ความสัมพันธ์ระหว่างพารามิเตอร์ทางเภสัชจลนศาสตร์ของยาเฟนิทอยน์และยาคาร์บามาเซพีนในผู้ป่วยโรคลมชัก

นางสาว จันทิมา เมทนีธร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชกรรมคลินิก ภาควิชาเภสัชกรรม
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2550

THE RELATIONSHIP BETWEEN PHARMACOKINETIC PARAMETERS OF PHENYTOIN AND CARBAMAZEPINE IN EPILEPTIC PATIENTS

Miss Janthima Methaneethorn

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Clinical Pharmacy
Department of Pharmacy
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2007

Thesis Title	THE RELATIONSHIP BETWEEN PHARMACOKINETIC PARAMETERS OF PHENYTOIN AND CARBAMAZEPINE IN EPILEPTIC PATIENTS
By	Miss Janthima Methaneethorn
Field of Study	Clinical Pharmacy
Thesis Advisor	Associate Professor Duangchit Panomvana Na Ayudhya, Ph.D.
Thesis Co-advisor	Somchai Towanabut, M.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

Pornpen Pramyothin ..... Dean of the Faculty of Pharmaceutical Sciences

(Associate Professor Pornpen Pramyothin, Ph.D.)

THESIS COMMITTEE

Prapapuck Silapachote ..... Chairman

(Associate Professor Prapapuck Silapachote)

Duangchit Panomv ..... Thesis Advisor

(Associate Professor Duangchit Panomvana Na Ayudhya, Ph.D.)

Somchai Towanabut ..... Thesis Co-advisor

(Somchai Towanabut, M.D.)

Narat Kasettratat ..... Member

(Assistant Professor Narat Kasettratat)

Supasil Sra-ium ..... Member

(Supasil Sra-ium, M.Sc.)

จันทิมา เมทนีธร : ความสัมพันธ์ระหว่างพารามิเตอร์ทางเภสัชจลนศาสตร์ของยาเฟนิทอยน์และยาคาร์บามาเซพีนในผู้ป่วยโรคลมชัก. (THE RELATIONSHIP BETWEEN PHARMACOKINETIC PARAMETERS OF PHENYTOIN AND CARBAMAZEPINE IN EPILEPTIC PATIENTS) อ. ที่ปรึกษา: รศ. ดร. ดวงจิต พนมวัน ณ อยุธยา, อ. ที่ปรึกษาร่วม: นพ. สมชาย โตวณะบุตร. 87 หน้า.

วัตถุประสงค์: ยาเฟนิทอยน์และยาคาร์บามาเซพีนมักถูกใช้ร่วมกันเพื่อรักษาโรคลมชักในผู้ป่วยที่ไม่สามารถคุมอาการชักด้วยยาเพียงชนิดเดียว ยาทั้ง 2 ชนิดนี้มีการขจัดยาส่วนใหญ่ผ่านทางตับโดยใช้ระบบเอนไซม์ cytochrome P450 จึงมีความเป็นไปได้ว่าค่าการขจัดยาทั้งสองชนิดนี้จะมีความสัมพันธ์กัน การวิจัยนี้จึงต้องการหาความสัมพันธ์ระหว่างค่าการขจัดยาทั้ง 2 ชนิดนี้ตลอดจนหาสมการทำนายค่าการขจัดยาของกันและกัน นอกจากนี้การวิจัยนี้ยังมีวัตถุประสงค์เพื่อเปรียบเทียบความแตกต่างระหว่างค่าการขจัดยาทั้ง 2 ชนิดนี้ในผู้ป่วยกลุ่มที่ได้รับยาทั้งสองชนิดนี้ร่วมกันกับผู้ป่วยกลุ่มที่ได้รับยาเฟนิทอยน์หรือคาร์บามาเซพีนเพียงชนิดเดียว

วิธีการศึกษา: เป็นการศึกษาเชิงสังเกตแบบย้อนหลังร่วมกับแบบไปข้างหน้าในผู้ป่วยโรคลมชัก 48 คน โดยผู้ป่วยทั้งหมดที่เข้าเกณฑ์การศึกษาจะถูกแบ่งเป็น 3 กลุ่มตามชนิดของยาที่ผู้ป่วยได้รับ คือ กลุ่มที่ได้รับยาเฟนิทอยน์ร่วมกับคาร์บามาเซพีน, กลุ่มที่ได้รับยาเฟนิทอยน์เพียงชนิดเดียว และกลุ่มที่ได้รับยาคาร์บามาเซพีนเพียงชนิดเดียว จากนั้นจะเก็บข้อมูลระดับยาในเลือดของผู้ป่วยหลังจากได้รับยาในขนาดคงที่เป็นเวลาไม่น้อยกว่า 1 เดือน นำระดับยาที่ได้ไปคำนวณหาค่าอัตราสูงสุดของการเมแทบอลิซึมยาเฟนิทอยน์ (V_{max}) และอัตราการกำจัดยาคาร์บามาเซพีน (Cl_{CBZ}) นำค่าที่ได้มาทดสอบความสัมพันธ์และหาสมการทำนายค่าพารามิเตอร์ของกันและกัน และหาค่าความแตกต่างของ V_{max} และ Cl_{CBZ} ระหว่างกลุ่มผู้ป่วยที่ได้รับยา 2 ชนิดนี้ร่วมกันกับกลุ่มผู้ป่วยที่ได้รับยาเฟนิทอยน์หรือคาร์บามาเซพีนเพียงชนิดเดียว

ผลการศึกษา: V_{max} และ Cl_{CBZ} มีความสัมพันธ์กันเชิงเส้นตรงในระดับสูง โดยค่าสัมประสิทธิ์ความสัมพันธ์ (r) มีค่าเท่ากับ 0.828 (P = 0.001) เมื่อพิจารณาถึงผลความแตกต่างของพารามิเตอร์ระหว่างกลุ่มที่ได้รับยาร่วมกันกับกลุ่มที่ได้รับยาเดี่ยวพบว่า V_{max} ไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ (362.80 ± 51.78 และ 331.64 ± 71.93; P = 0.170) แต่ Cl_{CBZ} ระหว่างทั้งสองกลุ่มแตกต่างกันอย่างมีนัยสำคัญทางสถิติ (105.35 ± 35.45 และ 76.45 ± 26.62; P = 0.014)

สรุปผลการศึกษา: V_{max} และ Cl_{CBZ} มีความสัมพันธ์กันเชิงเส้นตรงในระดับสูง และสามารถนำค่า V_{max} มาทำนายค่า Cl_{CBZ} (หรือทำนายในทางกลับกัน) ซึ่งวิธีนี้จะช่วยประหยัดค่าใช้จ่ายในการตรวจวัดระดับยาของผู้ป่วยได้

ภาควิชา.....เภสัชกรรม.....ลายมือชื่อนิสิต.....จันทิมา เมทนีธร.....

สาขาวิชา.....เภสัชกรรมคลินิก.....ลายมือชื่ออาจารย์ที่ปรึกษา .....

