) + 0.754	0.642	0.412	0.007

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Part 3 Effect of *CYP3A5* polymorphism on CBZ pharmacokinetics

Seventy patients who used CBZ as monotherapy or coadministration with PHT, PB or VPA and met the inclusion criteria were included into this study. A retro-prospective data, February 2010 - September 2010, were collected from electronic database and medical record at the epilepsy outpatient clinic of Prasat Neurological Institute.

Demographic data

Of the 70 patients included, 67 were diagnosed to be epilepsy and 3 were neuropathic pain. Of the 67 epileptic patients, 11 had a generalized seizure and 56 had a localized seizure. Among these, 36 patients used CBZ as monotherapy, 7 patients used CBZ combination with PHT, 11 patients used CBZ combination with PB and 16 patients used CBZ combination with VPA. The seizures of 51 patients (76%) among the 67 epileptic patients could be controlled with the current regimens. Most of the patients (83%) used folic acid as supplementation to prevent side effects; the details are shown in Table 26.

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Table 26: Demographic data of patients (N=70)

Characteristic	Frequency, (mean ± SD or median)	% (range)
Number of patients	70	100
Gender		
Male	31	44
Female	39	56
Age (years)	(42.63 ± 13.83)	(16.53-82.05)
Weight (kgs)	(62.57 ± 11.76)	(40.10-104.00)
Height (cm)	(161.61 ± 8.00)	(145-185)
BMI (kg/m^2)	(23.35)	(16.50-37.53)
Indication of CBZ used		
Epilepsy	67	96
Neuropathic pain	3	4
Type of epilepsy		
Generalized seizure	11	16
Localized seizure	56	84
Seizure controlled		
Controlled	51	76
Uncontrolled	16	24
Combination therapy of AEDs		
CBZ monotherapy	36	51
CBZ+PHT	7	10
CBZ+PB	11	16
CBZ+VPA	16	23
Underlying diseases		
No other disease	47	67
Diabetes Mellitus	3	4
Dyslipidemia	13	19
Hypertension	15	21
Thalassemia	3	4
Smoking status		
Never	61	87.14
Ever smoke	1	1.43
Smoking	8	11.43

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Table 26: Demographic data of patients (N=70) (continue)

Characteristic	Frequency, (mean ± SD or median)	% (range)
Alcohol consumption		
Never	67	96
Ever drink	1	1
Drinking	2	3
Adverse effect		
No adverse effect	66	94.3
Tremor	1	1.4
Dizziness	2	2.9
Ataxia	1	1.4
AST (IU/L), N= 29	(21.00)	(9-64)
ALT (IU/L), N= 29	(15.00)	(3-54)
Serum albumin (g/dL), N= 32	(4.10)	(2.5-4.7)
Serum creatinine (mg/dL), N= 27	(0.90)	(0.50-1.40)
Co-medications		
Folic acid	58	83
Simvastatin	10	14
Calcium carbonate	8	11
Enalapril	7	10
HCTZ	6	9
Vitamin B complex	6	9
Multivitamin	5	7
Clobazam	4	6
Atenolol	4	6
Amlodipine	3	4
Manidipine	3	4
Rosuvastatin	3	4
Metformin	2	3
Ezetrimide	1	1
Atorvastatin	1	1
Clopidogrel	1	1
Aspirin	1	1
Glibenclamide	1	1

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Table 27 presents CBZ pharmacokinetic parameters from the total patients included into the study. All patients included into this part were the same patients that included into part 1 except for the twelve patients who lack of the genetic data were excluded. The pharmacokinetic parameters of CBZ from total patients in this part were closed to previous part.

Table 27: Pharmacokinetic parameters of CBZ from total patients included (N=70)

PK parameters (N=70)	Minimum	Maximum	Mean ± SD or Median
CBZ dose (mg/day)	200	2,000	800
(mg/kg/day)	3.33	32.33	14.59 ± 5.90
CBZ level (mg/L)	2.10	11.90	7.74 ± 2.39
(mcg/L/mg)	2.63	22.00	9.51 ± 3.67
CBZ clearance (L/hr)	1.33	11.11	3.15
(L/day)	31.82	266.67	75.68
(L/kg/hr)	0.022	0.185	0.054
(L/kg/day)	0.53	4.44	1.29

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Population allelic frequencies

Genotyping of *CYP3A5* was obtained from 70 patients, 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. When characterized the patients into 3 groups by *CYP3A5* genotyping, there were 8 patients (11%) with homozygous **1/*1*, 28 patients (40%) with heterozygous **1/*3* and 34 patients (49%) with homozygous **3/*3*. The allele frequency of *CYP3A5*1* was 31% and *CYP3A5*3* was 69%. The details were shown in Table 28.

Table 28: Prevalence of *CYP3A5* genotype

(70 patients x 2 alleles)				Genotypes	Observed N=70	%	Predicted (HWE)
Alleles	N=140	%	95%CI				
*1	44	31	23.5-38.5	*1/*1	8	11	7
				*1/*3	28	40	30
*3	96	69	61.5-76.5	*3/*3	34	49	33
					Chi-square=0.306, p=0.858		

Allelic frequencies of *CYP3A5* genotypes were in Hardy-Weinberg Equilibrium (HWE), p =0.858. The calculation if allelic frequencies were in HWE:

The number of the *1 allele = (8 x 2) + (28 x 1) = 44 alleles

The number of the *3 allele = (34 x 2) + (28 x 1) = 96 alleles

The frequency of the *1 allele = p = 44 / (44 + 96) = 0.31

The frequency of the *3 allele = q = 96 / (44 + 96) = 0.69

The proportion of expected *1/*1, *1/*3 and *3/*3 genotypes could be predicted from HWE: p+q = 1 and $(p + q)^2 = 1$ or $p^2 + 2pq + q^2 = 1$

$p^2 = 0.31 \times 0.31 = 0.0961$

$2pq = 2 \times 0.31 \times 0.69 = 0.4278$

$q^2 = 0.69 \times 0.69 = 0.4761$

The total number of patients included to this study was 70

Expected number of *1/*1 = $0.0961 \times 70 = 6.73 \approx 7$

Expected number of *1/*3 = $0.4278 \times 70 = 29.95 \approx 30$

Expected number of *3/*3 = $0.4761 \times 70 = 33.32 \approx 33$

The observed number of *1/*1 = 8

The observed number of *1/*3 = 28

The observed number of *3/*3 = 34

Chi-square =0.306, p=0.858

Therefore, could not reject the null hypothesis that the population is in HWE.

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Effect of CYP3A5 polymorphism on CBZ pharmacokinetics

Seventy patients were categorized by *CYP3A5* genotypes into 3 groups; *CYP3A5*1/*1*, *CYP3A5**1/*3, and *CYP3A5*3/*3*. Patient's age, body weight, BMI, the frequency of patients when categorized by gender and coadministration drugs were not significantly different among these 3 groups. The details about demographic data of patients when categorized by *CYP3A5* genotypes are shown in Table 29.

Table 29: Demographic characteristics of patients when categorized patients into 3 groups based on *CYP3A5* genotypes

Demographic data	*CYP3A5*1/*1*	*CYP3A5*1/*3*	*CYP3A5*3/*3*	p-value
No. of patients	8	28	34	
Gender (male/female) [a]	3/5	12/16	16/18	0.602
Age (yr) [b]	50.96±20.61	38.97±11.47	43.68±13.16	0.078
(range)	(16.53-82.05)	(18.35-64.90)	(17.81-69.77)	
Body weight (kg) [b]	66.48±12.51	58.56±9.04	64.95±12.89	0.061
(range)	(52.00-88.00)	(40.10-77.00)	(43.30-104.00)	
BMI (kg/m^2) [b]	24.01±2.26	22.73±2.85	24.93±4.79	0.093
(range)	(21.37-27.85)	(17.26-29.34)	(16.50-37.53)	
Coadministration drugs [a]				
CBZ monotherapy	7	14	15	0.061
CBZ+PHT	0	3	4	0.897
CBZ+PB	0	4	7	0.521
CBZ+VPA	1	7	8	0.660

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

Table 30 shows the comparisons of patient's PK parameters of CBZ when categorized patients into 3 groups based on their *CYP3A5* genotypes. CBZ dose, CBZ level and CBZ clearance were not significantly different among these 3 groups.

Table 30: Pharmacokinetic parameters of CBZ when categorized patients into 3 groups based on *CYP3A5* genotypes

Parameter	Mean±SD or Median			p-value
	*CYP3A5*1/*1* (N=8)	*CYP3A5*1/*3* (N=28)	*CYP3A5*3/*3* (N=34)	
CBZ dose (mg/day) [a]	800	800	800	0.366
(range)	(400-800)	(400-1,600)	(200-2,000)	
(mg/kg/day) [b]	11.16±2.96	15.63±6.25	14.53±5.94	0.168
(range)	(6.67-15.38)	(5.19-30.19)	(3.33-32.33)	
CBZ level (mg/L) [a]	8.40	8.35	8.00	0.982
(range)	(3.70-9.70)	(2.10-11.80)	(2.20-11.90)	
(mcg/L/mg)	10.50	9.22	9.25	0.512
(range)	(6.17-21.50)	(2.63-18.60)	(3.70-22.00)	
CBZ clearance (L/hr) [a]	2.78	3.16	3.15	0.512
(range)	(1.36-4.73)	(1.57-11.11)	(1.33-7.88)	
(L/day)	66.71	75.88	75.68	0.518
(range)	(32.56-113.51)	(37.63-266.67)	(31.82-189.19)	
(L/kg/hr)	0.043	0.055	0.054	0.220
(range)	(0.023-0.074)	(0.028-0.185)	(0.022-0.111)	
(L/kg/day)	1.03	1.33	1.30	0.223
(range)	(0.54-1.78)	(0.68-4.44)	(0.53-2.66)	

[a] Kruskal-Wallis H test, [b] One-way ANOVA.

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When we categorized patients into 2 groups based on *CYP3A5* genotypes; the first group was *CYP3A5*1/*1* and *CYP3A5*1/*3*, and the second group was *CYP3A5*3/*3*. Patient's age, body weight, the frequency of patients based on gender and coadministration drugs were not significantly different between these 2 groups, while the mean BMI in the *CYP3A5*1/*1* and *CYP3A5*1/*3* group was significantly (p=0.047) lower than that of the *CYP3A5*3/*3* group. The details about demographic data of patients when categorized by *CYP3A5* genotypes are shown in Table 31.

Table 31: Demographic characteristics of patients when categorized patients into 2 groups based on *CYP3A5* genotypes

Demographic data	*CYP3A5*1/*1* and **1/*3*	*CYP3A5*3/*3*	p-value
No. of patients	36	34	
Gender (male/female) [a]	15/21	16/18	0.650
Age (yr) [b]	41.63±14.56	43.68±13.16	0.541
(range)	(16.53-82.05)	(17.81-69.77)	
Body weight (kg) [b]	60.32±10.27	64.95±12.89	0.100
(range)	(40.10-88.00)	(43.30-104.00)	
BMI (kg/m^2) [b]	23.01±2.75	24.93±4.79	0.047
(range)	(17.26-29.34)	(16.50-37.53)	
Coadministration drugs [a]			
CBZ monotherapy	21	15	0.234
CBZ+PHT	3	4	0.706
CBZ+PB	4	7	0.276
CBZ+VPA	8	8	0.896

[a] Chi-square test, [b] independent t-test.

Table 32 shows the comparisons of patient's PK parameters of CBZ when categorized patients into 2 groups based on their *CYP3A5* genotypes. CBZ dose, CBZ level and CBZ clearance were not significantly different between these 2 groups.

Table 32: Pharmacokinetic parameters of CBZ when categorized patients into 2 groups based on *CYP3A5* genotypes

Parameter	Mean±SD or Median		p-value
	*CYP3A5*1/*1* and **1/*3* (N=36)	*CYP3A5*3/*3* (N=34)	
CBZ dose (mg/day) [a]	800	800	0.516
(range)	(400-1,600)	(200-2,000)	
(mg/kg/day) [b]	14.64±5.95	14.53±5.94	0.940
(range)	(5.19-30.19)	(3.33-32.33)	
CBZ level (mg/L) [a]	8.35	8.00	0.991
(range)	(2.10-11.80)	(2.20-11.90)	
(mcg/L/mg) [b]	9.74±3.91	9.27±3.44	0.599
(range)	(2.63-21.50)	(3.70-22.00)	
CBZ clearance (L/hr) [a]	3.04	3.15	0.634
(range)	(1.36-11.11)	(1.33-7.88)	
(L/day) [a]	72.84	75.68	0.634
(range)	(32.56-266.67)	(31.82-189.19)	
(L/kg/hr) [a]	0.054	0.054	0.991
(range)	(0.023-0.185)	(0.022-0.111)	
(L/kg/day) [a]	1.29	1.30	1.00
(range)	(0.54-4.44)	(0.53-2.66)	

[a] Mann-Whitney U test, [b] independent t-test.

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The patient's characteristics and PK parameters of CBZ between different *CYP3A5* genotypes were further compared through sub groups analysis based on the coadministration drugs; CBZ monotherapy, CBZ in combination with PHT, CBZ in combination with PB, CBZ in combination with VPA and CBZ in combination with enzyme inducing AED (CBZ in combination with PHT or PB). The details were shown in Table 33-37.

Among the 36 patients of CBZ monotherapy group, there were 21 patients (58%) who are *CYP3A5*1/*1* and **1/*3*, and 15 patients (42%) who are *CYP3A5*3/*3*. Patient's age, body weight, BMI, CBZ dose, CBZ level and CBZ clearance were not significantly different between these 2 groups of different genotypes (Table 33A).

Among the 36 patients of CBZ monotherapy group, there were 7 patients (19%) who are *CYP3A5*1/*1*, and 29 patients (81%) who are *CYP3A5*1/*3* and **3/*3*. Patient's body weight, BMI, CBZ dose, CBZ level and CBZ clearance were not significantly different between these 2 groups of different genotypes, while the mean of age in patients who are *CYP3A5*1/*1* (54.33±19.73 yrs) was significantly higher (p=0.028) than the mean of age in patients who are *CYP3A5*1/*3* and **3/*3* (40.76±12.46 yrs) (Table 33B).

Among the 7 patients of CBZ in combination with PHT group, there were 3 patients (43%) who are *CYP3A5*1/*3*, and 4 patients (57%) who are *CYP3A5*3/*3*. Patient's age, body weight, BMI, CBZ dose, CBZ level and CBZ clearance were not significantly different between these 2 groups of different genotypes (Table 34). Figure 9 shows box and whisker plot of the median CBZ level (mcg/L/mg) and Figure 10 shows box and whisker plot of the median CBZ clearance (L/kg/day) between different genotypes.

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Table 33A: Comparison of patient's characteristics and pharmacokinetic parameters of CBZ in CBZ monotherapy group between *CYP3A5*1/*1* and **1/*3* VS *CYP3A5*3/*3*

Parameter	Mean±SD or Median		p-value
	*CYP3A5*1/*1* and **1/*3* (N=21)	*CYP3A5*3/*3* (N=15)	
Age (yr) [a]	43.47±15.62	43.30±14.25	0.974
(range)	(16.53-82.05)	(17.81-69.77)	
Body weight (kg) [a]	57.12±9.36	61.37±11.33	0.227
(range)	(40.10-80.50)	(45.00-89.00)	
BMI (kg/m^2) [a]	22.17±2.57	23.33±3.44	0.253
(range)	(17.26-27.85)	(16.73-30.80)	
CBZ dose (mg/day) [b]	800	800	0.300
(range)	(400-1,600)	(200-1,400)	
(mg/kg/day) [a]	13.98±5.72	14.29±5.46	0.871
(range)	(6.67-29.09)	(3.33-23.53)	
CBZ level (mg/L) [a]	8.02±2.29	8.39±2.51	0.645
(range)	(3.70-11.80)	(4.40-11.90)	
(mcg/L/mg) [a]	11.06±3.92	10.61±3.65	0.727
(mcg/L/mg) [b]	10.75	9.92	0.619
(range)	(5.40-21.50)	(6.75-22.00)	
CBZ clearance (L/hr) [a]	2.96±1.06	2.97±0.76	0.972
(range)	(1.36-5.40)	(1.33-4.32)	
(L/day) [a]	71.06±25.47	71.32±18.20	0.973
(range)	(32.56-129.63)	(31.82-103.70)	
(L/kg/hr) [a]	0.053±0.023	0.049±0.013	0.552
(range)	(0.023-0.129)	(0.022-0.071)	
(L/kg/day) [a]	1.28±0.55	1.18±0.32	0.552
(range)	(0.54-3.09)	(0.53-1.70)	

[a] independent t-test, [b] Mann-Whitney U test.

