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.

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 % 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.

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.