ปีการศึกษา.....2550.....ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4876557733 : MAJOR CLINICAL PHARMACY
KEY WORD: PHENYTOIN / CARBAMAZEPINE / RELATIONSHIP

JANTHIMA METHANEETHORN: THE RELATIONSHIP BETWEEN PHARMACOKINETIC PARAMETERS OF PHENYTOIN AND CARBAMAZEPINE IN EPILEPTIC PATIENTS. THESIS ADVISOR: ASSOC. PROF. DUANGCHIT PANOMVANA AYUDHYA, Ph.D., THESIS CO-ADVISOR: SOMCHAI TOWANABUT, M.D., 87 pp.

Objective: Phenytoin (PHT) and carbamazepine (CBZ) are commonly prescribed together for treatment of epilepsy in patients who cannot control seizures with single drug. Both drugs are mainly metabolized via cytochrome P450, it is therefore possible that their pharmacokinetic parameters would be related. This study was designed to determine the correlation between pharmacokinetic parameters of the two drugs and provide regression equations to predict pharmacokinetic parameters of PHT from CBZ, and vice versa. Moreover, this study was also designed to compare the maximum rate of metabolism of phenytoin (V_{max}) and carbamazepine clearance (Cl_{CBZ}) when used as a monotherapy with those obtained from incombination therapy.

Method: A retro-prospective observational pharmacokinetic design was performed in 48 epileptic patients. They were categorized into 3 groups: PHT-CBZ combination therapy group, PHT monotherapy group and CBZ monotherapy group. One blood sample was collected from each patient at steady state condition. Serum drug concentrations of both drugs were determined. V_{max} and Cl_{CBZ} were calculated, correlation coefficients and regression equation were performed. Comparisons of V_{max} and Cl_{CBZ} between monotherapy group and combination therapy group were also determined to evaluate the pharmacokinetic interaction between two drugs.

Results: There was a significant linear correlation between V_{max} and Cl_{CBZ} ($r=0.828$; $P = 0.001$). V_{max} between monotherapy group and combination therapy group were not significantly different (362.80 ± 51.78 and 331.64 ± 71.93; $P = 0.170$) while Cl_{CBZ} were significantly different (105.35 ± 35.45 and 76.45 ± 26.62; $P = 0.014$) between monotherapy group and combination therapy group.

Conclusion: V_{max} could be accurately predicted from Cl_{CBZ} and vice versa. This would save cost for patients.

Department.....Pharmacy.....Student's signature.....Janthima Methaneethorn.....
Field of study.....Clinical Pharmacy.....Advisor's signature.....Duangchit Panomvana.....
Academic year .....2007 .....Co-advisor's signature.....Somchai Towanabut.....

ACKNOWLEDGEMENTS

A number of individuals contributed towards much of this work. I would like to take this opportunity to thank for contribution.

First of all, I would like to express my sincere gratitude to my thesis advisor, Associate Professor Duangchit panomvana Na Ayudhya, Ph.D. of the Department of Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for invaluable advice, guidance, encouragement, and kindness throughout the course of this study.

To my thesis co-advisor, Somchai Towanabut, M.D. Prasart Neurological Institute, for his full support, valuable advice and the time he devoted to helpful discussion through the course of this study.

Sincere thanks are expressed to all physicians, pharmacists, nurses, ethic committee and laboratory staffs at Prasart Neurological Institute for their helpful cooperation.

Thanks are also contributed to the members of the thesis committee for their valuable scrutinizing and discussion.

I am also grateful to all patients who participate in this study and all of those whose name have not been mentioned for helping me in anyway for this study.

Finally, I would like to express my indefinite gratitude to my parents, my sister and all of my friends for their encouragement, understanding and supporting throughout my graduate study.

CONTENTS

PAGE

Thai abstract iv
English abstract v
Acknowledgement vi
Contents vii
Lists of tables viii
Lists of figures x
Abbreviations xi
Chapter I Introduction 1
Chapter II Literature review 4
1. Review of epilepsy 4
2. Review of phenytoin 7
3. Review of carbamazepine 16
4. Review of assay methods 25
5. Review of pharmacokinetic interaction between phenytoin and Carbamazepine 27
Chapter III Patients and Methods 28
Chapter IV Result 34
1. Study population 34
2. Plasma drug concentrations 40
3. Pharmacokinetic parameters 43
4. Correlation between maximum rate of metabolism of phenytoin and carbamazepine clearance 46
5. Comparison of pharmacokinetic parameters between combination therapy group and monotherapy group 50
6. Therapeutic outcome 52
Chapter V Discussion 54
Chapter VI Conclusion 60
References 63
Appendices 71
Vitae 87

LIST OF TABLES

TABLE **PAGE**

Table 1 International classification of seizure type.....5
Table 2 Antiepileptic drug of choice in Thailand 6
Table 3 Drug-drug interaction involving phenytoin.....12
Table 4 The loading dose of phenytoin 13
Table 5 The maintenance dose of phenytoin 13
Table 6 A practical guide for incrementally increasing the phenytoin maintenance regimen (at stead state) for an adult 14
Table 7 Drug-drug interaction involving carbamazepine..... 21
Table 8 Dosage information for carbamazepine tablets 22
Table 9 The loading dose of carbamazepine 23
Table 10 The non-dose related adverse reaction of carbamazepine 24
Table 11 Characteristics of patients receiving combination therapy of phenytoin and carbamazepine.....36
Table 12 Characteristics of patients receiving phenytoin monotherapy 37
Table 13 Characteristics of patients receiving carbamazepine monotherapy..... 38
Table 14 Comparison of patients' characteristics 39
Table 15 Plasma concentrations of phenytoin-carbamazepine combination therapy group and phenytoin monotherapy group..... 41
Table 16 Plasma concentrations of carbamazepine combination therapy group and carbamazepine monotherapy group 42
Table 17 Pharmacokinetic parameters of phenytoin and carbamazepine in combination therapy group 44
Table 18 Pharmacokinetic parameters of phenytoin and carbamazepine in monotherapy group..... 45
Table 19 Relationships between maximum rate of metabolism of phenytoin and carbamazepine clearance 49
Table 20 Comparison of age, weight, dosage and maximum rate of metabolism of phenytoin between combination therapy group and monotherapy group.....51

TABLE **PAGE**

Table 21 Comparison of age, weight, dosage and carbamazepine clearance between combination therapy group and monotherapy group 51
Table 22 Therapeutic outcome of patients receiving phenytoin-carbamazepine combination therapy 53
Table 23 Therapeutic outcome of patients receiving carbamazepine monotherapy ……..53
Table 24 Therapeutic outcome of patients receiving phenytoin monotherapy ……53
Table 25 Overview of Maximum rate of metabolism of phenytoin obtained from different population studies ……...56
Table 26 Overview of carbamazepine clearance obtained from different population studies.....56

LIST OF FIGURES

FIGURE **PAGE**

Figure 1 The structure of phenytoin 7
Figure 2 The structure of iminodibenzyl 16
Figure 3 The structure of carbamazepine..... 16
Figure 4 Flow chart of the study……..32
Figure 5 Scatterplots of maximum rate of metabolism of phenytoin (mg/d) versus carbamazepine clearance (L/D) (n = 16) ……47
Figure 5 Scatterplots of maximum rate of metabolism of phenytoin (mg/kg/d) versus carbamazepine clearance (L/kg/d) (n = 16)……48