Table 33B: Comparison of patient's characteristics and pharmacokinetic parameters of CBZ in CBZ monotherapy group between *CYP3A5*1/*1* VS *CYP3A5*1/*3* and **3/*3*

Parameter	Mean±SD or Median		p-value
	*CYP3A5*1/*1* (N=7)	*CYP3A5*1/*3* and **3/*3* (N=29)	
Age (yr) [a]	54.33±19.73	40.76±12.46	0.028*
(range)	(16.53-82.05)	(17.81-69.77)	
Body weight (kg) [a]	63.40±9.71	57.80±10.29	0.201
(range)	(52.00-80.50)	(40.10-89.00)	
BMI (kg/m^2) [a]	23.76±2.33	22.38±3.08	0.276
(range)	(21.37-27.85)	(16.73-30.80)	
CBZ dose (mg/day) [b]	800	800	0.360
(range)	(400-800)	(200-1,600)	
(mg/kg/day) [a]	11.46±3.07	14.74±5.84	0.161
(range)	(6.67-15.38)	(3.33-29.09)	
CBZ level (mg/L) [b]	8.20	8.70	0.263
(range)	(3.70-9.00)	(3.70-11.90)	
(mcg/L/mg) [b]	10.25	10.50	0.749
(mcg/L/mg) [a]	11.13±4.90	10.81±3.54	0.844
(range)	(6.17-21.50)	(5.40-22.00)	
CBZ clearance (L/hr) [a]	2.97±1.03	2.96±0.93	0.983
(range)	(1.36-4.73)	(1.33-5.40)	
(L/day) [a]	71.32±24.75	71.13±22.31	0.985
(range)	(32.56-113.51)	(31.82-129.63)	
(L/kg/hr) [b]	0.046	0.049	0.603
(L/kg/hr) [a]	0.048±0.017	0.053±0.020	0.543
(range)	(0.023-0.074)	(0.022-0.129)	
(L/kg/day) [b]	1.11	1.17	0.617
(range)	(0.54-1.78)	(0.53-3.09)	

* Statistical significant difference, [a] independent t-test, [b] Mann-Whitney U test.

Table 34: Comparison of patient's characteristics and pharmacokinetic parameters of CBZ in CBZ+PHT group between *CYP3A5 *1/*3* and *CYP3A5*3/*3*

Parameter	Mean±SD or Median		p-value
	*CYP3A5*1/*3* (N=3)	*CYP3A5*3/*3* (N=4)	
Age (yr) [a]	48.98±15.87	45.36±9.75	0.721
(range)	(33.16-64.90)	(35.09-57.97)	
Body weight (kg) [a]	68.00±8.54	74.00±10.61	0.461
(range)	(60.00-77.00)	(59.00-82.00)	
BMI (kg/m^2) [a]	26.10±2.81	29.47±5.01	0.348
(range)	(24.31-29.34)	(22.48-34.13)	
CBZ dose (mg/day) [a]	866.67±503.32	1,000±678.23	0.788
(range)	(400-1,400)	(500-2,000)	
(mg/kg/day) [a]	13.14±7.86	13.27±7.90	0.984
(range)	(5.19-20.90)	(6.76-24.69)	
CBZ level (mg/L) [a]	4.64±2.79	5.92±3.05	0.592
(mg/L) [b]	4.20	6.15	0.480
(range)	(2.10-7.62)	(2.20-9.20)	
(mcg/L/mg) [a]	6.19±3.99	6.84±4.32	0.846
(mcg/L/mg) [b]	5.44	5.26	0.724
(range)	(2.63-10.50)	(3.70-13.14)	
CBZ clearance (L/hr) [a]	6.42±4.26	5.37±2.46	0.696
(L/hr) [b]	5.36	5.70	0.724
(range)	(2.78-11.11)	(2.22-7.88)	
(L/day) [a]	153.98±102.39	128.96±59.11	0.697
(L/day) [b]	128.61	136.69	0.724
(range)	(66.67-266.67)	(53.26-189.19)	

[a] independent t-test, [b] Mann-Whitney U test.

Table 34: Comparison of patient's characteristics and pharmacokinetic parameters of CBZ in CBZ+PHT group between *CYP3A5 *1/*3* and *CYP3A5*3/*3* (continue)

Parameter	Mean±SD or Median		p-value
	*CYP3A5*1/*3* (N=3)	*CYP3A5*3/*3* (N=4)	
CBZ clearance (L/kg/hr) [a]	0.100±0.077	0.071±0.028	0.497
(L/kg/hr) [b]	0.080	0.074	1.00
(range)	(0.036-0.185)	(0.038-0.097)	
(L/kg/day) [a]	2.41±1.84	1.70±0.67	0.495
(L/kg/day) [b]	1.92	1.77	1.00
(range)	(0.87-4.44)	(0.90-2.34)	

[a] independent t-test, [b] Mann-Whitney U test.

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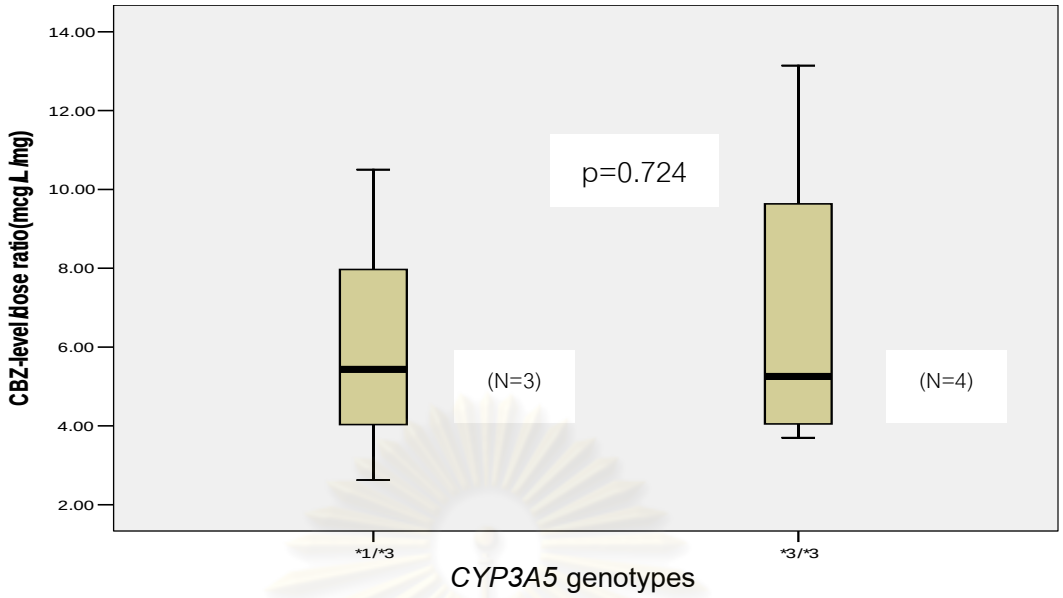

Figure 9: Box and whisker plot of the median CBZ level (mcg/L/mg) between different genotypes in CBZ+PHT group (N=7).

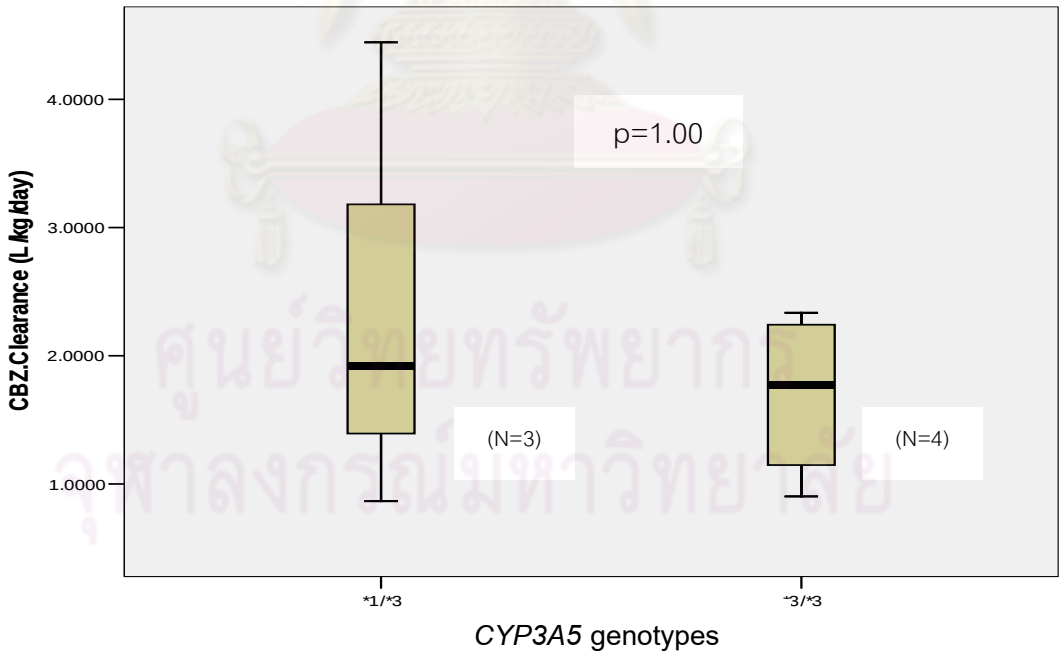

Figure 10: Box and whisker plot of the median CBZ clearance (L/kg/day) between different genotypes in CBZ+PHT group (N=7).

Among the 11 patients of CBZ concurrently used with PB group, there were 4 patients (36%) who are *CYP3A5*1/*3*, and 7 patients (64%) who are *CYP3A5*3/*3*. Patient's age, body weight, BMI, CBZ dose, CBZ level and CBZ clearance were not significantly different between these 2 groups of different genotypes (Table 35). Figure 11 shows box and whisker plot of the median CBZ level (mcg/L/mg) and Figure 12 shows box and whisker plot of the median CBZ clearance (L/kg/day) between different genotypes.

Among the 16 patients of CBZ in combination with VPA group, there were 8 patients (50%) who are *CYP3A5*1/*1* and **1/*3*, and 8 patients (50%) who are *CYP3A5*3/*3*. Patient's age, body weight, BMI, CBZ dose, CBZ level and CBZ clearance were not significantly different between these 2 groups of different genotypes (Table 36). Figure 13 shows box and whisker plot of the median CBZ level (mcg/L/mg) and Figure 14 shows box and whisker plot of the median CBZ clearance (L/kg/day) between different genotypes.

Among the 18 patients of CBZ in combination with enzyme inducing AED (CBZ+PHT and CBZ+PB) group, there were 7 patients (39%) who are *CYP3A5*1/*3*, and 11 patients (61%) who are *CYP3A5*3/*3*. Patient's age, body weight, BMI, CBZ dose, CBZ level and CBZ clearance were not significantly different between these 2 groups of different genotypes (Table 37). Figure 15 shows box and whisker plot of the median CBZ level (mcg/L/mg) and Figure 16 shows box and whisker plot of the median CBZ clearance (L/kg/day) between different genotypes.

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Table 35: Comparison of patient's characteristics and pharmacokinetic parameters of CBZ in CBZ+PB group between *CYP3A5 *1/*3* and *CYP3A5*3/*3*

Parameter	Mean±SD or Median		p-value
	*CYP3A5*1/*3* (N=4)	*CYP3A5*3/*3* (N=7)	
Age (yr) [a]	44.91±6.76	45.88±9.19	0.859
(range)	(39.26-53.82)	(32.58-61.69)	
Body weight (kg) [a]	59.85±10.45	63.89±8.32	0.496
(range)	(47.30-69.00)	(55.00-75.00)	
BMI (kg/m^2) [a]	22.65±2.31	23.68±2.30	0.495
(range)	(19.94-24.94)	(20.20-26.72)	
CBZ dose (mg/day) [a]	1,050±191.48	857.14±377.96	0.372
(range)	(800-1,200)	(400-1,400)	
(mg/kg/day) [a]	17.52±0.51	13.29±5.27	0.078
(range)	(16.91-18.12)	(6.23-20.00)	
CBZ level (mg/L) [a]	6.60±2.84	7.39±2.35	0.631
(range)	(3.80-10.50)	(3.70-9.90)	
(mcg/L/mg) [a]	6.23±2.10	9.28±2.40	0.064
(range)	(3.80-8.75)	(6.29-12.50)	
CBZ clearance (L/hr) [a]	5.14±1.88	3.34±0.89	0.055
(range)	(3.33-7.68)	(2.33-4.64)	
(L/day) [a]	123.32±44.95	80.06±21.46	0.055
(range)	(80.00-184.21)	(56.00-111.36)	
(L/kg/hr) [a]	0.089±0.038	0.053±0.016	0.050
(range)	(0.048-0.139)	(0.035-0.078)	
(L/kg/day) [a]	2.13±0.91	1.27±0.37	0.050
(range)	(1.16-3.34)	(0.83-1.87)	

[a] independent t-test.

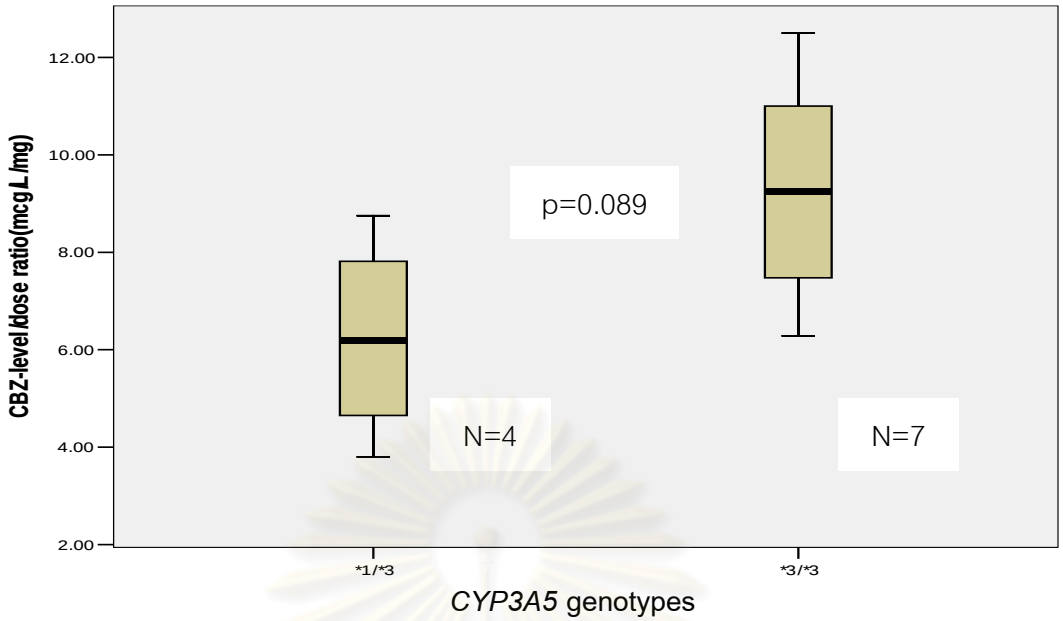

Figure 11: Box and whisker plot of the median CBZ level (mcg/L/mg) between different genotypes in CBZ+PB group (N=11).

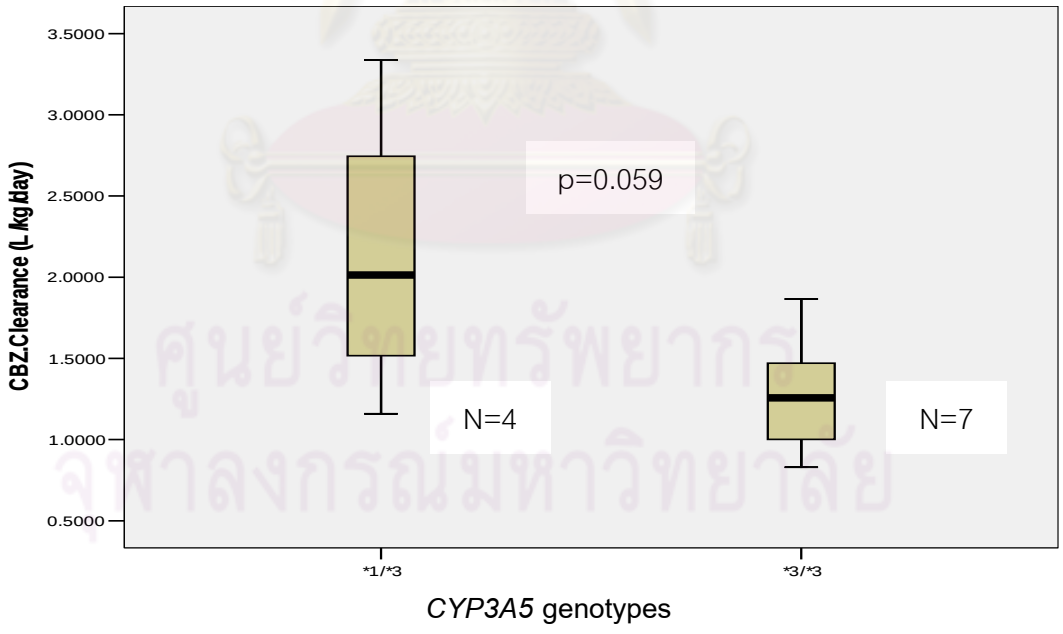

Figure 12: Box and whisker plot of the median CBZ clearance (L/kg/day) between different genotypes in CBZ+PB group (N=11).

