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]

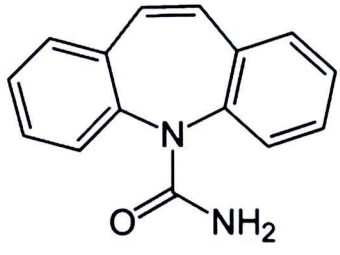

Figure 2: Chemical structure of CBZ.

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

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

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.

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 x Dose (mg/kg/day)$^{0.403}$ x TBW (kg) $^{0.697}$ x 1.45 ^{PHT} x 1.17 ^{PB} x 1.21 ^{VPA} x 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)* = $5.35[\text{DCBZ (mg/kg/day)}/15]^{0.591}$ x [1 + $0.414(\text{DPB(mg/kg/day)}/2)$] x $[\text{WT(kg)}/70]^{0.564}$ x 1.18^{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]

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

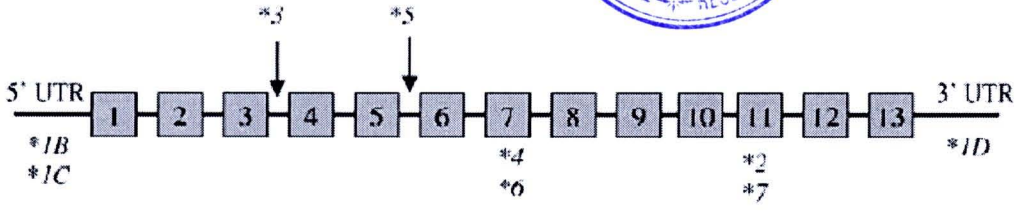

Figure 3: Distribution of mutation in the *CYP3A5* gene [30]

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]

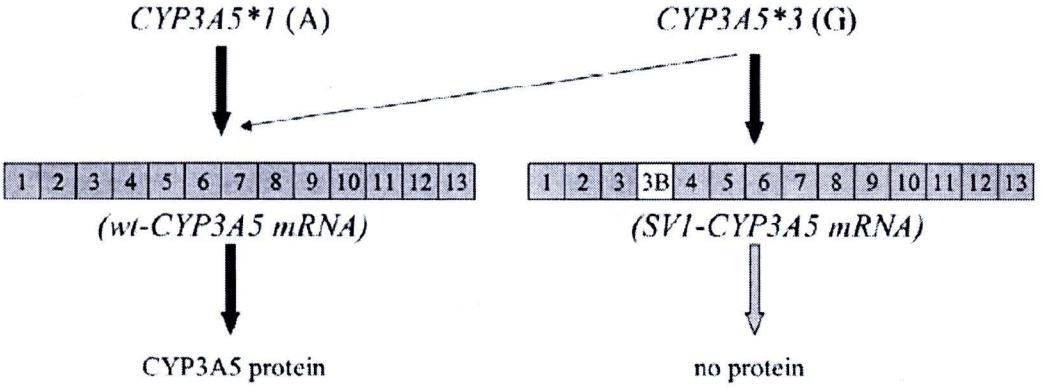

Figure 4: SNP in *CYP3A5* gene within intron 3 (A6986G) [30]

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

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)