ABBREVIATIONS

τ	Dosing interval
° C	Celsius
AEDs	Antiepileptic drugs
bid	Twice daily
CBZ	Carbamazepine
Cl_{CBZ}	Carbamazepine clearance
CBZ-E	Carbamazepine-10.11 epoxide
Cal	Calibration
Conc	Concentration
CPS	Complex partial seizure
CPS 2^{nd} GTC	Complex partial seizure with secondary generalize tonic clonic
C_{ss}	The average steady-state
CYP450	Cytochrome P450
CYP2C9	Cytochrome P450 isoenzyme 2C9
CYP2C19	Cytochrome P450 isoenzyme 2C19
CYP3A4	Cytochrome P450 isoenzyme 3A4
CYP2C8	Cytochrome P450 isoenzyme 2C8
d	Day
EEG	Electroencephalographic
F	Bioavailability factor
FPIA	Fluorescence polarization immunoassay
Freq	Frequency
g	Gram
GTC	Generalized tonic-clonic
hr	Hour
HTN	Hypertension
kg	Kilogram
K_m	Michaelis constant
L	Liter
µg	Microgram

mg	Milligram
min	Minute
mL	Milliliter
no	Number
PHT	Phenytoin
pt	Patient
qid	Four times a day
QC	Quality control
R_a	Dosing rate
r	Correlation coefficient
SD	Standard deviation
TLE	Temporal lobe epilepsy
tid	Three times a day
V_d	Volume of distribution
V_{max}	Maximum rate of metabolism
yr	Year

CHAPTER I

INTRODUCTION

Background and Rationale

About 50-70% of newly diagnosed patients with epilepsy may achieve an adequate seizure control with a single antiepileptic drug. However, the one third of epileptic patients, usually those with difficult to control epilepsy, remain refractory to their antiepileptic monotherapy. Uncontrolled epilepsy is associated with excess mortality, cognitive and behavioral dysfunction, and social disadvantage. In those patients, combination therapy must be initiated to control seizure. If two drugs have been unsuccessful, the patients, then, are given antiepileptic drug polytherapy to improve effectiveness*(1-4)*. To achieve an effective combination drug therapy, rationale criteria have been defined, such as 1) additive efficacy 2) broader spectrum 3) decrease adverse effect and 4) complementary mechanism*(5)*.

Both carbamazepine and phenytoin are thought to prevent sustained repetitive firing due to their inhibition at sodium channel. However, several studies have suggested that carbamazepine in combination with phenytoin increase effectiveness in clinical practice*(5)*. Patients not responding to phenytoin may benefit from carbamazepine, and vice versa. Also, an in vivo model, animals not responding to phenytoin may response to carbamazepine. This can be explained by the assumption that carbamazepine and phenytoin bind to different types of the α-subunit of the sodium channel, consequently leading to better effectiveness in clinical practice when used in combination*(4)*.

Carbamazepine and phenytoin have narrow therapeutic index and need monitoring plasma drug level to achieve an optimize drug therapy*(6)*. Both drugs are mainly metabolized by the liver via cytochrome p 450 mixed function oxidase*(7-12)*, it is highly possible that phenytoin pharmacokinetic parameters could be predicted from those of carbamazepine, and vice versa, if so, it would save cost of drug level monitoring. Despite all these facts, this topic has never been investigated.

Furthermore, both drugs are enzyme inducers. Numerous studies have suggested that phenytoin significantly decrease carbamazepine plasma level by inducing carbamazepine clearance*(13-16)*. A study by Ichiko et al. has found that total and free serum carbamazepine levels fell by nearly 0.29 μg/ml and 0.33 μg/ml, respectively, for each 1 mg/kg/day of phenytoin intake*(17)*. In contrast, addition of carbamazepine to phenytoin has been reported to either decrease or increase serum phenytoin concentration. This can be due to an increase in phenytoin clearance or a competitive inhibition of phenytoin hydroxylation by carbamazepine*(18-21)*. However, in Thailand, there has been no study clarifing the differences between carbamazepine clearance when used as a monotherapy and in combination with phenytoin, or vice versa.

This study was therefore designed to 1) determine a correlation between pharmacokinetic parameters of both drugs and provide a regression equation to predict pharmacokinetic parameters of phenytoin from carbamazepine, and vice versa. 2) compare maximum rate of metabolism of phenytoin and carbamazepine clearance when used as a monotherapy to those obtained in combination therapy.

Objective

1) To determine relationships between maximum rate of metabolism of phenytoin and carbamazepine clearance in epileptic patients.

2) To compare maximum rate of metabolism of phenytoin or carbamazepine clearance between a phenytoin or carbamazepine monotherapy group and a phenytoin-carbamazepine combination therapy group.

Significances of the study

1) This study will provide an equation to predict patient- specific carbamazepine pharmacokinetic parameters from phenytoin pharmacokinetic parameters (and vice versa). This will help producing rapid attainment of carbamazepine (or phenytoin) target concentration in an individual patient who receives concomitant therapy of carbamazepine and phenytoin.

2) The differences of pharmacokinetic parameters of phenytoin and carbamazepine between monotherapy and combination therapy will be known.

CHAPTER II

REVIEW OF LITERATURE

Review of epilepsy

Epilepsy is a periodic recurrence of seizures with or without convulsions*(22)*. A seizure consists of a train of paroxysmal depolarizing shifts lasting for several seconds to minutes in which the patient may exhibit a variety of symptoms depending on the localizing of this discharge, the number of neurons involved, and the duration of the discharge*(23)*.

Epilepsy affects more than 50 million people worldwide, 5 million of whom have seizures more than once per month*(24)*. Data from the World Health Organization suggests that as many as 1 in 20 of the population may have an epileptic seizure during their lives and 1 in 200 will have epilepsy. These epilepsy figures exclude febrile convulsions which occur in about 5% of children*(25)*.

Epilepsy can be classified in several ways such as by seizure type, electroencephalographic (EEG) changes, etiology, pathophysiology, anatomy, or age. The 1981 classification scheme by the International League Against Epilepsy is widely accepted. In this scheme, seizures are divided into three groups: generalized, partial, and unclassifiable. Subdivisions of the classification are showed in Table 1. Generalized seizures are those in which epileptic discharges involve both cerebral hemispheres widely and simultaneously from the onset of the seizure, whereas partial seizures are those in which epileptic activity is confined to a focal area of the brain. The epileptic activity of partial seizures may spread to become generalized, in which case the seizure is said to be secondarily generalized*(25, 26)*.