Table 36: Comparison of patient's characteristics and pharmacokinetic parameters of CBZ in CBZ+VPA group between *CYP3A5 *1/*1* and**1/*3* VS *CYP3A5*3/*3*

Parameter	Mean±SD or Median		p-value
	*CYP3A5*1/*1* and **1/*3* (N=8)	*CYP3A5*3/*3* (N=8)	
Age (yr) [a]	32.43±11.65	41.62±16.88	0.226
(range)	(18.35-54.65)	(23.18-65.51)	
Body weight (kg) [a]	66.09±10.73	68.09±18.21	0.793
(range)	(53.00-88.00)	(43.30-104.00)	
BMI (kg/m^2) [b]	25.42	25.73	0.529
(range)	(20.02-27.06)	(16.50-37.53)	
CBZ dose (mg/day) [a]	1,000±427.62	1,100±385.45	0.631
(range)	(400-1,600)	(600-1,600)	
(mg/kg/day) [a]	15.50±7.63	16.72±6.96	0.745
(range)	(7.08-30.19)	(9.30-32.33)	
CBZ level (mg/L) [a]	8.52±2.17	7.96±0.93	0.510
(range)	(3.70-10.90)	(6.60-9.30)	
(mcg/L/mg) [a]	9.34±2.87	7.96±2.63	0.335
(range)	(5.81-13.83)	(5.36-13.17)	
CBZ clearance (L/hr) [a]	3.41±1.10	3.98±1.13	0.325
(range)	(2.11-5.02)	(2.22-5.44)	
(L/day) [a]	81.85±26.46	95.50±27.19	0.326
(range)	(50.60-120.43)	(53.16-130.67)	
(L/kg/hr) [a]	0.054±0.022	0.061±0.023	0.511
(range)	(0.027-0.095)	(0.034-0.111)	
(L/kg/day) [a]	1.28±0.52	1.46±0.54	0.511
(range)	(0.66-2.27)	(0.82-2.66)	

[a] independent t-test, [b] Mann-Whitney U Test..

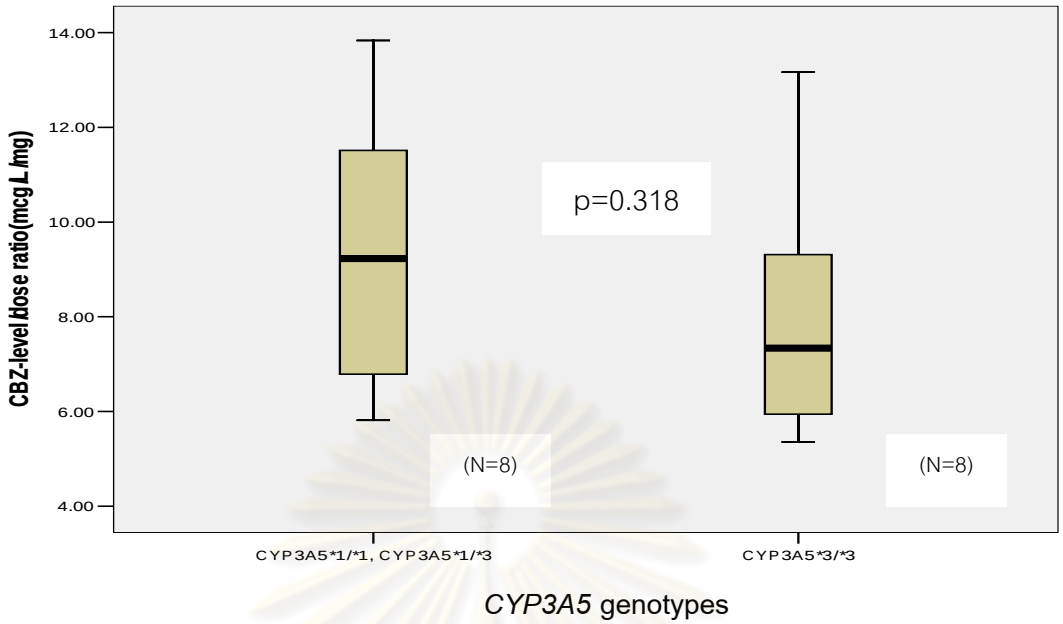

Figure 13: Box and whisker plot of the median CBZ level (mcg/L/mg) between different genotypes in CBZ+VPA group (N=16).

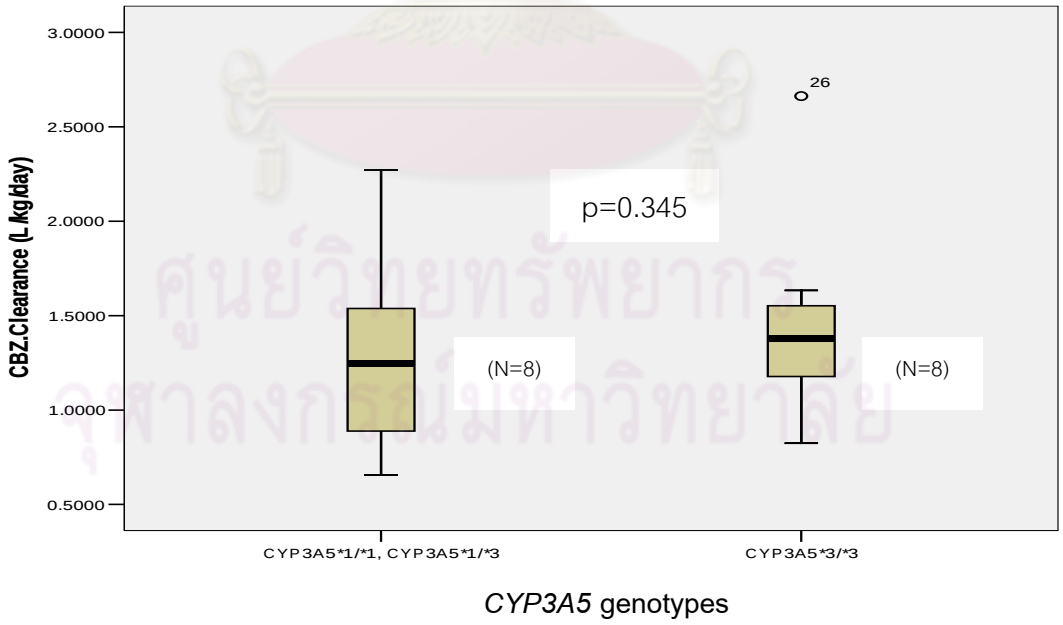

Figure 14: Box and whisker plot of the median CBZ clearance (L/kg/day) between different genotypes in CBZ+VPA group (N=16).

Table 37: Comparisons of patient's characteristics and pharmacokinetic parameters of CBZ in CBZ in combination with enzyme inducing AED group (PHT and PB) between *CYP3A5 *1/*3* and *CYP3A5*3/*3*

Parameter	Mean±SD or Median		p-value
	*CYP3A5*1/*3* (N=7)	*CYP3A5*3/*3* (N=11)	
Age (yr) [a]	46.66±10.56	45.69±8.90	0.838
(range)	(33.16-64.90)	(32.58-61.69)	
Body weight (kg) [a]	63.34±9.90	67.56±10.07	0.396
(range)	(47.30-77.00)	(55.00-82.00)	
BMI (kg/m^2) [a]	24.13±2.95	25.78±4.39	0.394
(range)	(19.94-29.34)	(20.20-34.13)	
CBZ dose (mg/day) [a]	971.43±335.23	909.09±478.44	0.768
(range)	(400-1,400)	(400-2,000)	
(mg/kg/day) [b]	17.39	11.86	0.497
(range)	(5.19-20.90)	(6.23-24.69)	
CBZ level (mg/L) [a]	5.76±2.78	6.85±2.58	0.406
(range)	(2.10-10.50)	(2.20-9.90)	
(mcg/L/mg) [a]	6.21±2.74	8.40±3.25	0.161
(mcg/L/mg) [b]	5.50	8.25	0.189
(range)	(2.63-10.50)	(3.70-13.14)	
CBZ clearance (L/hr) [a]	5.69±2.88	4.08±1.83	0.164
(range)	(2.78-11.11)	(2.22-7.88)	
(L/day) [a]	136.46±69.09	97.84±43.96	0.164
(range)	(66.67-266.67)	(53.26-189.19)	
(L/kg/hr) [a]	0.094±0.052	0.059±0.021	0.139
(range)	(0.036-0.185)	(0.035-0.097)	
(L/kg/day) [a]	2.25±1.25	1.43±0.51	0.139
(L/kg/day) [b]	1.92	1.35	0.135
(range)	(0.87-4.44)	(0.83-2.34)	

[a] independent t-test, [b] Mann-Whitney U test.

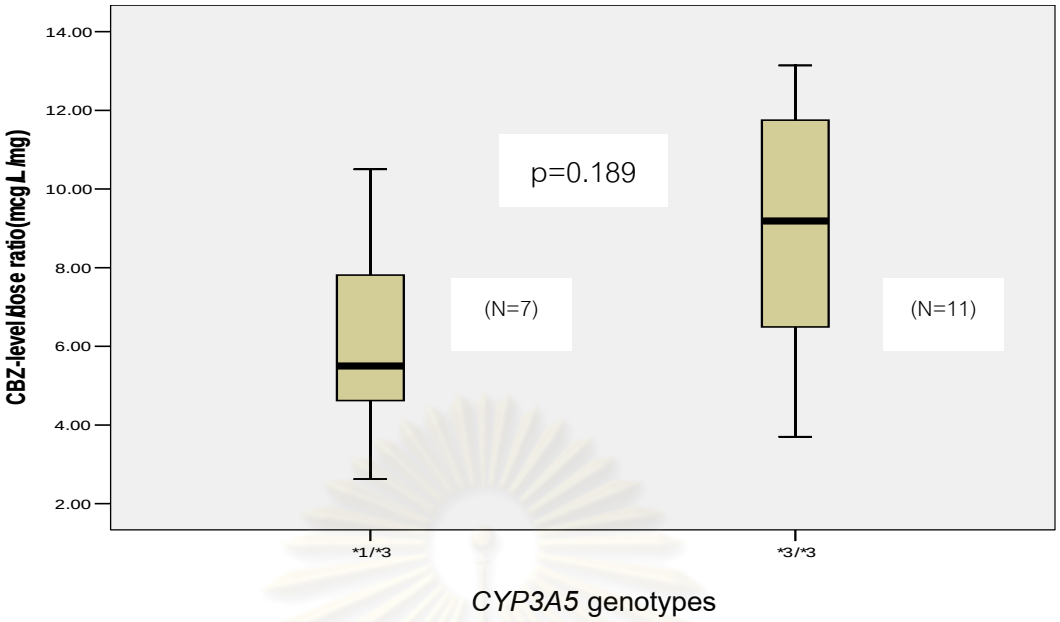

Figure 15: Box and whisker plot of median CBZ level (mcg/L/mg) between different genotypes in CBZ concurrently used with enzyme inducing AED group (N=18).

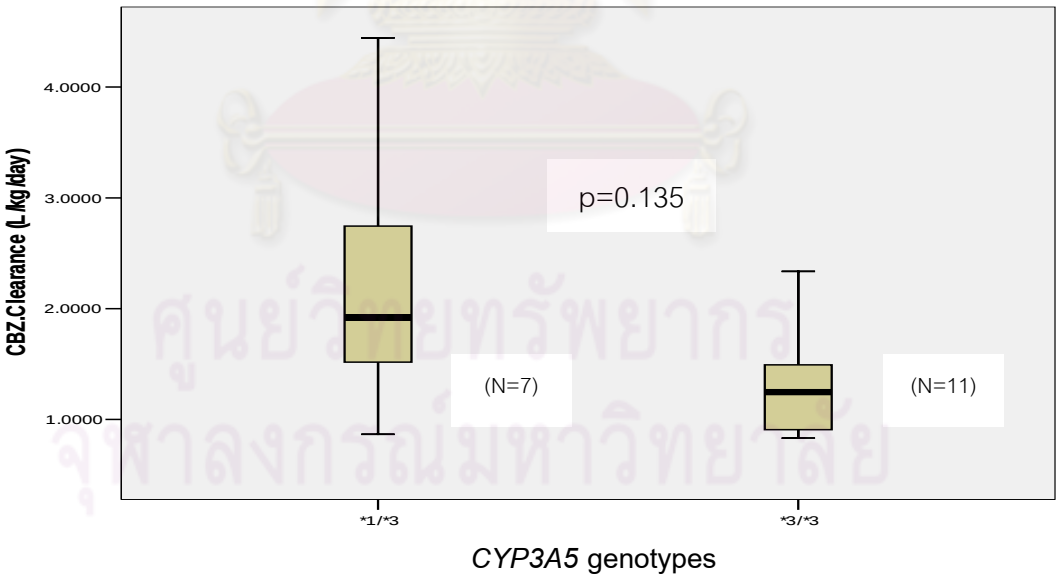

Figure 16: Box and whisker plot of median CBZ clearance (L/kg/day) between different genotypes in CBZ concurrently used with enzyme inducing AED group (N=18).

Table 38 shows comparisons of PK parameters of other AEDs used in combination with CBZ when categorized patients into 2 groups based on *CYP3A5* genotypes; the first group was *CYP3A5*1/*1* or *CYP3A5*1/*3*, and the second group was *CYP3A5*3/*3*. The PK parameters of PHT, PB and VPA (dose, level, PHT Vmax, PB clearance and VPA clearance) were not significantly different between these 2 groups of different genotypes.

Table 38: Comparisons of PK parameters of other AEDs used in combination with CBZ when categorized patients into 2 groups based on *CYP3A5* genotypes

PK parameters of other AEDs	Mean±SD or Median		p-value
	*CYP3A5*1/*1* and *CYP3A5*1/*3*	*CYP3A5*3/*3*	
CBZ+PHT (N=7)	(N=3)	(N=4)	
PHT dose (mg/day) [a]	300	325	0.150
(mg/kg/day) [b]	3.90±0.58	4.40±0.68	0.352
PHT level (mg/L) [b]	10.47±7.92	15.14±8.31	0.487
(mg/L/mg) [b]	0.0379±0.0239	0.0462±0.0209	0.646
PHT Vmax (mg/day) [b]	367.13±73.20	403.13±84.62	0.583
(mg/kg/day) [b]	5.45±1.22	5.43±0.66	0.975
CBZ+PB (N=11)	(N=4)	(N=7)	
PB dose (mg/day) [b]	120.00±48.99	83.57±45.71	0.246
(mg/kg/day) [b]	2.06±0.82	1.32±0.78	0.173
PB level (mg/L) [b]	22.75±7.24	16.50±9.13	0.273
(mg/L/mg) [b]	0.22±0.13	0.20±0.02	0.788
PB clearance (L/day) [b]	5.17±2.67	4.55±0.52	0.677
(L/kg/day) [b]	0.0935±0.0633	0.0719±0.0091	0.545
CBZ+VPA (N=16)	(N=8)	(N=8)	
VPA dose (mg/day) [b]	1,137.50±370.09	1,331.25±319.53	0.281
(mg/kg/day) [b]	17.61±6.93	20.89±8.50	0.412
VPA level (mg/L) [b]	56.70±24.57	68.41±16.00	0.278
(mg/L/mg) [b]	0.0520±0.0257	0.0545±0.0193	0.828
VPA clearance (L/day) [b]	24.12±11.76	20.89±8.75	0.543
(L/kg/day) [a]	0.36	0.27	0.834

[a] Mann-Whitney U test, [b] independent t-test.

Table 39, 40 show the comparisons of PK parameters of CBZ in the same genotype groups (*CYP3A5*1/*1* or *CYP3A5*1/*3* and *CYP3A5*3/*3*) when categorized patients into 4 groups based on other AEDs used in combination with CBZ; CBZ monotherapy, CBZ+PHT, CBZ+PB and CBZ+VPA.