Table 1 International classification of seizure type*(26)*

I. Partial seizures A Simple partial seizures 1. With motor signs: focal motor without march, versive, postural, phonatory 2. With somatosensory or special sensory symptomps: somatosensory, visual, auditory, olfactory, gustatory, vertiginious 3. With autonomic symptoms and signs (including epigastric sensation, pallor, sweating, flushing, piloerection and papillary dilation) 4. With psychic symptoms: dysphasic, dysmnesic, cognitive, affective, illusion, structured hallucinations B Complex partial seizures 1. Simple partial onset followed by impairment of conscious a. with the simple partial features followed by impaired consciousness b. with automatisms 2. With impaired consciousness at onset a. with impairment of consciousness only b. with automatisms C Partial seizure evolving to secondarily generalized seizures (tonic-clonic, tonic, or clonic) 1. Simple partial seizures evolving to generalized seizures 2. Complex partial seizures evolving to generalized seizures 3. Simple partial seizures evolving to complex partial seizures evolving to generalized seizures
II. Generalized seizures A Absence seizures 1. Absence seizure with impairment of consciousness only, mild clonic components, atonic components, tonic components, automatism, autonomic components 2. Atypical absence B Myoclonic seizures C Clonic seizures D Tonic seizures E Tonic-clonic seizures F Atonic seizures (combinations of the above may occur e.g. B and F, B and D)
III. Unclassifiable epileptic seizures

Treatment of seizure

Once it has been determined that recurring seizures are not the result of a correctable problem, antiepileptic drug therapy should be initiated*(24)*. Antiepileptic drugs (AEDs) for seizure types used in Thailand are shown in Table 2. Treatment of epilepsy begins with the use of a single agent as monotherapy*(5)*. Usually a drug given as monotherapy is titrated to a maximally tolerated dose before the decision is made to try another drug. If two drugs have thus been unsuccessful as monotherapy, the patient is given AED polytherapy in an

attempt to improve effectiveness*(4)*. Other options, including second-line drugs, or surgery should be considered when standard drugs have failed*(24)*.

Table 2 Antiepileptic drug of choice in Thailand*(27)*

Seizure type	**First-line**	**Alternative**
Generalized tonic-clonic	Valproic acid, Phenytoin, Carbamazepine Carbamazepine	Clonazepam, clobazam
Partial	Carbamazepine Carbamazepine	Clonazepam, Clobazam
Absence	Valproic acid	Clonazepam, Acetazolamide
Myoclonic, Atonic, Tonic	Valproic acid	Clonazepam, Nitrazepam
Infantile spasms	ACTH, Prednisolone, Vigabatrin, Valproic acid	Nitrazepam, Clonazepam, Clobazam

Review of phenytoin

1. Chemistry

Phenytoin is an antiepileptic drug whose structure is related to that of barbiturates, but has a five-membered ring. The chemical name is 5,5-diphenyl-2,4-imidazolidinedione and the chemical structure is shown in Figure 1. It occurs as a white crystalline material with a molecular weight of 252.3 with an empirical formula $C_{15}H_{12}N_2O_2$. Practically insoluble in water, phenytoin acid has a pKa value of 8.06-8.33. Its sodium salt is a white hygroscopic powder with a molecular weight of 274.3, so that 100 mg of it is equivalent to 91.8 mg of phenytoin acid. Unlike acid form, phenytoin sodium is freely soluble in water. Its aqueous solutions gradually absorb carbon dioxide, and the drug undergoes partial hydrolysis to phenytoin.*(8, 28)*

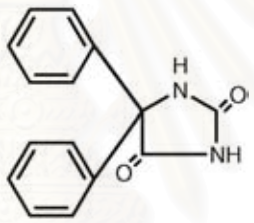


Figure 1 The structure of phenytoin.

2. Indications

Phenytoin is used to prevent generalized tonic-clonic seizures and partial seizures with or without generalization. It is not recommended for the treatment of pure absence (petit mal) seizures since the drug may increase the frequency of these seizures*(8)*. In addition, phenytoin may be used for the prevention of seizures secondary to neurosurgery or head trauma *(29, 30)*. The drug may also be used parenterally in the treatment of status epilepticus*(31)*. For non-FDA approved indications, phenytoin may be useful in the treatment of ventricular arrhythmias and convulsions associated with preeclampsia. Moreover, it may have beneficial effects in the treatment of trigeminal neuralgia in some patients*(28, 32)*.

3. Mechanism of action

Phenytoin exerts its anticonvulsant effect mainly by reducing post-tetanic potentiation and limiting the spread of seizure activity. Post-tetanic potentiation is a physiological synaptic mechanism that enhances the channeling of nerve impulses of normal activity. Reducing the post-tetanic potentiation at each synaptic site in a highly active polysynaptic pathway results in compound attenuation of the impulse. Thus it can prevent the spread of seizure activity yet has little or no effect on normal functions. These effects are mediated by the blocking of sodium channel in neural cell membrane.*(3, 4, 8, 12)*

Phenytoin at high concentration also blocks intracellular uptake of calcium by presynaptic organelles and mitochondria in synaptosome preparations. This may increase excitability temporarily and may account for the increase in seizures which are seen in some patients with high phenytoin plasma levels*(8, 12)*.

4. Pharmacokinetic

a. Absorption

Phenytoin is administered orally and parenterally. The bioavailability of phenytoin is variable with different formulations and certain foods or drugs in the intestinal tract, generally it is about 90%.*(12)*. Prompt release capsules are rapidly absorbed and generally reach peak serum concentrations in 1.5 - 3 hours, while extended phenytoin sodium capsules are more slowly absorbed and generally reach peak serum concentration in 4-12 hours*(28)*. However, extremely large oral doses (for example 1600 mg) required more time to reach a peak concentration. Phenytoin sodium contains 8% less phenytoin than does phenytoin acid. Thus, adjustment the dose to account for this 8% difference is necessary for patients who switch dosage forms*(8)*.

b. Distribution

At the pH of plasma (7.4), phenytoin exists predominantly in an unionized form and distributes freely through the body into cerebrospinal fluid, saliva, semen, gastrointestinal fluid, and bile. Phenytoin concentrations in the cerebrospinal fluid usually equal the

unbound concentrations of the drug in plasma, while those in saliva can be somewhat higher. Phenytoin crosses the placenta and is distributed into breast milk. Fetal plasma concentrations are similar to maternal concentrations. The apparent volume of distribution ranges from 0.5-0.8 L/kg in adults and from 0.8-1.2 L/kg in children *(8, 12)*.

Phenytoin is rapidly and reversibly bound to proteins. The percent bound to plasma proteins is about 90%, but less in patients with renal failure (due to displacement of the drug at its protein binding sites by uremic products), chronic liver disease, pregnancy, hypoproteinemia and other conditions which cause reduced serum albumin levels*(8, 12, 28)*.

c. Elimination

Metabolism

Phenytoin is eliminated almost entirely by metabolism. The metabolism of phenytoin involves extensive hydroxylation, mostly at the C5 position of the hydantoin ring via an arene oxidation, resulting in an arene oxide of phenytoin which converts spontaneously into p-hydroxyl-phenylhydantoin (HPPH). These metabolites, then, are major mediated by cytochrome P450C 2 subfamily isoenzymes CYP2C9 (90% of the dose) and minor mediated by CYP2C19 (10% of the dose). A similar portion of the arene-oxide becomes phenytoin-epoxide and this quickly converted to a dihydrodiol (DHD) by epoxide hydrolase. In therapeutic doses, approximately 1% is excreted unchanged in urine*(8, 10, 33)*

The capacity to metabolize phenytoin is highly variable among individuals because of their genetic backgrounds and environmental factors. In general, the efficiency of phenytoin metabolism is low in neonates, increase considerably in children, in adolescent and in pregnancy, and decrease again with advance age*(8, 12, 33-39)*.

Elimination parameters

Clearance values for phenytoin varies among individuals, ranging from 0.015 to 0.065 L/kg/h. Children tend to have higher values than adults. The published values for the maximum rate of metabolism (V_{max}) have ranged from 6-16 mg/kg/d (average 7 mg/kg/d) and those for the Michaelis constant (K_m) from 3-30 mg/L (average 4 mg/L). The V_{max} values increase in pregnancy and are usually higher in children than in adults. Phenytoin

half life can range from 7-42 hours (average 22 hours), depending on the drug dose and the drug's preexisting plasma concentration*(8)*.

5. Pharmacokinetic model

An open one-compartment pharmacokinetic model with a single capacity limited metabolism may provide an adequate description of phenytoin pharmacokinetics. Below the saturation point, phenytoin is eliminated in a linear, first-order process. Above the saturation point, elimination is much slower and occurs via a zero-order process. Because of this saturable mechanism, small increases in dose can produce large increases in plasma concentrations.

The model that appears to fit the metabolic pattern for phenytoin elimination is the one originally proposed by Michaelis-Menten. Equation below is the Michaelis-Menten equation.

$$R_a = \frac{(V_{max})(C_{ss})}{(K_m) + (C_{ss})}$$

In this equation R_a is the dosing rate, V_{max} is the maximum rate of metabolism (metabolic capacity), K_m, Michaelis-menten constant, is the plasma concentration at which the rate of metabolism is one-half the maximum and Css is the average steady-state phenytoin concentration. The individual values of V_{max} and K_m appear to be constant over time, but there is considerable interindividual variability*(40, 41)*.

6. Drug interaction

The pharmacokinetic interactions between phenytoin and other drugs are attributable largely to the following mechanisms:

Absorption

Oral absorption of phenytoin can be reduces by any of the followings: antacids containing magnesium or aluminium, calcium salts, antineoplastic agents, sulcrafate and enteral feeding products. This reduction can result from a change in either the rate or extent of absorption. Separating the administration of phenytoin and enteral feeding products, antacids, or calcium salts by at least 2 hours will help avoid this interaction*(42-44)*.

Plasma protein binding

A large number of strongly protein binding drugs, such as salicylates, ibuprofen, sulfadiazine and valproate can displace phenytoin from plasma protein binding sites, leading to an increased phenytoin unbound fraction which may lead to phenytoin toxicity, however, the total phenytoin concentration is unlikely to be altered or will be reduced due to the increase of phenytoin clearance as the free drug reaches the liver*(42, 43)*

Metabolism

Phenytoin clearance can be affected by drugs that either stimulate or inhibit hepatic microsomal enzymes. The role of CYP2C9 and CYP2C19 in phenytoin metabolism helps to anticipate the extent and intensity of its interactions. Inhibitors or co-substrates of CYP2C9 which catalyze the biotransformation of the larger part of a phenytoin dose, may cause extensive rises in plasma phenytoin levels. Inhibitors or co-substrates of CYP2C19, which handle a small portion of phenytoin dose, might be expected to cause smaller changes. In contrast, drugs that stimulate hepatic metabolism of phenytoin will lower its plasma level*(8, 42, 43, 45, 46)*.

Phenytoin itself is an enzyme inducer, thus it can stimulate hepatic microsomal enzyme. This action enhances the clearance of other drugs metabolized via this pathway*(8)*. Drugs that interact with phenytoin are shown in Table 3

Table 3 Drug-drug interaction involving phenytoin*(8, 33, 43-47)*

Drugs reported to evaluate phenytoin levels
A. *Inhibitors/co-substrates of CYP2C9* amiodarone, cotrimoxazole, fluconazole, metronidazole, miconazole, phenylbutazone, propoxyphene, warfarin, celecoxib, diclofenac, ibuprofen,naproxen, piroxicam, valproic acid, fluoxetine, fluvoxamine
B. *Inhibitors/co-substrates of CYP2C19* diazepam, felbamate, fluoxetine, imipramine, phenobarbital, propranolol, cimetidine, topiramate, fluvoxamine, omeprazole, amitriptylline citralopram, ticlopidine, clomipramine,
C. Mechanism not yet established but likely to be inhibit of CYP2C isoenzymes chloramphenical, dicoumarol, diltiazem, ethanol (acute intake), isoniazid, trazodone, ranitidine, sulfonamides, allopurinol
Drugs reported to lower phenytoin levels
D. *Interfere with phenytoin absorption, protein binding, induction of metabolism or combination mechanism* antacids, antineoplastics (abs), dexamethasone, ethanol, folic acid, phenobarbital, rifampicin, clonazepam, nitrofurantoin, chlordiazepoxide (met), salicylates (pb)
Drugs reported to be affected by phenytoin
E. *Phenytoin reduces the level of the following drugs, probably by inducing P450 isoenzymes* clonazepam, cyclosporine, dexamethasone, dicoumarol, digitalis, disopyramide, doxycycline, folic acid, haloperidol, lamotrigine, methadone, nortriptyline, oral contraceptives, pethidine, praziquantel, prednisolone, primidone, quinidine, theophylline, thyroxine, valproate, vitamins D and K, zonisamide
F. *Phenytoin may increase the level of the following drugs* chloramphenical, phenobarbital, warfarin

abs = absorption, met = metabolism, pb = protein binding

7. Dosage regimen and clinical application

Loading dose

Loading is indicated when the risk of seizures is so great that adequate serum levels of the drug must be reached rapidly. Depending on the degree of emergency, a loading dose can be accomplished with intravenous phenytoin or with oral phenytoin*(48)*. Intramuscular route should be avoided because the absorption is slow and erratic*(40)*. If the loading dose is to be given by the intravenous route, it should be administered slowly to avoid cardiac toxicities (The maximum intravenous infusion rate in neonates, children, and adolescents is 1-3 mg/kg/min and no more than 50 mg/min in adults). If the loading dose is to be given

orally, it usually divided (three increments of approximately 5 mg/kg) and administered at 2-hour interval to avoid the gastrointestinal distress associated with large doses of phenytoin and to decrease the time required for the concentration to peak*(49, 50)*. The recommended loading dose is shown in Table 4

Table 4 The loading dose of phenytoin *(50)*

Age	Loading dose (mg/kg)
Neonates and infants (<1 year)	15-20
Children (1-<12 years)	15-18
Adolescents (≥ 12 years), adults, and geriatrics	15-18

Maintenance dose

A useful maintenance dose formula yields the dose ordinarily needed to achieve a specified serum level or maintain it after loading. The initial maintenance dose is shown in Table 5. The steady-state serum phenytoin concentration may acheive in 5-14 days. Because of the capacity-limited metabolism, dose adjustments should be small increment base on clinical response*(49)*. A practical guide for incrementally increasing the phenytoin maintenance regimen (at stead state) for an adult is shown in Table 6.

Table 5 The maintenance dose of phenytoin*(50)*

Age	Maintenance dose (mg/kg/day)
Neonates (<4 weeks)	3-5
Infants (4 weeks-<1 year)	4-8
Children (1-<12 years)	4-10
Adolescents (12-<18 years)	4-8
Adults and geriatrics (≥ 18 years)	4-7

Table 6 A practical guide for incrementally increasing the phenytoin maintenance regimen (at stead state) for an adult*(48)*

Serum level (µg/ml)	Increase daily maintenance dose (mg)
<7	100
7-11	50
≥12	30

Optimum control without clinical sign of toxicity occurs more often with serum levels between 10-20 µg/ml, although there are patients who are seizure free at plasma concentration outside the usually accepted therapeutic range*(40, 49)*. Schmidt and Haenel reported 21% of the patients were controlled with plasma concentrations of less than 10 µg/ml and 30% of the patients required plasma concentrations more than 20 µg/ml for seizure control*(51)*. The individual's effective level depends mainly on the type and the severity of the individual epilepsy, as indicated by the seizure frequency at the onset of the epilepsy or prior to the treatment*(8)*.