Among the *CYP3A5*1/*1* and *CYP3A5*1/*3* genotypes group, CBZ dose (mg/day, mg/kg/day), CBZ level (mg/L), and CBZ clearance (L/kg/hr, L/kg/day) were not significantly different among the 4 groups categorized based on other AEDs used in combination with CBZ, while the median of CBZ level-to-dose ratio (mcg/L/mg) and the median of CBZ clearance (L/hr, L/day) were significantly different (p=0.018) between CBZ monotherapy group and CBZ+PB group (10.75 mcg/L/mg, 2.71 L/hr and 65.12 L/day VS 6.19 mcg/L/mg, 4.77 L/hr and 114.54 L/day, respectively). The details were shown in Table 39.

Among the *CYP3A5*3/*3* genotype group, CBZ dose (mg/day, mg/kg/day), CBZ level (mg/L, mcg/L/mg), and CBZ clearance (L/hr, L/day, L/kg/hr, L/kg/day) were not significantly different among the 4 groups categorized based on other AEDs used in combination with CBZ. The details were shown in Table 40.

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Table 39: Comparisons of pharmacokinetic parameters of CBZ among CBZ monotherapy group and difference combination therapy groups (*CYP3A5*1/*1* and *CYP3A5*1/*3* genotypes)

Parameter	Mean±SD or median				P- value
	CBZ (N=21)	CBZ+PHT (N=3)	CBZ+PB (N=4)	CBZ+VPA (N=8)	
CBZ dose (mg/day) [a]	800	800	1,100	1,000	0.169
(mg/kg/day) [b]	13.98±5.72	13.14±7.86	17.52±0.51	15.50±7.63	0.687
CBZ level (mg/L) [a]	8.60	4.20	6.05	9.25	0.158
(mcg/L/mg) [a]	10.75 [C]	5.44	6.19 [C]	9.22	0.030*
CBZ clearance (L/hr) [a]	2.71 [C]	5.36	4.77 [C]	3.16	0.030*
(L/day) [a]	65.12 [C]	128.61	114.54 [C]	75.88	0.028*
(L/kg/hr) [a]	0.048	0.080	0.084	0.052	0.153
(L/kg/day) [a]	1.16	1.92	2.01	1.25	0.153

* Statistical significant difference, [a] Kruskal- Wallis H test, [b] One-way ANOVA, [C] Mann-Whitney U test between CBZ VS CBZ+PB group; p-value = 0.018.

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Table 40: Comparisons of pharmacokinetic parameters of CBZ among CBZ monotherapy group and difference combination therapy groups (*CYP3A5*3/*3* genotype)

Parameter	Mean±SD or median				P- value
	CBZ (N=15)	CBZ+PHT (N=4)	CBZ+PB (N=7)	CBZ+VPA (N=8)	
CBZ dose (mg/day) [a]	866.67±335.23	1,000±678.23	857.14±377.96	1,100±385.45	0.550
(mg/kg/day) [b]	14.49	10.81	14.54	14.72	0.786
CBZ level (mg/L) [a]	8.39±2.51	5.92±3.05	7.39±2.35	7.96±0.93	0.281
(mcg/L/mg) [b]	9.92	5.26	9.25	7.34	0.107
CBZ clearance (L/hr) [b]	2.94	5.70	3.15	4.04	0.108
(L/day) [b]	70.59	136.69	75.68	96.87	0.109
(L/kg/hr) [a]	0.049±0.013	0.071±0.028	0.053±0.016	0.061±0.023	0.168
(L/kg/day) [a]	1.18±0.32	1.70±0.67	1.27±0.37	1.46±0.54	0.170

[a] One-way ANOVA, [b] Kruskal- Wallis H test.

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Model for prediction of carbamazepine clearance and level-to-dose ratio

Multiple regression analysis with forward-inclusion method was performed to create the model for prediction of CBZ clearance and level-to-dose ratio (mcg/L/mg) from demographic data and *CYP3A5* genotypes. Among the 70 patients participated in this study, there were only 4 factors related to CBZ clearance (L/hr and L/day) including CBZ dose (mg/kg), PHT dose (mg/kg), PB dose (mg/kg) and body weight (kg). It was found that when generated the equation to predict CBZ clearance from the related factors, the error (observed value – predicted value) was not normal distribution, when the CBZ clearance was transformed using log transformation (ln CBZ clearance), then, the error was normally distributed. Table 41A shows the entire significant models for prediction of CBZ clearance from forward-inclusion linear regression, the model 4 was the best fit equation.

There were only 4 factors related to CBZ clearance (L/kg/day) including CBZ dose (mg/kg), PHT dose (mg/kg), PB dose (mg/kg) and body weight (kg). It was found that when generated the equation to predict CBZ clearance from the related factors, the error (observed value – predicted value) was not normal distribution, then the CBZ clearance was transformed using log transformation (ln CBZ clearance) and the error was normally distributed. Table 41B shows the entire significant models for prediction of CBZ clearance from forward-inclusion linear regression, the model 4 was the best fit equation.

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Table 41A: Model summary of forward stepwise linear regression for prediction of ln CBZ Clearance (L/hr and L/day)

Model	Variable entered	R	R-square	R-square change	Sig (F change)	Model Sig (ANOVA)
1	CBZ dose (mg/kg)	0.502	0.252	0.252	<0.001	<0.001
2	CBZ dose (mg/kg) PHT dose CBZ dose	0.646	0.417	0.165	<0.001	<0.001
3	CBZ dose (mg/kg) PHT dose (mg/kg) PB dose (mg/kg)	0.685	0.470	0.053	0.013	<0.001
4	CBZ dose (mg/kg) PHT dose (mg/kg) PB dose (mg/kg) Body weight (kg)	0.725	0.525	0.055	0.008	<0.001

Table 41B: Model summary of forward stepwise linear regression for prediction of ln CBZ Clearance (L/kg/day)

Model	Variable entered	R	R-square	R-square change	Sig (F change)	Model Sig (ANOVA)
1	CBZ dose (mg/kg)	0.639	0.408	0.408	<0.001	<0.001
2	CBZ dose (mg/kg) PHT dose CBZ dose	0.674	0.455	0.046	0.020	<0.001
3	CBZ dose (mg/kg) PHT dose (mg/kg) PB dose (mg/kg)	0.714	0.510	0.056	0.008	<0.001
4	CBZ dose (mg/kg) PHT dose (mg/kg) PB dose (mg/kg) Body weight (kg)	0.740	0.547	0.037	0.024	<0.001

The coefficients and p-value of each variables which entered by forward-inclusion method of model 4 to predict CBZ clearance (L/hr and L/day) were presented in Table 42A. Multicolinearity of independent factors was determined (data not shown).

The coefficients and p-value of each variables which entered by forward-inclusion method of model 4 to predict CBZ clearance (L/kg/day) were presented in Table 42B. Multicolinearity of independent factors was determined (data not shown).

Table 42A: Coefficients of factors in the best fit equation for prediction of ln CBZ Clearance (L/hr and L/day)

Factor	B	Sig (p-value)	95% CI
For predict ln CBZ CL (L/hr)			
Constant	0.01	0.964	(-0.436)-(0.457)
CBZ dose (mg/kg)	0.04	<0.001	0.028-0.051
PHT dose (mg/kg)	0.117	<0.001	0.062-0.171
PB dose (mg/kg)	0.142	0.007	0.04-0.244
Body weight (kg)	0.008	0.008	0.002-0.014
For predict lnCBZ CL (L/day)			
Constant	3.188	< 0.001	2.741-3.635
CBZ dose (mg/kg)	0.04	< 0.001	0.028-0.051
PHT dose (mg/kg)	0.117	<0.001	0.062-0.172
PB dose (mg/kg)	0.142	0.007	0.040-0.244
Body weight (kg)	0.008	0.008	0.002-0.014

Table 42B: Coefficients of factors in the best fit equation for prediction of ln CBZ Clearance (L/kg/day)

Factor	B	Sig (p-value)	95% CI
Constant	-0.018	0.934	(-0.458)-(0.421)
CBZ dose (mg/kg)	0.042	< 0.001	0.031-0.054
PHT dose (mg/kg)	0.091	0.001	0.038-0.145
PB dose (mg/kg)	0.138	0.008	0.038-0.239
Body weight (kg)	-0.007	0.024	(-0.013)-(-0.001)

The estimation equations of CBZ clearance were shown below:

ln CBZ clearance (L/hr) = (0.04) [CBZ dose (mg/kg)] + (0.117) [PHT dose (mg/kg)] + (0.142) [PB dose (mg/kg)] + (0.008)(BW) + 0.01

ln CBZ clearance (L/day) = (0.04) [CBZ dose (mg/kg)] + (0.117) [PHT dose (mg/kg)] + (0.142) [PB dose (mg/kg)] + (0.008)(BW) + 3.188

ln CBZ clearance (L/kg/day) = (0.042) [CBZ dose (mg/kg)] + (0.091) [PHT dose (mg/kg)] + (0.138) [PB dose (mg/kg)] - (0.007)(BW) - 0.018

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As shown in Figure 17, the correlation between observed ln CBZ clearance and predicted ln CBZ clearance (L/hr) was moderately significant (R-square=52.5%, $p<0.001$).

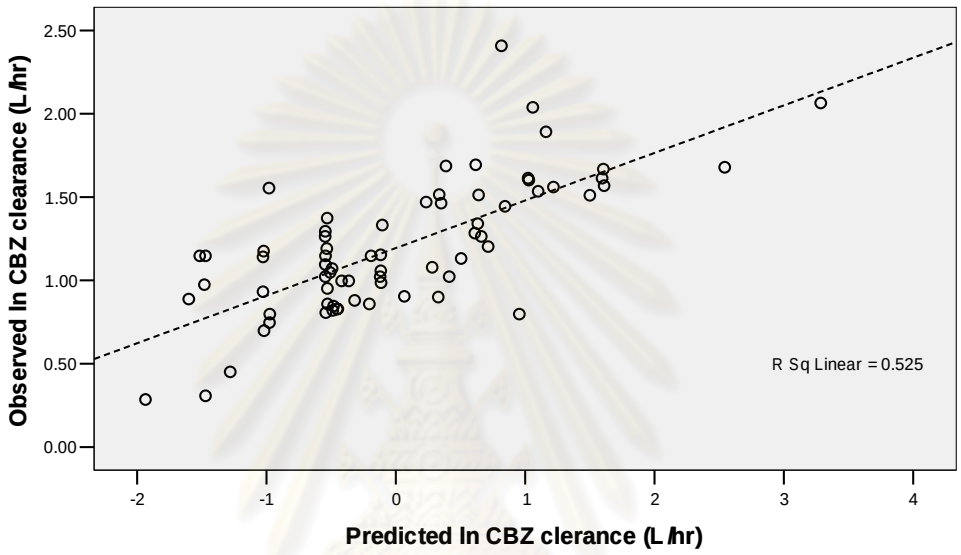

Figure 17: Scatter plot of observed ln CBZ clearance and predicted ln CBZ clearance (L/hr)

There were only 4 factors related to CBZ level-to-dose ratio including CBZ dose (mg/kg), body weight (kg), PHT dose (mg/kg) and PB dose (mg/kg).

Table 43 shows the entire significant model for prediction of CBZ level-to-dose ratio from forward stepwise linear regression, the model 4 was the best fit equation.

Table 43: Model summary of forward stepwise linear regression for prediction of CBZ level-to-dose ratio (mcg/L/mg)

Model	Variable entered	R	R-square	R-square change	Sig (F change)	Model Sig (ANOVA)
1	CBZ dose (mg/kg)	0.527	0.277	0.277	<0.001	<0.001
2	CBZ dose (mg/kg) Body weight (kg)	0.614	0.377	0.100	0.002	<0.001
3	CBZ dose (mg/kg) Body weight (kg) PHT dose (mg/kg)	0.661	0.436	0.059	0.011	<0.001
4	CBZ dose (mg/kg) Body weight (kg) PHT dose (mg/kg) PB dose (mg/kg)	0.698	0.487	0.051	0.014	<0.001

The coefficients and p-value of each variables which entered by forward stepwise method of model 4 were presented in Table 44. Multicolinearity of independent factors was determined (data not shown).

Table 44: Coefficients of factors in the best fit equation for prediction of CBZ level-to-dose ratio (mcg/L/mg)

Factor	B	Sig (p-value)	95% CI
Constant	20.964	< 0.001	16.643-25.286
CBZ dose (mg/kg)	-0.382	< 0.001	(-0.495)-(-0.269)
Body weight (kg)	-0.084	0.006	(-0.142)-(-0.025)
PHT dose (mg/kg)	-0.8	0.004	(-1.33)-(-0.27)
PB dose (mg/kg)	-1.254	0.014	(-2.243)-(-0.265)

The estimation equation of CBZ level-to-dose ratio was show below:

CBZ level-to-dose ratio (mcg/L/mg) = (-0.382) [CBZ dose (mg/kg)] - (0.084) [BW (kg)] - (0.8) [PHT dose (mg/kg)] - (1.254) [PB dose (mg/kg)] + 20.964

As shown in Figure 18, the correlation between observed CBZ level-to-dose ratio and predicted CBZ level-to-dose ratio was moderately significant (R-square=48.7%, $p<0.001$).

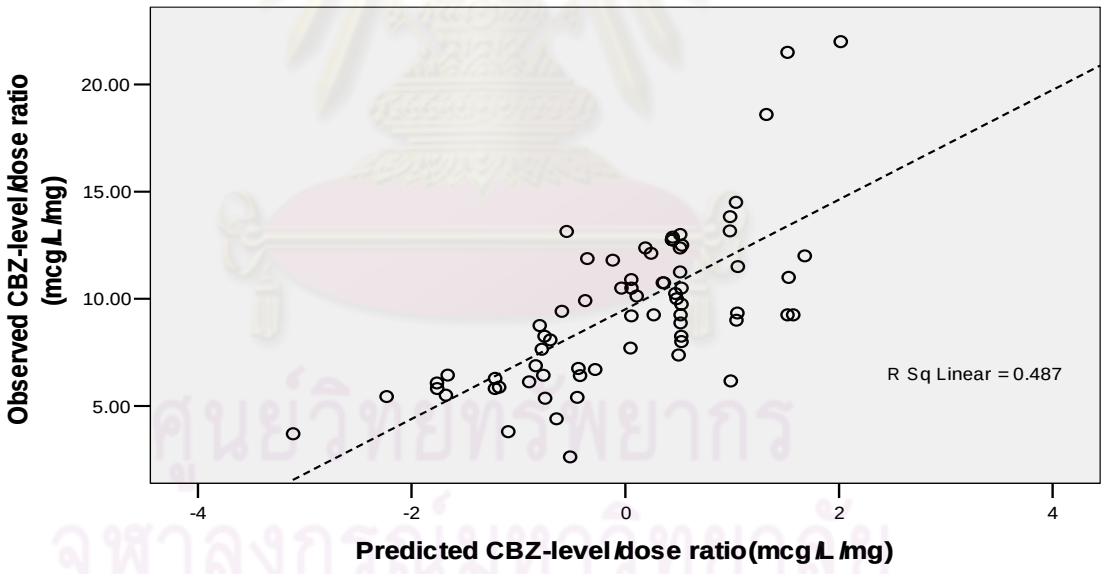

Figure 18: Scatter plot of observed CBZ level-to-dose ratio and predicted CBZ level-to-dose ratio

Interethnic variability of CYP3A5 polymorphism in Asia

Allelic frequencies of *CYP3A5* polymorphism in Asian population were different from Caucasian and African-American population. [17, 34-38] Among Asian population the allelic frequencies of *CYP3A5* polymorphism were not different (Table 45).

Table 45: Comparison of *CYP3A5* allele frequencies among Asians

Ethnicity	Number of subject	% Allele frequency		p-value (compared to this study)
		**1*	**3*	
Thai (This study)	**70**	**31**	**69**	-
Thai [17]	150	34	66	0.65
Chinese [34]	302	22	78	0.15
Indian [35]	90	41	59	0.14
Malaysian [35]	98	39	61	0.24
Japanese [36]	200	23	77	0.21

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CHARPTER V
DISCUSSION AND CONCLUSION

Part 1 Clinical pharmacokinetics of carbamazepine as monotherapy and in combination with classical antiepileptic drugs

The mean daily dose of CBZ calculated from the total patients included in this study was 15.45 ± 6.53 mg/kg/day which was within the recommended dose range of 15–25 mg/kg/day for seizure controlled. [9] Even though the daily dose of CBZ used in several patients was lower than that of the recommendation, especially in patients who used CBZ as monotherapy, but most of the patient's CBZ levels were within the therapeutic range. The mean daily dose of PHT from the patients who used CBZ in combination with PHT was 5.01±1.07 mg/kg/day which was within the recommended dose range of 4–7 mg/kg/day. [48] The mean daily dose of PB from the patients who used CBZ in combination with PB was 1.53±0.73 mg/kg/day which was within the recommended dose range of 1.1–2.0 mg/kg/day [49] The mean daily dose of VPA from the patients who used CBZ in combination with VPA was 19.25±7.68 mg/kg/day, while the recommended dose range of VPA in the absence of enzyme inducer drug is 7–18 mg/kg/day. [50] This indicated that when VPA was used concurrently with CBZ which is an enzyme inducer, the VPA dose had been increased. The median level-to-dose ratio of CBZ in patients who used CBZ as monotherapy was significantly higher than those obtained after combination therapy with PHT, PB or VPA, even though the median daily dose per body weight of CBZ was not significantly different. This indicated that when CBZ was used with PHT, PB or VPA the dose of CBZ had not been changed, even though the level of CBZ was decreased, especially when used CBZ with PHT which is the strongest inducer.

The CBZ clearance (L/kg/hr) in patients who used CBZ in combination with PB was 31% increased, which was consistent with previous studies who reported the increment of CBZ clearance to be within the range of 16-44% when concurrently used with PB. [13, 14, 51, 52] The CBZ clearance (L/kg/hr) in patients who used CBZ in combination with PHT was 98% increased, while previous studies reported the increment to be 42-45

%. [13, 51] Previous study reported CBZ clearance in patients who used CBZ as polytherapy (in combination therapy with enzyme-inducing AED, for instance PHT, PB) to be 0.1 L/kg/hr [9]. The median of CBZ clearance in patients who used CBZ in combination with PHT in this study was 0.097 L/kg/hr which was close to that reported in previous study, however, the median of CBZ clearance in patients who used CBZ in combination with PB was lower than that reported previously (0.064 L/kg/hr). There are conflicting results on the effect of VPA on CBZ clearance; increase, decrease, or no change. [13, 14, 20, 21, 24, 52] In this study, we found that CBZ when used in combination with VPA, the clearance of CBZ, after accounted for the body weight of the patients, did not change significantly. The mean daily dose of VPA was greater than 18 mg/kg which had been claimed by previous study that this high dose could increase CBZ clearance by 21%. [13] The average CBZ clearance in patients who received CBZ as monotherapy in this study was lower than those reported by previous studies (Table 46). This can be attributed to the reasons that CBZ clearance might be decreased with increasing age, while in contrary CBZ clearance might be increased with the size of the dose, the average age of patients in previous studies was all lower and the dose size was mostly higher as compared to this study. [13, 14, 52]

There were 19 patients (24%) of the 79 epileptic patients who had uncontrolled seizures and 4 patients (5%) had mild adverse effects. Patients with uncontrolled seizure without any precipitating factors, the doses of AEDs were adjusted or the second or third AED were added, for instance topiramate, lamotrigine which are the newer AEDs with different mechanism of actions, then, the seizures were better controlled. When considered the levels of AEDs, we found that majority of the patients had their drug levels within the therapeutic ranges (58 of the 79 patients, 74%), 16 patients (20%) had their drug levels lower than the therapeutic ranges, while the remainder 5 patients (6%) had their PHT levels higher than the therapeutic ranges. Recommended therapeutic ranges are the good guideline especially when the drug is used as monotherapy, however, when AEDs were used in combination, the therapeutic ranges might be decreased since the seizures could sometimes be controlled with lower therapeutic levels of each drug and adverse effect could be found in some patients even at subtherapeutic or therapeutic ranges.

Table 46: Overview of CBZ clearance estimations from CBZ monotherapy reported by different ethnicity

Population	CBZ clearance (L/kg/hr)	Characteristics		
		Age (yrs)	Weight (kg)	Dose (mg/kg/day)
Chinese [13]	0.0539	23.6	52.3	9.47
American [51]	0.0611	35.0	75.0	12.90
Japanese [52]	0.0554	14.0	39.3	7.36
Singaporean [53]	0.0636	12.5	34.9	16.70
Omani [54]	0.0540	27.8	60.8	9.70
Thai [18]	0.0610	34.5	51.75	17.03
Thai (This study)	0.049	43.38	58.70	13.33

In conclusion the CBZ clearance in patient who used CBZ as monotherapy was significantly lower than that in patient who used CBZ with PHT or PB, but was not significantly different from patient who used CBZ with VPA. Therapeutic ranges are the good guideline especially in monotherapy, however, these recommended ranges should be adjusted when the drugs are used in combination. TDM of the classical AEDs has the role to identify an individual's optimum concentrations and thus establish a reference level in that patient.

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Part 2 Correlation between pharmacokinetic parameters of carbamazepine and other classical antiepileptic drugs when used in combination

The correlation between PHT Vmax (mg/kg/day) and CBZ clearance (L/kg/day) was highly significant (R = 0.883, R-square =78%, p < 0.001), while the correlation between VPA clearance (L/kg/day) and CBZ clearance (L/kg/day) was moderately significant (R = 0.642, R-square =41.2%, p = 0.007), but the correlation between PB clearance (L/kg/day) and CBZ clearance (L/kg/day) was not reach statistically significant level (R = 0.332, R-square =11%, p = 0.227). Since we set the correlation coefficient to be 0.6 or higher in the part of calculation for the sample size to find a significant correlation. Therefore, the correlation coefficient of 0.332 would require a bigger sample size to be significant at $\alpha \leq 0.05$, power $\geq$ 80% while the sample size of 15 as we could recruit into this part of study could not.

CBZ is approximately 99% metabolized by oxidation, hydroxylation, direct conjugation with glucuronic acid, and sulfur conjugation pathways. Oxidation and hydroxylation pathway account for about 65% of its metabolism. The isoenzymes that catalyze 10, 11-oxidation of CBZ in the liver are *CYP3A4*, *CYP3A5, CYP2C8,* and *CYP1A2*; *CYP3A4* and *CYP3A5* are the most important of them. [11, 21] Phenytoin is eliminated 90% primarily by hepatic metabolism via Cytochrome P450 mixed function oxidase isoenzymes *(CYP 450)* (90% by *CYP2C9* and 10% by *CYP2C19*). [48, 55] PB is eliminated via hepatic metabolism and unchanged in the urine. The isoenzymes involved in PB elimination are *CYP2C9* and *CYP2C19*. About 20-40% of a dose of PB is excreted unchanged in the urine [20] VPA is primarily eliminated by hepatic metabolism (about 95%). Glucuronidation, oxidation and hydroxylation are the main metabolic pathways of VPA. Approximately 60% of the recovered dose of VPA in urine is metabolized via glucuronidation which is mediated by UDPGT1A6, UDPGT1A9, and UDPGT2B7. [50, 56, 57] The high correlation between CBZ clearance and PHT Vmax may attribute to the reason that the elimination process of both CBZ and PHT are involved hydroxylation by an arene oxidase enzyme which is also the rate limiting step of PHT metabolism. [55, 58] VPA appears to competitively inhibit the glucuronidation of CBZ metabolite (CBZ-10, 11-trans-diol) which might be the reason for detecting moderate correlation between CBZ clearance and VPA clearance. [55, 56] While CBZ clearance and

PB clearance was not significantly correlated, even though PB is metabolized via the same hepatic isoenzymes as CBZ (*CYP 450*), but the sub-families are different (PB is metabolized by *CYP2C9* and *CYP2C19*) and 20-40% of PB is excreted unchanged in the urine. [7, 20]

In conclusion there was highly significant linear correlation between CBZ clearance and PHT Vmax, while CBZ clearance and VPA clearance was moderately significant linear correlated, and CBZ clearance and PB clearance was less correlated and was not reach the significant level with the small sample size recruited into this part of study. The regression equations which showed significant and high correlations between CBZ pharmacokinetic parameters and PHT or VPA pharmacokinetic parameters might be useful to apply in the therapeutic drug monitoring, however, validation of each equation may be required.

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Part 3 Effect of *CYP3A5* polymorphism on CBZ pharmacokinetics

This study determined the effect of the polymorphic *CYP3A5* genotype on pharmacokinetics of CBZ in Thai patients. The CBZ level, CBZ clearance were the pharmacokinetic parameters evaluated in this study. The observed allelic frequencies of *CYP3A5*1* and *CYP3A5*3* in 70 patients were 31% and 69%, respectively. These frequencies are similar to previous study in Thai population and in all Asians, including Chinese, Indian, Malaysian and Japanese populations [17, 34-36], but are different from those reported for other populations, including Caucasian and African-American populations. [37, 38] The expected allelic frequencies of *CYP3A5* estimated at Hardy-Weinberg equilibrium were quite similar to the observed distributions in the population (Chi-square =0.306, p=0.858).

CBZ is metabolized by *CYP3A4/5*, *CYP2C8* and *CYP1A2* with *CYP3A4/5* play the most important role. [11, 20] *CYP3A5* is a hepatic, intestinal and kidney drug-metabolizing enzyme that is closely related in structure and function to *CYP3A4*. [11, 20] One of the *CYP3A5* polymorphism, *CYP3A5*3* allele that has a SNP in intron 3 (A6986G) and causes alternative splicing and protein truncation, thereby affecting *CYP3A5* expression. [30, 32, 33] The functional defect in *CYP3A5* cause the interindividual variability in the disposition of various *CYP3A* substrates, including amlodipine, tracolimus, cyclosporine, saquinavir, simvastatin and alprazolam. [39-44] However, other studies have also shown that the polymorphic of *CYP3A5* is not the major factor that affects the disposition of *CYP3A* substrates, including midazolam, nifedipine, diltiazem and clopidogrel. [59-62] Previous study by Seo et al. [15] in Japanese epileptic patients reported that patients with *CYP3A5*3/*3* exhibited CBZ clearance which was 8% higher than patients without *CYP3A5*3/*3*; this result was conflicted with the result from the study by Park et al. [16] in 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 a *CYP3A5*1* allele. Seo et al. [15] recruited patients who used CBZ either monotherapy or concurrently with 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 due to the number of patients who used CBZ concurrently with potent inducer was also higher in

that group. Park et al. [16] included only patients who used CBZ as monotherapy, the result from their study indicated that *CYP3A5*3/*3* would result in lower CBZ clearance might be more valid.

In this study, when the total patients were categorized into 3 groups based on their *CYP3A5* genotypes, i.e. *CYP3A5*1/*1*, *CYP3A5*1/*3, and *CYP3A5*3/*3*, CBZ level and CBZ clearance were not significantly different among these 3 groups. The median of CBZ clearance in patients with *CYP3A5*1/*1* (1.03 L/kg/day) was lower than the median of CBZ clearance in patients with *CYP3A5*1/*3, and *CYP3A5*3/*3* (1.33 and 1.30 L/kg/day, respectively), but not reaching the statistically significantly different level (p=0.223). One of the important confounding factor was that most of the patients with *CYP3A5*1/*1* used CBZ as monotherapy, while some of patients with *CYP3A5*1/*3, and *CYP3A5*3/*3* used CBZ concurrently with enzyme inducing AEDs; i.e. PHT or PB. When we categorized the total patients into 2 groups based on *CYP3A5* genotypes; the first group was *CYP3A5*1/*1* and *CYP3A5*1/*3*, and the second group was *CYP3A5*3/*3*, the medians of CBZ level and the medians of CBZ clearance of these 2 groups were nearly equal and were not statistically significantly different.

To avoid the confounding effect from enzyme inducing factor, the effects of *CYP3A5* polymorphism on CBZ pharmacokinetic parameters were determined by grouped patients into CBZ monotherapy, CBZ+PHT, CBZ+PB, CBZ+VPA and CBZ in combination with enzyme inducing AED (CBZ in combination with PHT or PB).

Comparisons of CBZ pharmacokinetic parameters between the 2 groups of different genotypes among the 36 patients who used CBZ as monotherapy, either categorized patients into 2 groups as *CYP3A5*1/*1* and **1/*3* VS *CYP3A5*3/*3*, or *CYP3A5*1/*1* VS *CYP3A5*1/*3* and **3/*3*, CBZ level and CBZ clearance showed no significantly different between the 2 groups of different genotypes. These results conflict with the results reported by Park et al. [16] , they reported that the mean of CBZ level-to-dose ratio in patients with *CYP3A5*1/*1* and **1/*3* (9.94±3.38 mcg/L/mg) was significantly lower (p=0.032) than the mean of CBZ level-to-dose ratio in patients with *CYP3A5*3/*3* (13.07±4.46 mcg/L/mg), while the mean of CBZ clearance in patients with *CYP3A5*1/*1* and **1/*3* (0.056±0.017 L/kg/hr) was significantly higher (p=0.004) than the mean of CBZ clearance in patients with *CYP3A5*3/*3* (0.040±0.014 L/kg/hr). In our

study, the mean of CBZ level-to-dose ratio in patients with *CYP3A5*1/*1* and **1/*3* was 11.06±3.92 mcg/L/mg, while the mean of CBZ level-to-dose ratio in patients with *CYP3A5*3/*3* was 10.61±3.65 mcg/L/mg which were nearly equal and were not statistically significantly different (p=0.727). At the same time, the mean of CBZ clearance in patients with *CYP3A5*1/*1* and **1/*3* was 0.053±0.023 L/kg/hr while the mean of CBZ clearance in patients with *CYP3A5*3/*3* was 0.049±0.013 L/kg/hr which was 8% lower, but was not statistically significantly different (p=0.552) from *CYP3A5*1/*1* and **1/*3*. Actually the mean and standard deviation of CBZ level-to-dose ratio and CBZ clearance obtained from our study were quite similar to those from Park et al. study. However, small variation in either group resulted in opposite conclusion which means that the power of the test might be low due to the small number of patients participated in this study.

Comparisons of CBZ pharmacokinetic parameters between 2 groups of different genotypes among the 7 patients who used CBZ in combination with PHT were performed by categorized the patients into 2 groups as *CYP3A5*1/*3* VS *CYP3A5*3/*3*. The mean of CBZ level-to-dose ratio in patients with *CYP3A5*1/*3* was 6.19±3.99 mcg/L/mg while the mean of CBZ level-to-dose ratio in patients with *CYP3A5*3/*3* was 6.84±4.32 mcg/L/mg, which was 11% higher, but was not significantly different (p=0.846) from *CYP3A5*1/*3*. The mean of CBZ clearance in patients with *CYP3A5*1/*3* was 0.100±0.077 L/kg/hr while the mean of CBZ clearance in patients with *CYP3A5*3/*3* was 0.071±0.028 L/kg/hr which was 29% lower, but was not significantly different (p=0.497) from *CYP3A5*1/*3*. The comparisons of CBZ level-to-dose ratio and CBZ clearance between these 2 groups of genotype were not significantly different due to much too small number of patients included into the study.

Comparisons of CBZ pharmacokinetic parameters between 2 groups of different genotypes among the 11 patients who used CBZ in combination with PB were performed by categorized patients into 2 groups as *CYP3A5*1/*3* VS *CYP3A5*3/*3*. The mean of CBZ level-to-dose ratio in patients with *CYP3A5*1/*3* was 6.23±2.10 mcg/L/mg while the mean of CBZ level-to-dose ratio in patients with *CYP3A5*3/*3* was 9.28±2.40 mcg/L/mg which was 33% higher, but was not significantly different (p=0.064) from *CYP3A5*1/*3*. The mean of CBZ clearance in patients with *CYP3A5*1/*3* was

0.089±0.038 L/kg/hr while the mean of CBZ clearance in patients with *CYP3A5*3/*3* was 0.053±0.016 L/kg/hr which was 40% lower, and was border significantly different (p=0.05) from *CYP3A5*1/*3*. The comparisons of CBZ level-to-dose ratio and CBZ clearance between these 2 groups of genotype were border significantly different, further study with higher number of patients are required.

Comparisons of CBZ pharmacokinetic parameters between 2 groups of different genotypes among the 16 patients who used CBZ in combination with VPA were performed by categorized patients into 2 groups as *CYP3A5*1/*1* and **1/*3* VS *CYP3A5*3/*3*. The mean of CBZ level-to-dose ratio in patients with *CYP3A5*1/*1* and **1/*3* was 9.34±2.87 mcg/L/mg while the mean of CBZ level-to-dose ratio in patients with *CYP3A5*3/*3* was 7.96±2.63 mcg/L/mg which was 17% lower, but was not significantly different (p=0.335) from *CYP3A5*1/*1* and **1/*3*. The mean of CBZ clearance in patients with *CYP3A5*1/*1* and **1/*3* was 0.054±0.022 L/kg/hr while the mean of CBZ clearance in patients with *CYP3A5*3/*3* was 0.061±0.023 L/kg/hr which was 13% higher, but was not significantly different (p=0.511) from *CYP3A5*1/*1* and **1/*3*.