8. Adverse reaction and Toxicity

a. Acute toxicity

The acute systemic toxicity of phenytoin is characterized by central nervous system effects, such as nystagmus, ataxia, dystonia, dizziness, drowsiness, and lethargy. These adverse reactions increase as serum concentrations of phenytoin increase*(8)*. Far-lateral nystagmus occurs in some patients with phenytoin concentration below 15 µg/ml but is usually observed in the majority of patients at concentrations exceeding 20 µg/ml. Ataxia and diminished mental capacity are usually observed when phenytoin concentrations are above 30 and 40 µg/ml, respectively*(40, 49)*. As the blood concentration exceeds 50 µg/ml, extreme lethargy and comatose states may result*(28)*. However, there may be a significant variation for individual's toxic level.

Parenteral phenytoin, when administered by the intravenous route, can produce bradycardia, hypotension, and widening of the QRS and QT intervals. These cardiovascular symptoms can be avoided by injecting the intravenous solution slowly. Infusion rate of

phenytoin should not exceed 50 mg/min in adults and 1-3 mg/kg/min in neonates, children, and adolescents*(50)*.

b. Idiosyncratic adverse effects

Adverse dermatological reactions, such as maculopapular rash, erythema multiforme, and exfoliative dermatitis, are the most frequent idiosyncratic effects of phenytoin, and occur in 5-10% of patients*(8)*. Phenytoin may produce lymphadenopathy associated with fever, rash, and liver involvement; however, this is an uncommon occurrence. In addition, various blood dyscrasis can develop from phenytoin use. These include thrombocytopenia, leucopenia, agranulocytosis, and pancytopenia. Macrocytosis and megaloblastic anemia which usually respond to folic acid therapy may also occur. Adverse GI effects of phenytoin therapy include nausea, vomiting, constipation, abdominal pain, and anorexia also have been reported*(28, 52, 53)*.

c. Chronic toxicity

Gingival hyperplasia occurs in up to 40% of patients taking phenytoin and, if present, is evident in the first few months of therapy. It occurs more often in children and adolescents and can be minimized by good oral hygiene and gum massage. Hypertrichosis, coarsening of the facial features can also occur in some patients. In addition, osteomalacia secondary to phenytoin's interference with vitamin D metabolism may occur in patient receiving long-term therapy with phenytoin*(8, 28)*.

Review of carbamazepine

1. Chemistry

Iminodibenzyl, shown in Figure 2, may be considered the precursor of carbamazepine. Considerable anticonvulsant effect occurs when a carbamyl group is added at the 5 position of iminodibenzyl. The carbamyl side chain combined with iminostilbene, a structure similar to iminodibenzyl but having a double bond between the 10 and 11 positions, shows the strongest anticonvulsant properties and become known as carbamazepine (Figure 3). The double bond between positions 10 and 11 in the carbamazepine molecule is somewhat unstable and provides a site of action for the biotransformation enzyme. Carbamazepine has a molecular weight of 236.26 with an empirical formula $C_{15}H_{12}N_2O$. It occurs as a white to off-white lipophilic material which dissolves in certain organic solvents such as ethanol, chloroform, and acetone but practically insoluble in water*(9, 54, 55)*.

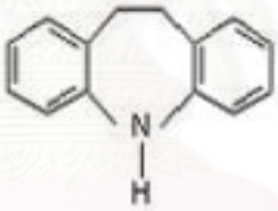


Figure 2 The structure of iminodibenzyl

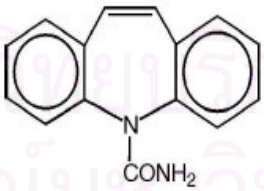


Figure 3 The structure of carbamazepine

2. Indications

In the treatment of epilepsy, carbamazepine is effective in preventing simple and complex partial seizures with or without secondary generalization. It also effective in preventing generalized tonic-conic seizures of generalized epilepsy, but is not useful in treating absence, atonic and myoclonic seizures*(9)*. In addition, carbamazepine is also used

in the treatment of neuropathic pain such as trigeminal neuralgia and has been reported useful in managing various involuntary movement disorders such as chorea, dystonia, restless legs and diaphragmatic flutter*(56)*. In clinical trials it has proved useful in managing benzodiazepine and alcohol withdrawal*(57)*. In psychiatry, carbamazepine has been shown to be effective in the treatment of bipolar disorder (manic-depressive psychosis) and explosive aggression*(55)*. Finally, the drug has also be used for its antidiuretic effects in the management of neurogenic diabetes insipidus; however, other less toxic agents are available*(58)*.

3. Mechanism of action

The basis of the antiepileptic action of carbamazepine is accepted to be the blockade of voltage-gated sodium channels in cell membranes. Like phenytoin, carbamazepine inhibits sustained repetitive firing and reduces post-tetanic potentiation of synaptic transmission in the spinal cord. The effect may explain its ability to limit the spread of seizures*(9)*.

4. Pharmacokinetic

a. Absorption

Carbamazepine is marketed only for oral use. Absorption of carbamazepine from gastrointestinal tract is slow and variable. Following chronic oral administration of carbamazepine 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*(55)*.

The absolute bioavailability of carbamazepine in man has not been determined, because a preparation for intravenous injection is not available. However, based on the recovery of radio-labeled carbamazepine in urine and feces, the oral bioavailability of the drug is more than 70%*(15)*. The size of the administered dose may play a role in the rate and extent of absorption of carbamazepine. A delay in peak time as well as an apparent reduced absorption when daily dose higher than 20 to 25 mg/kg are employed*(7)*.

b. Distribution

Carbamazepine is lipophilic and widely distributed in the body. It appears in cerebrospinal fluid, brain, duodenal fluids, bile, and saliva. Carbamazepine has been shown to accumulate in the fetus after crossing the placenta, and it is distributed in to breast milk. Cerebrospinal fluid concentrations of carbamazepine may range from 17% to 31% of those in plasma. Similar data have been reported for amniotic fluid, whereas breast milk concentrations are about 60% of total plasma drug levels*(7)*.

Carbamazepine and its 10, 11-epoxide are bound to α_1-acid glycoprotein and to albumin. In vitro and in vivo studies with healthy volunteers have shown that carbamazepine at concentrations of 1-50 μg/ml is 75-90% bound to plasma protein. The percentage of protein binding may be decrease in newborns and in children. Its epoxide metabolite is about 50% bound to plasma protein. Carbamazepine has a free fraction of approximately 0.2 to 0.3. The increase in free carbamazepine concentrations in patients with hepatic or uremic disease has no practical importance, even if significant on a statistical basis. This may be because of the low affinity constant of carbamazepine and the amount of free fraction in normal conditions*(7, 9, 54, 55)*.