Comparisons of CBZ pharmacokinetic parameters between 2 groups of different genotypes among the 18 patients who used CBZ in combination with enzyme inducing AED were performed by categorized patients into 2 groups as *CYP3A5*1/*3* VS *CYP3A5*3/*3*. The mean of CBZ level-to-dose ratio in patients with *CYP3A5*1/*3* was 6.21±2.74 mcg/L/mg while the mean of CBZ level-to-dose ratio in patients with *CYP3A5*3/*3* was 8.40±3.25 mcg/L/mg which was 26% higher, but was not significantly different (p=0.161) from *CYP3A5*1/*3*. The mean of CBZ clearance in patients with *CYP3A5*1/*3* was 0.094±0.052 L/kg/hr while the mean of CBZ clearance in patients with *CYP3A5*3/*3* was 0.059±0.021 L/kg/hr which was 37% lower, but was not significantly different (p=0.139) from *CYP3A5*1/*3*. This study has not sufficient statistical power to detect significant different of CBZ pharmacokinetic parameters between different genotypes, further study with higher number of patients are required.

When compared the CBZ pharmacokinetic parameters between CBZ monotherapy, CBZ+PHT, CBZ+PB and CBZ+VPA in the *CYP3A5*1/*1* and *CYP3A5*1/*3* genotypes group, the median of CBZ level-to-dose ratio in patients who used CBZ as monotherapy (10.75 mcg/L/mg) was significant higher (42%, p=0.018) than the median

of CBZ level-to-dose ratio in patients who used CBZ in combination with PB (6.19 mcg/L/mg), while the median of CBZ level-to-dose ratio in patients who used CBZ in combination with PHT (5.44 mcg/L/mg) was 49% lower than the median of CBZ level-to-dose ratio in patients who used CBZ as monotherapy, but was not significantly different (p=0.067). The median of CBZ clearance in patients who used CBZ as monotherapy (2.71 L/hr) was significantly lower (76%, p=0.018) than the median of CBZ clearance in patients who used CBZ in combination with PB (4.77 L/hr), while the median of CBZ clearance in patients who used CBZ in combination with PHT (5.36 L/hr) was 49% higher than the median of CBZ clearance in patients who used CBZ as monotherapy, but was not significantly different (p=0.067). Among the patients with *CYP3A5*3/*3* genotype, the pharmacokinetic parameters of CBZ were not significantly different among 4 groups (CBZ monotherapy, CBZ+PHT, CBZ+PB and CBZ+VPA). The effects of enzyme inducing AEDs (PHT, PB) were more potent in the *CYP3A5*1/*1* and *CYP3A5*1/*3* genotypes group as compared to the *CYP3A5*3/*3* genotype group.

Multiple regression analysis shows that the factors related to CBZ clearance (L/hr, L/day and L/kg/day) were CBZ dose (mg/kg), PHT dose (mg/kg), PB dose (mg/kg) and body weight (kg) which produced the best model for estimating CBZ clearances (R-square for CBZ clearance in L/hr and L/day =52.5%, R-square for CBZ clearance in L/kg/day = 54.7%, p<0.001). This study shows that the *CYP3A5*3/*3* genotype was not correlate to CBZ clearance, inconsistent to previous study by Seo et al. who incorporated *CYP3A5*3/*3* genotype into the equation generated to predict CBZ clearance. [15] The linear regression model generated to predict CBZ clearance (L/kg/day) from PHT Vmax (part 2; R-square = 78%) showed a better correlation compared to the linear regression model which included CBZ dose (mg/kg), PHT dose (mg/kg), PB dose (mg/kg) and body weight (kg) (part 3, which could explain 54.7% of the variance in CBZ clearance). The linear regression model generated to predict CBZ clearance from VPA clearance (part 2; R-square = 41.2%) showed less correlation compared to the model which included demographic data.

The factors related to CBZ level-to-dose ratio (mcg/L/mg) were CBZ dose (mg/kg), body weight (kg), PHT dose (mg/kg) and PB dose (mg/kg) which produced the best model for estimating CBZ level-to-dose ratio (R-square=48.7%, p<0.001).

In conclusion *CYP3A5* genotype did not substantially affect the pharmacokinetics of CBZ. However, in patients who used CBZ in combination with enzyme inducing AED (PHT or PB), individuals carrying *CYP3A5*1* allele yielded the trend toward more susceptible to changes in CBZ clearance and showed lower CBZ-level-to-dose ratio as compared to individuals carrying *CYP3A5*3*. The results suggest that the presence of the *CYP3A5*3* allele play a minor role in causing interindividual variability in the disposition of CBZ.

Limitation

1. Comparisons of pharmacokinetic parameters between CBZ monotherapy and combination therapy were performed in different patients groups, variations among individual due to their genetic and environment factors may interfere with the result.
2. This study retrieved some information from retrospective data especially the AEDs level, the exact time and date of sample obtaining may be varied and not so accurate.
3. This study included only patients with normal liver and kidney function, therefore, using the equations obtained from this study should be applied with caution in patients with poor liver and kidney function.
4. The number of patients recruited into the combination therapy of CBZ and PHT or PB group in order to study the effect of *CYP3A5* on pharmacokinetics of CBZ was too few, higher numbers of patients are needed to increase the power of statistical analysis before any strong conclusion could be made.

Further study

1. Higher number of patients should be recruited for the study about the effect of *CYP3A5* on pharmacokinetics of CBZ when used CBZ in combination therapy with PHT or PB.
2. The equations for predict CBZ clearance from demographic data, PHT Vmax or VPA clearance obtained from this study should be validated and evaluated to determine the accuracy and precision.

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APPENDICES

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APPENDIX A

แบบบันทึกข้อมูลของผู้ป่วย (Demographic data)

ตอนที่ 1: ลักษณะทั่วไปของผู้ป่วย

1. เพศ ❒ ชาย❒ หญิง 2. วัน เดือน ปี เกิด ……/……/……อายุ.....ปี

3. ส่วนสูง.....เซนติเมตร 4. น้ำหนักปัจจุบัน.....กก 5. BMI…….kg/m^2

6. ท่านสูบบุหรี่❒ ไม่สูบบุหรี่ ❒ สูบบุหรี่ ปริมาณที่สูบ.....มวน/วัน

7. ท่านดื่มสุรา ยาดอง เบียร์ ไวน์

❒ ไม่ดื่ม/ดื่มแต่ปัจจุบันเลิกแล้ว ❒ ดื่มเป็นครั้งคราว ❒ ดื่มเป็นประจำปริมาณ.....

8. ประวัติการแพ้ยา ❒ ไม่มี ❒ มี ระบุ.....

9. ประวัติโรคประจำตัว ❒ ไม่มีโรคประจำตัว ❒ มีโรคประจำตัวระบุ……

10. ยารักษาโรคประจำตัว มีจำนวน.....ชนิดได้แก่

.....

.....

.....

ตอนที่ 2: ข้อมูลเกี่ยวกับโรคลมชักหรือโรคทางระบบประสาทอื่น ๆและการรักษา

11. โรคทางระบบประสาทที่เป็นในปัจจุบันคือ ❒ ลมชัก ❒ อื่นๆ คือ.....

กรณีเป็นโรคลมชัก

12. ลักษณะและชนิดของโรคลมชัก

.....

13. ประวัติการรักษาด้วยยากันชัก

.....

.....

14. สูตรยาที่ใช้ในปัจจุบัน

❒ CBZ ❒ CBZ+PHT ❒ CBZ+PB ❒ CBZ+VPA

15. ระยะเวลาที่ใช้ยาสูตรปัจจุบัน.....

16. กรณีเป็นโรคลมชักผลการรักษาด้วยยากันชักในปัจจุบัน

❒ ควบคุมอาการชักได้ ❒ ยังมีอาการชักอยู่.....ครั้ง/เดือน นานครั้งละ.....นาที

17. ผลข้างเคียงจากยา ❒ ไม่มี ❒ มี ระบุ.....

18. การรักษาอื่นๆที่ไม่ได้ใช้ยา.....

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รูปแบบและการบริหารยา

วันที่	ยากันชัก	ขนาดและวิธีการบริหารยา

ตอนที่ 3: **ข้อมูลเกี่ยวการตรวจวัดระดับยาในเลือด**

ลำดับที่	วันที่	เวลาที่รับประทานยา		เวลาที่เจาะเลือด		ระดับยาในเลือด	
		CBZ		CBZ		CBZ (mg/L)	(mg/L)
1							
2							
3							

ตอนที่ 4: **ข้อมูลเกี่ยวการตรวจยีน** *CYP3A5*

ลักษณะของอัลลีล	
*CYP3A5 *1/*1*	
*CYP3A5 *1/*3*	
*CYP3A5 *3/*3*	

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APPENDIX B

เอกสารชี้แจงข้อมูล/คำแนะนำแก่ผู้เข้าร่วมการวิจัย

(Patient/Participant Information Sheet)

ชื่อโครงการ ผลของภาวะพหุสัณฐานของยีน CYP3A5 ต่อเภสัชจลนศาสตร์ของยาคาร์บามาซีพีนในผู้ป่วยไทย เมื่อใช้เป็นยาเดี่ยวหรือใช้ร่วมกับยาเฟนิทอยน์ ฟีโนบาร์บิทาล หรือวาลโพรอิกแอซิด

ชื่อผู้วิจัย เภสัชกรหญิงธราธร ไตรยวงค์ นิสิตระดับปริญญาโท ภาควิชาเภสัชกรรมปฏิบัติ
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

สถานที่วิจัย สถาบันประสาทวิทยา

บุคคลและวิธีการติดต่อเมื่อมีเหตุฉุกเฉินหรือความผิดปกติที่เกี่ยวข้องกับการวิจัย

1. เภสัชกรหญิงธราธร ไตรยวงค์

ที่อยู่ ภาควิชาเภสัชกรรมปฏิบัติ สาขาเภสัชกรรมคลินิก
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

โทรศัพท์ติดตามตัว 08-9574-3712

2. นายแพทย์สมชาย โตวณะบุตร

ที่อยู่ สถาบันประสาทวิทยา

โทรศัพท์ที่ทำงาน 02-3547075 ต่อ 1138

ท่านได้รับเชิญให้เข้าร่วมการศึกษาวิจัยนี้เนื่องจากมีภาวะโรคลมชักหรือโรคทางระบบประสาทอื่นๆ และได้รับยาคาร์บามาซีพีนในการรักษาโดยท่านจะได้อ่านข้อมูลข้างล่างก่อน (หรือทีมแพทย์ผู้ศึกษาวิจัยอ่านให้ท่านรับทราบ) ถ้าท่านมีข้อข้องใจสงสัยใดๆ เกี่ยวกับการศึกษาวิจัยนี้ สามารถซักถามผู้ทำการศึกษาวิจัยหรือแพทย์ที่ทำการศึกษาวิจัยได้ หากท่านตัดสินใจเข้าร่วมการศึกษาวิจัย ท่านจะได้รับสำเนาใบยินยอมที่ท่านเซ็นชื่อกำกับเก็บไว้ 1 ฉบับ

ความเป็นมาของโครงการ

คาร์บามาซีพีนเป็นยาที่รับรองให้ใช้เป็นยาหลักในการรักษาโรคลมชักชนิดที่มีอาการชักเฉพาะที่หรืออาการชักเกร็งกระตุกทั้งตัวโดยใช้เป็นยาเดี่ยวหรือใช้ร่วมกับยากันชักชนิดอื่น เช่น ยาเฟนิทอยน์ ฟีโนบาร์บิทาล วาลโพรอิกแอซิด และนอกจากนี้ยังใช้ในการรักษาโรคทางระบบประสาทอื่นๆ คาร์บามาซีพีนถูกกำจัดทางตับร้อยละ 99 โดย CYP3A4/5 จะเป็นเอนไซม์หลักที่สำคัญที่สุด ระดับยาคาร์บามาซีพีนในเลือดที่อยู่ในช่วงของการรักษาคือ 4-12 มิลลิกรัมต่อลิตรซึ่งเป็นระดับยาที่ผู้ป่วยส่วนใหญ่ได้ผลในการรักษา

การศึกษาเกี่ยวกับอัตราการกำจัดยาคาร์บามาซีพีนมีความสำคัญในการนำมาใช้ติดตามระดับยาในเลือด การศึกษาทางเภสัชพันธุศาสตร์ในปัจจุบันพบว่าผู้ที่มีภาวะพหุสัณฐานของยีน CYP3A5 แตกต่างกันจะมีอัตราการกำจัดยาคาร์บามาซีพีนเร็วช้าแตกต่างกัน ในต่างประเทศการศึกษาผลของภาวะพหุสัณฐานของยีน CYP3A5 ต่ออัตราการกำจัดยาคาร์บามาซีพีนกรณีที่ใช้ร่วมกันกับยากันชักชนิดอื่นที่เกิดปฏิกิริยาระหว่างยายังไม่มีการศึกษาและรายงานผลที่ชัดเจน และยังไม่เคยมีการศึกษาในประเทศไทย

ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของภาวะพหุสัณฐานของยีน CYP3A5 ต่ออัตราการกำจัดยาคาร์บามาซีพีน ตลอดจนหาความสัมพันธ์ระหว่างอัตราการกำจัดยาคาร์บามาซีพีนกับอัตราการกำจัดยาเฟนิทอยน์ ฟีโนบาร์บิทาลและวาลโพรอิกแอซิด และพัฒนาสมการสำหรับทำนายอัตราการกำจัดยาคาร์บามาซีพีนในผู้ป่วยโรคลมชักหรือโรคทางระบบประสาทอื่นๆที่มีภาวะพหุสัณฐานของยีน CYP3A5 ที่แตกต่างกันทั้งในผู้ป่วยที่ใช้ยาคาร์บามาซีพีนเป็นยาเดี่ยวหรือใช้สองตัวร่วมกับ เฟนิทอยน์ ฟีโนบาร์บิทาลหรือวาลโพรอิกแอซิดซึ่งจะนำไปสู่การคำนวณขนาดยาให้ผู้ป่วยแต่ละรายได้อย่างมีประสิทธิผลยิ่งขึ้น

วัตถุประสงค์

1. เปรียบเทียบอัตราการกำจัดยาและสัดส่วนระดับยาต่อขนาดยาคาร์บามาซีพีนในผู้ป่วยที่มีภาวะพหุสัณฐานของยีน CYP3A5 ต่างกัน คือ CYP3A5*1 กับ CYP3A5*3 เมื่อใช้เป็นยากันชักแบบเดี่ยว หรือใช้ร่วมกับยาเฟนิทอยน์ ฟีโนบาร์บิทาลหรือ วาลโพรอิกแอซิด
2. สร้างสมการทำนายอัตราการกำจัดยาคาร์บามาซีพีนจากข้อมูลพื้นฐานของผู้ป่วย ภาวะพหุสัณฐานของยีน CYP3A5
3. ศึกษาความสัมพันธ์ระหว่างอัตราการกำจัดยาคาร์บามาซีพีนกับอัตราการกำจัดยาเฟนิทอยน์ ฟีโนบาร์บิทาลและวาลโพรอิกแอซิด

รายละเอียดที่จะปฏิบัติต่อผู้เข้าร่วมการวิจัย

หากท่านตัดสินใจเข้าร่วมการศึกษาวิจัยนี้กรุณาเซ็นชื่อลงในใบยินยอม ท่านจะได้รับการตรวจดังต่อไปนี้

เมื่อท่านมาพบแพทย์ตามนัดท่านจะได้รับการชั่งน้ำหนัก วัดส่วนสูง และได้รับการเจาะเลือดดังต่อไปนี้ ในตอนเช้าก่อนที่ท่านจะรับประทานยากันชักในมื้อเช้า (ให้นำยากันชักที่ต้องรับประทานในมื้อเช้ามาด้วย)

- ท่านจะได้รับการเจาะเลือดปริมาณ 10-15 มิลลิลิตร (2-3 ช้อนชา) เพื่อตรวจหา
  - ระดับยากันชัก
  - ลักษณะของยีน CYP3A5

และท่านจะได้รับการสอบถามข้อมูลพื้นฐานทั่วไปโดยใช้แบบสอบถาม

หมายเหตุ ในการนัดเจาะเลือดจะทำในวันที่ท่านต้องมาพบแพทย์อยู่แล้ว และท่านไม่ต้องเสียค่าใช้จ่ายใด ๆ ที่นอกเหนือไปจากค่ารักษาพยาบาลของท่านตามปกติ ระยะเวลาที่ท่านต้องเกี่ยวข้องในการศึกษาวิจัยนี้คือ 1-3 เดือนตามระยะเวลาในการนัดหมายพบแพทย์ตามปกติ