The volume of distribution (V_d) of carbamazepine, calculated in most cases assuming complete bioavailability, may range from 0.8 to 2.0 L/kg. This variability is probably due to alterations in plasma protein binding and calculation of the V_d from oral administration data. On average, the V_d for carbamazepine is approximately 1.4 L/kg. These figures have been calculated assuming complete bioavailability of the drug and the real figures may therefore be lower*(7, 15, 59)*.

c. Elimination

Metabolism

Carbamazepine is mainly metabolized in the liver by four major pathways: epoxidation of the 10,11 double bond of the azepine ring, 40%; hydroxylation of the six membered aromatic rings, 25%; direct *N*-glucuronidation at the carbamoyl side chain, 15%; and substitution of the six-membered rings with sulfur-containing groups, 5%. About 3% of an oral carbamazepine is excreted in urine as the intact molecule*(11)*.

The epoxidation pathway leading to carbamazepine-10,11-epoxide is catalysed by the microsomal cytochrome P450 isoenzymes CYP3A4, and to a lesser extent, CYP2C8. The epoxide undergoes hydration catalysed by microsomal epoxide hydrolase to form carbamazepine-10,11-trans-diol. The trans-diol is then conjugated to form a 10-hydroxy-o-glucuronide in a reaction catalysed by UDP-glucuronosyl transferase. The diol and its conjugate are then excreted in urine*(9)*.

The metabolism of carbamazepine is autoinducible in humans. A major part of the autoinduction is a result of the formation of the trans-carbamazepine-diol. The formation of 9-hydroxymethyl-10-carbamoyl acridan is also induced, but this is a quantitatively less important metabolic reaction*(60)*. The autoinduction of carbamazepine metabolism is already apparent during the first dose of the drug and seems to be complete during the first 3 to 5 weeks of treatment*(13)*.

Elimination Parameters

- Half – life

The first dose half–life of the drug in humans is approximately 30-35 hours. The half–life at steady state is approximately 15 hours in adult patients receiving carbamazepine monotherapy and approximately 10 hours in patients receiving other enzyme-inducing antiepileptic drugs concurrent. Children have shorter half–life than adults with reported steady state half–life of 4-12 hours*(59)*.

- Clearance

After chronic intake of carbamazepine monotherapy, the clearance of the drug is approximately 0.064 L/kg/hr while, in patents receiving other enzyme-inducing anticonvulsants, the clearance is approximately 0.1 L/kg/hr. The clearance decreases with age in childhood and increasing body weight. In children receiving carbamazepine monotherapy, the average clearance is 0.11 L/kg/hr*(9, 59)*.

5. Pharmacokinetic model

After single oral doses of carbamazepine, the absorption is fairly complete and the elimination half-life is about 30-35 hours. During multiple doses, the half-life decreased to

10-20 hours, probably due to autoinduction of the oxidative metabolism of the drug. However, at steady state, the elimination of carbamazepine follows dose-dependent first order kinetics model in which the value of clearance increases with increasing dosage of carbamazepine*(15)*.

6. Drug interaction

Concomitant drugs may affect pharmacokinetics of carbamazepine by modifying its absorptions, plasma protein binding, or metabolism. The most important interaction occurs at the site of metabolism.

Absorption

The bioavailability of carbamazepine may be decreased by activated charcoal, enteral feeding, and some cytotoxic drugs such as doxorubicin, cisplatin*(9, 16)*.

Plasma protein binding

Salicylates, valproic acid, and verapamil was found to displace carbamazepine from plasma protein binding sites, but the magnitude of these interactions are generally small and without clinical significance*(9, 16, 61)*.

Metabolism

Drugs that inhibit metabolism of carbamazepine will increase its plasma level. Conversely, drugs that stimulate hepatic metabolism of carbamazepine will lower its plasma level. The first set of inhibition mechanism involves the inhibition of CYP3A4, the main enzyme system responsible for 30-50% of total carbamazepine clearance. A second set of clinically important interactions is mediated by the inhibition of epoxide hydrolase, the enzyme responsible for the conversion of CBZ-E to the inactive diol. This mechanism results in increased serum CBZ-E concentrations, which may cause carbamazepine intoxication in the absence of any change in parent drug concentration*(16)*.

Carbamazepine probably induces cytochrome P450 isoforms and thus enhances the biological oxidations of numerous co-administered drugs. Drugs that interact with carbamazepine are shown in Table 7.

Table 7 Drug-drug interaction involving carbamazepine*(9, 16, 46, 61)*

Drugs reported to effect carbamazepine metabolism
A. Drugs which inhibit carbamazepine metabolism
remacemide, progabide, acetazolamide, clobazam, fluoxetine, lithium fluvoxamine,certraline, imipramine, cimetidine , erythromycin, nicotinamide clarithromycin, isoniazid, quinidine, metronidazole, naproxen, verapamil, diltiazem, , danazol, influenza vaccine, lamotrigine[a], valproate[a]
B. Drugs which induce carbamazepine metabolism
Phenytoin, phenobarbitone, felbamate, cyclosporine A, progabide, zonisamide
Drugs reported to be affected by carbamazepine
C. Carbamzepine reduces the level of the following drugs, probably by inducing P450 isoenzymes
Alprazolam, clobazam, clozapine, fluconazole, itraconazole, doxycycline, ketoconazole, coumarin, cyclosporine A, dexamethasone, doxepine, warfarin erythromycin, oral contraceptive, haloperidol, lamotrigine, mebendazole, midazolam, nimodipine, nortriptyline, opiates, praziquantel, prednisolone, rifampicin, trazodone, theophylline, thioridazine, valproate, vancuronium,
D. Carbamazepine may increase the level of the following drugs
Phenytoin

[a] Lamotrigine and valproic acid do not alter plasma carbamazepine concentration but raise plasma CBZ-E concentration, probably due to inhibition of epoxide hydrolase

7. Dosage regimen and clinical applications

The therapeutic range established for the treatment of seizures is 4-12 mg/L when the drug is used in monotherapy. However, some patients will suffer from disturbance of gait, headache, dizziness, and disturbance of vision with plasma concentration of 8.5-10 mg/L. For this reason, many clinicians prefer to use a therapeutic range of approximately 4-8 mg/L*(59, 62)*.

Dosage of carbamazepine must be carefully and slowly adjusted according to individual requirements and response, since rapid increases may cause patients to develop adverse GI or nervous system effects. It is generally started at one-fourth to one-third of the expected maintenance dose to allow for completion of the autoinduction of metabolism and for tolerance to CNS effects. The dose is titrated to a balance between occurrence of side effects and the cessation of seizures*(63)*. Dosage information for carbamazepine tablets is

shown in Table 8. However, in critically ill patients, loading dose is indicated for the rapid control of seizures. Loading dose in patients using carbamazepine is shown in Table 9.

Table 8 Dosage information for carbamazepine tablets*(55)*

Indication	Initial dose	Subsequent dose	Maximum daily dose
Epilepsy Under 6 yr.	10-20 mg/kg/d bid or tid	Increase weekly to achieve optimal clinical response tid or qid	35 mg/kg/d
6-12 yr.	100 mg bid	Add up to 100 mg/d at weekly intervals, tid or qid	1000 mg/d
Over 12 yr.	200 mg bid	Add up to 200 mg/d at weekly intervals, tid or qid	1000mg/d (12-15 yr) 1200mg/d(>15 yr) 1600mg/d (adults, in rare instances)
Trigeminal neuralgia	100 mg bid	Add up to 200 mg/d in increments of 100 mg every 12 hr	1200 mg/d
Bipolar disorder	200-600 mg/d tid or qid	Titrate upward according to patient response and tolerability	1600 mg/d

When adequate seizure control is achieved, dosage should be adjusted to the minimum effective level, which is usually 800-1200 mg/d in adults and children older than 12 years of age and 400-800 mg/d in children 6-12 years of age. For the treatment of trigeminal neuralgia, the dosage necessary to relieve pain may range from 200-1200 mg/d. After control of pain is achieved, maintenance dosage of 400-800 mg/d is usually adequate; however, some patients may require as little as 200 mg/d while others may require 1200 mg/d*(55)*.