ประโยชน์ที่จะเกิดแก่ผู้เข้าร่วมการวิจัยและประโยชน์ในทางวิชาการต่อส่วนรวม

1. ได้ทราบลักษณะของยีน CYP3A5 ของตัวท่านเอง ซึ่งเกี่ยวข้องกับการกำจัดยา
2. ได้ข้อมูลระดับยากันชักของท่านเมื่อได้รับขนาดยาในปัจจุบันและสามารถใช้เป็นค่าอ้างอิงต่อไป
3. ข้อมูลการศึกษาที่ได้สามารถนำไปสร้างสมการทำนายอัตราการกำจัดยาคาร์บามาซีพีนซึ่งจะช่วยให้บุคลากรทางการแพทย์นำมาพิจารณาวางแผนการรักษาเพื่อนำไปสู่การคำนวณขนาดยาที่เหมาะสมให้กับผู้ป่วย

ความเสี่ยงจากการเข้าร่วมการวิจัย

ความเสี่ยงในการเจาะเลือดคือ อาจมีอาการปวด หรือมีจ้ำเลือดบริเวณที่เจาะ แต่มีความเสี่ยงน้อยมากที่จะเกิดการติดเชื้อจากการเจาะเลือด ถ้าหากเกิดขึ้น ท่านจะได้รับการรักษาพยาบาลโดยแพทย์ผู้ทำหัตถการหรือแพทย์และบุคลากรทางการแพทย์คนอื่นที่ได้รับมอบหมาย

การเก็บตรวจดีเอ็นเอ อาจทำให้ท่านเกิดความกังวลว่าความลับในส่วนนี้จะถูกเปิดเผย งานวิจัยนี้จะตรวจเฉพาะยีนหรือสารทางพันธุกรรมที่เกี่ยวข้องกับการกำจัดยาที่ทำการศึกษาวิจัยคือยีน CYP3A5 ข้อมูลของท่านจะถูกเก็บไว้เป็นความลับและจะใช้สำหรับงานวิจัยนี้เท่านั้น เลือดหรือสารสกัดดีเอ็นเอที่เหลือจากการวิจัยจะไม่มีการเก็บไว้

หากท่านไม่ต้องการเข้าร่วมการศึกษาวิจัย หรือเปลี่ยนใจระหว่างร่วมศึกษาวิจัย

ท่านไม่จำเป็นต้องเข้าร่วมการศึกษาวิจัยนี้หากท่านไม่สมัครใจ หลังจากตัดสินใจเข้าร่วมการศึกษาแล้ว ท่านสามารถถอนตัวได้ตลอดเวลา การตัดสินใจของท่านจะไม่มีผลต่อการรักษาในอนาคตหรือการดูแลอื่นใด หากท่านไม่ต้องการเข้าร่วมการศึกษาหรือต้องการหยุดการศึกษา ณ เวลาใดก็ตาม

การเก็บข้อมูลเป็นความลับ

ข้อมูลของท่านที่ถูกบันทึกไว้ระหว่างการศึกษาจะถูกเก็บไว้เป็นความลับตลอดเวลาเช่นเดียวกับข้อมูลที่เกี่ยวข้องจากแฟ้มเวชระเบียนของโรงพยาบาล คณะกรรมการจริยธรรมการวิจัยและพนักงานหรือผู้วิจัยสามารถที่จะขอตรวจสอบข้อมูลเหล่านี้ได้ โดยข้อมูลเหล่านี้จะยังเก็บรักษาไว้เป็นเรื่องลับเฉพาะ

ข้อมูลส่วนตัวที่ท่านไม่ต้องการเปิดเผยจะถูกเก็บรวบรวมไว้ในฐานข้อมูล และนำมาใช้เพื่อวัตถุประสงค์ทางการวิจัยทางการแพทย์เฉพาะในส่วนที่เกี่ยวข้องกับการศึกษา โดยจะมีการกำหนดสิทธิการเข้าถึงการใช้งานเฉพาะแพทย์ผู้ศึกษาวิจัยและบุคคลที่แพทย์ผู้ศึกษาวิจัยอนุญาตเท่านั้นที่จะมีรหัสผ่านในการเข้าถึงข้อมูล ทั้งนี้เพื่อวัตถุประสงค์ทางการศึกษาวิจัยทางการแพทย์ **โดยไม่มีการอ้างถึงชื่อและเลขประจำตัวผู้ป่วยของท่าน ทางสถาบันประสาทวิทยาจะทำทุกวิถีทางเพื่อให้เกิดความมั่นใจว่าข้อมูลส่วนตัวของท่านจะถูกปกป้องไว้**

หากท่านได้รับการปฏิบัติที่ไม่ตรงตามที่ได้ระบุไว้ในเอกสารชี้แจงนี้ ท่านสามารถแจ้งให้ประธานคณะกรรมการจริยธรรมฯ ทราบได้ที่ สำนักงานคณะกรรมการจริยธรรมการวิจัยสถาบันประสาทวิทยา ตึกกุมารประสาทวิทยา ชั้น 4 โทร. 02-3547076 ต่อ 2402

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX C

หนังสือแสดงความยินยอมเข้าร่วมโครงการวิจัย

(Informed Consent Form)

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย รวมทั้งประโยชน์ที่เกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว

ผู้วิจัยรับรองว่าจะตอบคำถามต่างๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ ข้าพเจ้าเข้าร่วมโครงการวิจัยนี้โดยสมัครใจ และมีสิทธิ์ที่จะบอกเลิกการเข้าร่วมโครงการวิจัยเมื่อใดก็ได้ โดยการบอกเลิกการเข้าร่วมการวิจัยนี้จะไม่มีผลต่อการรักษาโรคและการรับบริการต่างๆที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับและจะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่างๆ ที่เกี่ยวข้องกระทำได้เฉพาะกรณีจำเป็นด้วยเหตุผลทางวิชาการเท่านั้น

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ และได้ลงนามในใบยินยอมนี้ด้วยความสมัครใจต่อหน้าพยาน เพื่อเป็นหลักฐานสำคัญ

ลงชื่อ.....ผู้เข้าร่วมโครงการวิจัย/
ผู้แทนโดยชอบธรรม
(..... ชื่อ-นามสกุล ตัวบรรจง)

ลงชื่อ.....ผู้ดำเนินการโครงการวิจัย
(..... ชื่อ-นามสกุล ตัวบรรจง)

ลงชื่อ.....พยาน
(..... ชื่อ-นามสกุล ตัวบรรจง)

ลงชื่อ.....พยาน
(..... ชื่อ-นามสกุล ตัวบรรจง)

ในกรณีที่ผู้เข้าร่วมโครงการวิจัยไม่สามารถลงลายมือชื่อด้วยตนเองได้ ให้ผู้แทนโดยชอบตามกฎหมายซึ่งมีส่วนเกี่ยวข้องเป็น.....ของผู้เข้าร่วมโครงการวิจัยเป็นผู้ลงนามแทน

วันที่ลงนาม.....

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

ใบแสดงเจตนายินยอมให้เก็บตัวอย่างเพื่อการตรวจทางเวชพันธุศาสตร์

วันที่.....เดือน.....พ.ศ. 2553

ข้าพเจ้า.....อายุ.....ปี.....

อนุญาตให้นายแพทย์/แพทย์หญิง.....เก็บตัวอย่างตรวจคือ เลือด จากข้าพเจ้า เพื่อประโยชน์ในการศึกษาวิจัยเรื่อง “ผลของภาวะพหุสัณฐานของยีน CYP3A5 ต่อเภสัชจลนศาสตร์ของยาคาร์บามาซีพีนในผู้ป่วยไทย เมื่อใช้เป็นยาเดี่ยวหรือใช้ร่วมกับยาเฟนิทอยน์ ฟีโนบาร์บิทาล หรือวาลโพรอิกแอซิด” ที่ข้าพเจ้าเข้าร่วมในการวิจัย

ข้าพเจ้าได้รับทราบข้อมูลเกี่ยวกับการวิจัยดังกล่าวดังนี้

1. วัตถุประสงค์ในการวิจัย
2. ประโยชน์ที่คาดว่าจะได้รับ
3. การตรวจดังกล่าวจะกระทำโดยไม่เปิดเผยข้อมูลส่วนตัวของข้าพเจ้าแก่บุคคลอื่น ที่ไม่เกี่ยวข้องกับการวิจัย
4. การเก็บตัวอย่างตรวจนี้กระทำโดยการเจาะเลือดดำ ซึ่งมีผลข้างเคียงคือ ความเจ็บปวด เลือดซึม หรือการติดเชื้อ ซึ่งเกิดได้น้อยมาก และถ้าหากเกิดขึ้น ข้าพเจ้าจะได้รับการรักษาพยาบาลโดยแพทย์ผู้ทำหัตถการหรือแพทย์และบุคลากรทางการแพทย์คนอื่นที่ได้รับมอบหมาย
5. การตรวจดีเอ็นเอจะตรวจเฉพาะยีน CYP3A5 เลือดหรือสารสกัดดีเอ็นเอที่เหลือจากการทำวิจัยจะไม่มีการเก็บไว้

ข้าพเจ้าได้รับทราบข้อมูลในเอกสารให้ความยินยอมนี้ และได้มีโอกาสซักถามแพทย์จนเข้าใจดี ข้าพเจ้าจึงลงนามไว้ข้างท้ายนี้เพื่อเป็นหลักฐาน

ลงชื่อ.....ผู้ยินยอม

( ) หรือผู้แทนโดยชอบธรรม (ระบุความเกี่ยวข้อง)

ลงชื่อ.....พยาน

( )

ลงชื่อ.....พยาน

( )

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

ใบส่งเจาะเลือดเพื่อตรวจวัดระดับยาและเก็บเลือดไว้ตรวจยีน CYP3A5

ชื่อโครงการวิจัย ผลของภาวะพหุสัณฐานของยีน CYP3A5 ต่อเภสัชจลนศาสตร์ของยาคาร์บามาซีพีนในผู้ป่วยไทย เมื่อใช้เป็นยาเดี่ยวหรือใช้ร่วมกับยาเฟนิทอยน์ ฟีโนบาร์บิทาล หรือวาลโพรอิกแอซิด

ชื่อ-สกุลผู้ป่วย.....HN.....

วันนัดเจาะเลือด.....

การส่งตรวจเลือด

❒ **วัดระดับยา** Carbamazepine

❒ **วัดระดับยา** Phenytoin

❒ **วัดระดับยา** Phenobarbital

❒ **วัดระดับยา** Valproic acid

❒ **เก็บเลือดปริมาณ** 5 ml ใส่ EDTA tube **แล้วแช่ที่อุณหภูมิ** 2-8 **องศาเซลเซียส** เพื่อให้ผู้วิจัยนำไปตรวจยีน CYP3A5 ต่อไป

แพทย์ผู้สั่ง.....

( )

ผู้วิจัย.....

(เภสัชกรหญิงธราธร ไตรยวงค์)

เบอร์โทรศัพท์ติดต่อ 0895743712

ข้อควรปฏิบัติ: ในวันนัดหมาย ให้ท่านงดยากันชักในมื้อเช้าก่อนเจาะเลือด ภายหลังเจาะเลือดให้ท่านรับประทานยากันชักได้ตามปกติ โดยต้องนำยากันชักที่จะรับประทานมาเองด้วย

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX D

TaqMan® Drug Metabolism Genotyping Assays

(TaqMan® MGB probes, FAM™ and VIC® dye-labeled)

Assay ID: C_26201809_30

rs: 776746

Chemical and reagents

1. TaqMan® Drug Metabolism Genotyping Assays Mix
   Applied Biosystems USA
2. TaqMan® Genotyping Master Mix
   Applied Biosystems USA

Apparatus

1. MicroAmp Optical 96-well reaction plate
2. MicroAmp Optical Adhesive Film kit
3. Vortex mixer
4. Real-Time PCR system (Applied Biosystems 7500) USA

Supplies

1. Disposable gloves
2. Pipette tip 10 mcL (White) Scientific Plastics USA
3. Micropipette 10 mcL Eppendorf Germany

Overview

TaqMan® Drug Metabolism Genotyping Assays consist of a 20X mix of unlabeled PCR primers and TaqMan® MGB probes (FAM™ and VIC® dye-labeled). These assays are designed for the allelic discrimination of specific Single Nucleotide Polymorphisms (SNPs) and insertion/deletions (indels). Each assay enables scoring of both alleles of a biallelic polymorphism in a single well. All assays are optimized to work with TaqMan® Universal PCR Master Mix No AmpErase® UNG (P/N 4324018)† and with genomic DNA. These products utilize the modified thermal cycling parameters described below in Table B.

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Procedure

To prepare the reaction components for one reaction refer to the table below. The ABI PRISM® 7900HT Sequence Detection System uses 5 mcL in a 384 well plate. The Applied Biosystems 7300 and 7500 Real-Time PCR System and ABI PRISM® 7000 Sequence Detection System use 25 mcL reactions in a 96 well plate.

Table A. Allelic Discrimination PCR Reaction

Reaction Components	Volume/Well (10 mcL volume reaction) *	Final concentration
TaqMan® Universal PCR Master Mix (2 X)	5 mcL	1 X
20 X TaqMan® Drugmetabolism Genotyping Assay Mix	0.5 mcL	1 X
Genomic DNA (20 ng/mcL) **	1 mcL	-
dH_2O	3.5 mcL	-
Total	10 mcL	-

* If different reaction volumes are used, amounts should be adjusted accordingly.

** 3-20 ng of genomic DNA per well. All wells on a plate should have equivalent amounts of genomic DNA.

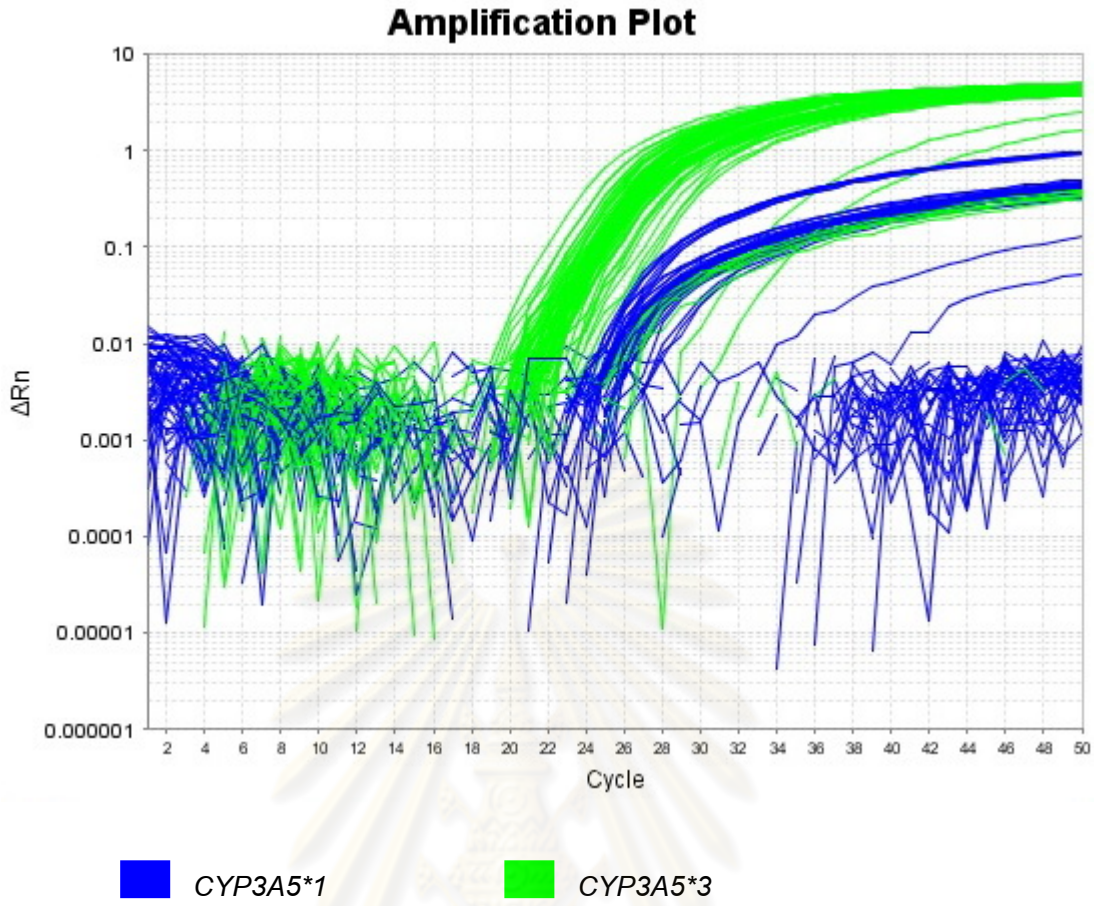
Table B. Thermal Cycler Conditions

Times and Temperatures		
Initial Steps	Denature	Anneal/Extend
HOLD	50 CYCLES	
10 min 95 ^{0}C	15 sec 92 ^{0}C	90 sec 60 ^{0}C

† Note: If using TaqMan® Universal Master Mix (P/N 4304437), add a 2 min @ 50°C HOLD step prior to the initial 10 min @ 95°C HOLD step.

Storage

Store between -15°C and -20°C; minimize freeze thaw cycles.



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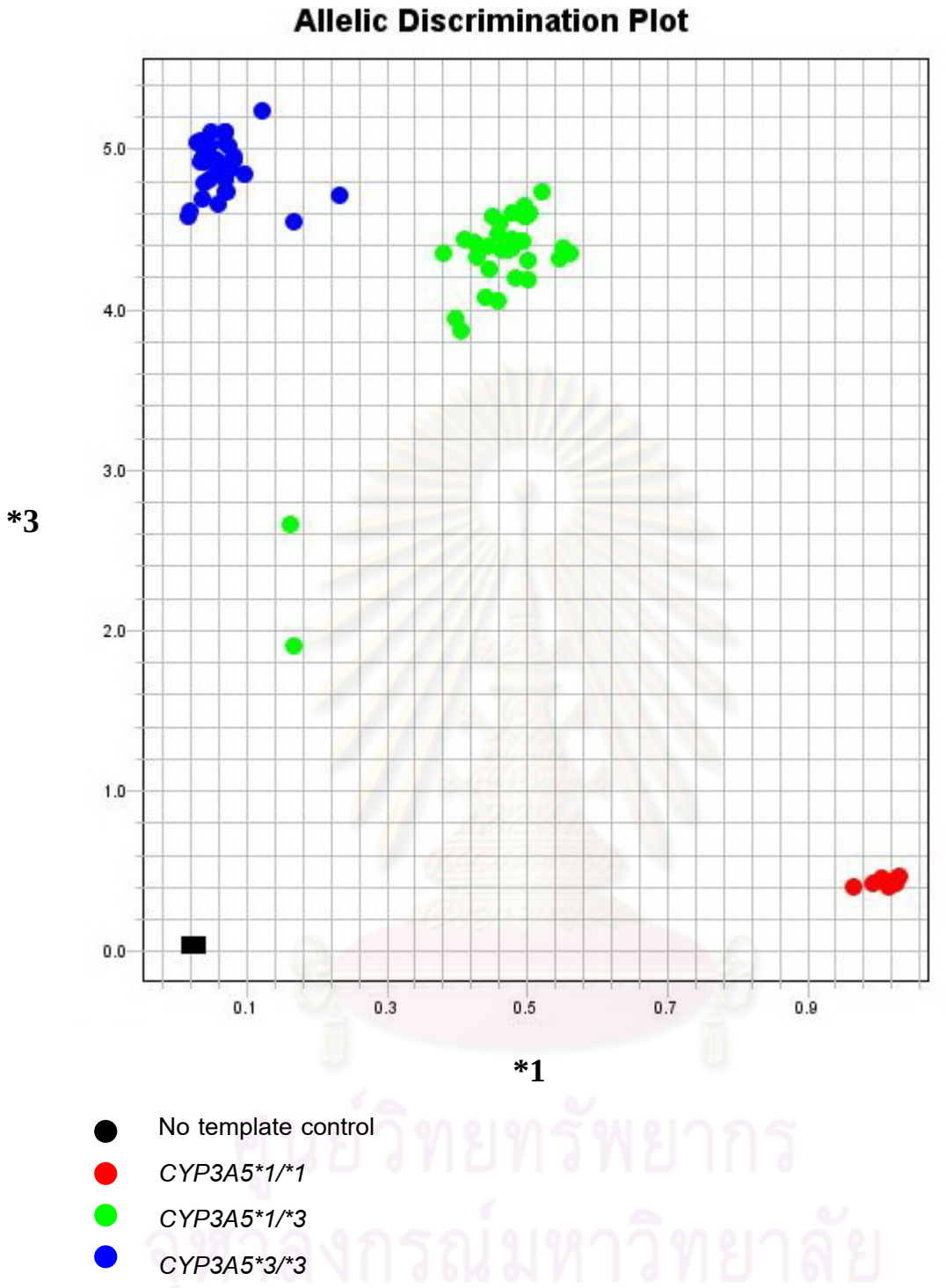

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APPENDIX E

Data of individual patient

Patient No	Gender	*CYP3A5*	Age (yr)	Weight (kg)	BMI (kg/m^2)	Combination	CBZ dose (mg/kg)	Other AED dose (mg/kg)	CBZ level (mg/L)	CBZ level/dose ratio (mcg/L/mg)	CBZ CL (L/day)	CBZ CL (L/kg/day)
1	Male	*1/*3	33.16	67.00	24.31	CBZ+PHT	20.90	4.48	7.62	5.44	128.61	1.92
2	Female	*3/*3	42.43	49.00	19.63	CBZ	16.33	0	10.20	12.75	54.90	1.12
3	Male	*1/*1	27.33	88.00	25.71	CBZ+VPA	9.09	11.36	9.70	12.13	57.73	0.66
4	Male	*1/*1	56.25	80.50	27.85	CBZ	9.94	0	8.60	10.75	65.12	0.81
5	Female	*3/*3	34.97	74.00	29.27	CBZ+VPA	18.92	23.65	9.00	6.43	108.89	1.47
6	Female	*1/*3	48.88	77.00	29.34	CBZ+PHT	5.19	3.90	4.20	10.50	66.67	0.87
7	Male	*3/*3	24.05	60.00	22.04	CBZ+VPA	13.33	16.67	7.80	9.75	71.79	1.20
8	Male	*3/*3	50.00	64.00	25.00	CBZ	12.50	0	7.40	9.25	75.68	1.18
9	Male	*3/*3	57.97	59.00	22.48	CBZ+PHT	11.86	4.24	9.20	13.14	53.26	0.90
10	Male	*1/*3	47.42	64.90	23.84	CBZ	12.33	0	10.40	13.00	53.85	0.83
11	Male	*1/*3	18.85	58.00	18.94	CBZ	10.34	0	8.70	14.50	48.28	0.83
12	Female	*3/*3	43.07	56.00	21.60	CBZ+PB	17.86	0.54	6.70	6.70	104.48	1.87
13	Female	*1/*1	57.35	60.00	24.34	CBZ	13.33	0	6.40	8.00	87.50	1.46
14	Male	*1/*3	40.13	55.00	20.70	CBZ	29.09	0	10.30	6.44	108.74	1.98
15	Male	*3/*3	45.45	54.40	22.64	CBZ	22.06	0	11.30	9.42	74.34	1.37
16	Female	*3/*3	47.11	64.20	26.72	CBZ+PB	6.23	1.87	5.00	12.50	56.00	0.87
17	Female	*1/*1	47.22	56.00	23.01	CBZ	14.29	0	9.00	11.25	62.22	1.11
18	Female	*1/*3	34.60	66.00	25.46	CBZ+VPA	15.15	15.15	9.20	9.20	76.09	1.15

Patient No	Gender	*CYP3A5*	Age (yr)	Weight (kg)	BMI (kg/m^2)	Combination	CBZ dose (mg/kg)	Other AED dose (mg/kg)	CBZ level (mg/L)	CBZ level/dose ratio (mcg/L/mg)	CBZ CL (L/day)	CBZ CL (L/kg/day)
19	Female	*1/*3	37.02	55.00	23.81	CBZ	9.09	0	9.30	18.60	37.63	0.68
20	Female	*1/*3	29.19	44.20	21.02	CBZ	9.05	0	4.80	12.00	58.33	1.32
21	Female	*1/*1	16.53	52.00	21.37	CBZ	15.38	0	8.00	10.00	70.00	1.35
22	Female	*3/*3	25.10	62.00	24.22	CBZ	19.35	0	8.10	6.75	103.70	1.67
23	Male	*3/*3	34.43	69.00	22.02	CBZ	17.39	0	11.90	9.92	70.59	1.02
24	Female	*1/*1	60.39	63.80	22.08	CBZ	9.40	0	3.70	6.17	113.51	1.78
25	Male	*1/*1	60.52	71.50	22.07	CBZ	11.19	0	8.20	10.25	68.29	0.96
26	Male	*3/*3	24.62	43.30	16.50	CBZ+VPA	32.33	39.26	8.50	6.07	115.29	2.66
27	Male	*3/*3	50.83	62.00	22.77	CBZ+VPA	12.90	24.19	6.60	8.25	84.85	1.37
28	Male	*1/*3	36.27	73.00	24.11	CBZ+VPA	21.92	20.55	9.40	5.88	119.15	1.63
29	Female	*3/*3	32.58	55.00	20.20	CBZ+PB	14.54	0.82	8.10	10.13	69.14	1.26
30	Female	*1/*3	29.30	65.00	27.06	CBZ+VPA	15.38	15.38	10.90	10.90	64.22	0.99
31	Female	*3/*3	69.77	74.00	28.91	CBZ	10.81	0	10.30	12.88	54.37	0.73
32	Female	*3/*3	56.41	55.00	24.12	CBZ	10.91	0	5.60	9.33	75.00	1.36
33	Female	*1/*3	40.08	47.30	19.94	CBZ+PB	16.91	2.54	5.50	6.88	101.82	2.15
34	Male	*1/*3	38.19	49.30	18.56	CBZ	16.23	0	10.20	12.75	54.90	1.11
35	Male	*3/*3	51.03	69.00	25.34	CBZ	14.49	0	10.50	10.50	66.67	0.97
36	Male	*1/*3	19.85	53.00	20.70	CBZ+VPA	30.19	30.19	9.30	5.81	120.43	2.27
37	Male	*1/*3	24.07	60.00	22.04	CBZ	13.33	0	8.40	10.50	66.67	1.11
38	Male	*3/*3	39.11	71.00	24.57	CBZ+PB	11.27	1.69	9.50	11.88	58.95	0.83
39	Male	*3/*3	61.69	70.00	23.39	CBZ+PB	20.00	0.86	8.80	6.29	111.36	1.59
40	Female	*1/*3	54.65	64.20	25.39	CBZ+VPA	9.35	15.58	8.30	13.83	50.60	0.79

Patient No	Gender	*CYP3A5*	Age (yr)	Weight (kg)	BMI (kg/m^2)	Combination	CBZ dose (mg/kg)	Other AED dose (mg/kg)	CBZ level (mg/L)	CBZ level/dose ratio (mcg/L/mg)	CBZ CL (L/day)	CBZ CL (L/kg/day)
41	Female	*1/*3	51.71	56.00	22.15	CBZ	14.29	0	9.90	12.38	56.57	1.01
42	Female	*1/*1	82.05	60.00	25.63	CBZ	6.67	0	8.60	21.50	32.56	0.54
43	Female	*3/*3	65.51	64.50	26.17	CBZ+VPA	9.30	15.50	7.90	13.17	53.16	0.82
44	Female	*3/*3	40.98	51.00	20.96	CBZ	23.53	0	9.70	8.08	86.60	1.70
45	Female	*3/*3	45.60	62.00	24.84	CBZ+PHT	6.76	5.41	7.30	9.13	76.71	1.24
46	Female	*3/*3	53.47	56.90	25.29	CBZ+VPA	14.06	17.57	7.10	8.88	78.87	1.39
47	Female	*1/*3	50.13	40.10	18.31	CBZ	19.95	0	9.90	12.38	56.57	1.41
48	Male	*3/*3	38.94	59.40	21.82	CBZ	6.73	0	4.40	11.00	63.64	1.07
49	Female	*1/*3	39.07	63.00	25.56	CBZ+VPA	15.87	23.81	7.70	7.70	90.91	1.44
50	Male	*3/*3	23.18	104.00	34.35	CBZ+VPA	15.38	11.54	9.30	5.81	120.43	1.16
51	Female	*3/*3	47.20	82.00	34.13	CBZ+PHT	9.76	3.96	4.90	6.13	114.29	1.39
52	Female	*1/*3	46.49	55.20	21.56	CBZ+PB	18.12	2.17	3.80	3.80	184.21	3.34
53	Male	*1/*3	18.35	56.50	20.02	CBZ+VPA	7.08	8.85	3.70	9.25	75.68	1.34
54	Male	*3/*3	35.09	81.00	31.64	CBZ+PHT	24.69	4.01	7.40	3.70	189.19	2.34
55	Female	*3/*3	47.61	60.00	21.77	CBZ	3.33	0	4.40	22.00	31.82	0.53
56	Male	*3/*3	54.95	89.00	30.80	CBZ	15.73	0	10.70	7.64	91.59	1.03
57	Female	*1/*3	27.74	60.00	22.04	CBZ	6.67	0	3.70	9.25	75.68	1.26
58	Female	*1/*3	37.78	42.00	17.26	CBZ	23.81	0	5.40	5.40	129.63	3.09
59	Female	*1/*3	39.26	67.90	24.94	CBZ+PB	17.67	2.65	6.60	5.50	127.27	1.87
60	Female	*3/*3	53.91	45.00	16.73	CBZ	17.78	0	8.60	10.75	65.12	1.45
61	Female	*1/*3	44.67	63.20	25.32	CBZ	18.99	0	7.70	6.42	109.09	1.73
62	Male	*3/*3	50.70	75.00	25.95	CBZ+PB	16.00	0.80	9.90	8.25	84.85	1.13

Patient No	Gender	*CYP3A5*	Age (yr)	Weight (kg)	BMI (kg/m^2)	Combination	CBZ dose (mg/kg)	Other AED dose (mg/kg)	CBZ level (mg/L)	CBZ level/dose ratio (mcg/L/mg)	CBZ CL (L/day)	CBZ CL (L/kg/day)
63	Female	*3/*3	46.92	56.00	23.31	CBZ+PB	7.14	2.68	3.70	9.25	75.68	1.35
64	Female	*3/*3	17.81	52.00	23.11	CBZ	11.54	0	6.90	11.50	60.87	1.17
65	Male	*1/*3	47.27	57.00	22.83	CBZ	10.53	0	5.40	9.00	77.78	1.36
66	Male	*1/*3	53.82	69.00	24.16	CBZ+PB	17.39	0.87	10.50	8.75	80.00	1.16
67	Female	*1/*3	38.33	51.00	22.37	CBZ	19.61	0	11.80	11.80	59.32	1.16
68	Male	*1/*3	64.90	60.00	24.65	CBZ+PHT	13.33	3.33	2.10	2.63	266.67	4.44
69	Male	*3/*3	20.69	67.70	22.88	CBZ	11.82	0	5.90	7.38	94.92	1.40
70	Female	*3/*3	56.30	80.00	37.53	CBZ+VPA	17.50	18.75	7.50	5.36	130.67	1.63
71	Female	-	17.56	50.00	-	CBZ+PHT	20.00	4.00	6.20	6.20	112.90	2.26
72	Female	-	15.06	47.00	-	CBZ+PHT	19.15	6.38	4.70	5.22	134.04	2.85
73	Female	-	15.92	37.00	-	CBZ+PHT	8.11	5.41	2.20	7.33	95.45	2.58
74	Female	-	35.77	45.00	-	CBZ+PHT	26.67	6.67	7.50	6.25	112.00	2.49
75	Female	-	37.27	43.00	-	CBZ+PHT	27.91	5.81	6.70	5.58	125.37	2.92
76	Female	-	14.39	70.00	-	CBZ+PHT	25.71	5.71	2.90	1.61	434.48	6.21
77	Female	-	35.29	71.80	-	CBZ+PHT	11.14	5.57	5.14	6.43	108.95	1.52
78	Male	-	14.13	52.00	-	CBZ+PHT	19.23	6.25	4.40	4.40	159.09	3.06
79	Female	-	28.96	53.00	-	CBZ+PB	30.19	1.13	10.80	6.75	103.70	1.96
80	Female	-	29.88	52.00	-	CBZ+PB	30.77	1.15	6.60	4.13	169.70	3.26
81	Male	-	13.87	82.00	-	CBZ+PB	12.20	1.46	5.90	5.90	118.64	1.45
82	Male	-	14.79	68.00	-	CBZ+PB	17.65	1.76	8.10	6.75	103.70	1.53

VITA

Miss Tharathorn Traiyawong was born on the 10th of May in 1980 at Mukdaharn. She graduated Bachelor degree in Pharmaceutical Science (1st Class Honours) from The Faculty of Pharmaceutical Science, Khonkaen University in 2004. She started to work as hospital pharmacist in Nongpok Hospital, Roi-et Province for two years and then work in Nongsung Hospital, Mukdaharn province in May 2006. She had been enrolled in a study program for Master degree of Pharmacy Practice Department, Faculty of Pharmaceutical Sciences, Chulalongkorn University since June 2008.

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