Table 9 The loading dose of carbamazepine*(55, 63)*

Age	Loading dose (mg/kg)
Children (≤ 12 years)	10
Adults (≥ 12 years)	8

There is significant inter-subject variability in the pharmacokinetics of carbamazepine that impacts on the frequency of daily dosing. Some patients may be dosed

twice a day, others may require dosing as often as four times a day with immediate release tablets. The dosage form will also affect the frequency of daily dosing. Controlled- and sustained- release dosage forms are designed for twice-a-day dosing*(63)*.

8. Adverse reaction and Toxicity

a. Biochemical abnormalities

Such abnormalities associated with carbamazepine intake include: raised serum concentrations of ALP, HDL, LDL, and reduced serum levels of : bilirubin, folate, thyroxine, uric acid, 24-hydroxy-vitamin D, sodium*(9)*.

b. Dose-determined adverse effects

Concentration-related side effects include lethargy, dizziness, drowsiness, headache, blurred-vision, diplopia, ataxia, nystagmus, and incoordination. These side effects are more common at concentrations greater than 8-12 mg/l*(63)*. Side effects generally occurred at the time of peak plasma concentration of carbamazepine.

A decrease in fluctuations of plasma results in a substantial reduction of carbamazepine side effects*(64, 65)*.

c. Idiosyncratic adverse effects

The severity of idiosyncratic adverse effects is not dose-proportional and they may affect various organs such as skin, nervous system, hematological system, cardiovascular, respiratory, alimentary tract, liver, pancreas, kidney, bone, and thyroid. The non-dose related adverse reactions of carbamazepine are shown in table 10.

Table 10 The non-dose related adverse reaction of carbamazepine*(9, 55)*

Dermatologic
Alopecia, alteralion in skin pigmentation, erythema multiforme, exfoliative dermatitis pruritis, SJS, rash, urticaria
Cardiovascular
Arrhythmia, AV block, bradycardia, chest-pain, edema, thromboembolism, lymphadenopathy, syncope
Respiratory
Pneumonitis, eosinophilia
Alimentary tract
Diarrhea, colitis, nausea, vomiting, constipation, abdominal pain, anorexia
Liver
Hepatitis, cholangitis, jaundice
Kidney
Intestinal nephritis
Haematological
Leucopenia (2.1%), agranulocytosis, aplastic anemia, pure red cell aplasia, thrombocytopenia, eosinophilia, megaloblastic anemia,

Review of assay methods

Various techniques have been used to determine the concentrations of phenytoin and carbamazepine in biological fluids. These include: spectrophotometry, gas-liquid chromatography, enzyme-mediated immunoassay, radioimmunoassay, fluorescense polarization immunoassay, and high-performance liquid chromatography*(54, 66)*.

The fluorescence polarization immunoassay (FPIA) and the turbidimetric inhibition immunoassay are chosen in this study because it has rapid turnaround time, calibration stability, acceptable accuracy and precision. In addition, the method requires a minimum of reagent and sample and is simple to perform*(67)*.

The Abbott TDx is a fully automated system for drug-level monitoring which uses FPIA method for determination of phenytoin and carbamazepine quantity. The fundamental principle of the method can be summarized as follows: A fluorescent derivatives of the analyte (tracer), when excited by linearly polarized light, emits fluorescence that is polarized in inverse proportion to its rotational relaxation time: the faster the rate of rotation, the less polarized is the emitted light. However, when the tracer binds to the antibody, the rate of rotation of the tracer becomes that of the large antibody molecule, which is much slower than that of the smaller tracer molecule. The polarization of the emitted light is, therefore, much higher. The drug in patient's sample and the tracer compete for limiting antibody-combining sites specific for the drug. The more drug present, the lower is the measured fluorescence polarization of the tracer. A standard curve can be prepared from data obtained by assaying calibrators of known drug concentration. Unknown are determined from this standard curve*(68)*.

The principle mechanism of turbidimetric inhibition immunoassay method can be summarized as follows: A particle-bound drug (PBD) binds to drug-specific antibody (Ab) to form insoluble aggregates causing light scatter. Nonparticle- bound drug in the patient sample competes with the PBD for the antibody binding site, inhibiting the formation of insoluble particle-drug antibody aggregates. The rate particle aggregation is inversely proportional to the concentration of the drug in the sample.

Reaction Scheme

$$\text{Drug}_{\text{sample}} + \text{PBP+Ab} = \text{PBP-Ab}_{\text{Aggregates}} + \text{Drug}_{\text{sample}}\text{-Ab}$$

Review of pharmacokinetic interaction between phenytoin and carbamazepine

Phenytoin and carbamazepine are frequently prescribed together in patients who cannot control seizures with single drug therapy. Although both drugs have similar mechanism of action (modification of sodium conductance), some studies have suggested that combination of both drugs improve seizure control. One assumption used to explain this is both drugs bind to different type of the α-subunit of the sodium channel, therefore using both drugs in combination can increase effectiveness*(4, 5)*.

Combination of both drugs has been reported to cause pharmacokinetic interactions. Previous studies have been reported that phenytoin causes the decrease in carbamazepine plasma concentrations since it increases carbamazepine metabolism*(14, 16)*. In contrast, the effects of carbamazepine on pharmacokinetics of phenytoin are controversial. Some studies have suggested that carbamazepine induces phenytoin metabolism resulting in the decrease of phenytoin plasma concentrations*(18, 19, 21)*. Others have reported that carbamazepine increases phenytoin plasma concentrations due to the competitive inhibition of phenytoin hydroxylation by carbamazepine (route of metabolism of both drugs)*(20, 69)*.

CHAPTER III

PATIENTS AND METHODS

The study was conducted from January to July 2007 at Prasat Neurological Institute, Bangkok, Thailand

1. Patient

Patients were recruited for this study based on the following criteria:

1.1 Inclusion criteria

The patients who had all of these characteristics were enrolled in this study.

a. The patients who received phenytoin or carbamazepine monotherapy and in combination, and had a steady state concentration (both seizure controlled and seizure uncontrolled patient).

b. All prospective patients consented to enroll in the study.

1.2 Exclusion criteria

The patients who had either one of these characteristics were excluded from this study.

a. The patients with serum albumin out of normal range (3.5-5.0 g/dl).

b. The patients with hepatic enzyme (ALT and AST) out of normal range (5-40 IU/L).

c. The patients who received drugs that interact with phenytoin or carbamazepine metabolism such as phenobarbital, valproic acid.

1.3 Sample size determination

1) The sample size for the first objective was calculated from the following equation:

$$N = \frac{(z_\alpha + z_\beta)^2}{z_0^{\,2}} + 3$$

When $Z_0^{\,2} = \frac{1}{2}\log_e \frac{1+r}{1-r}$

$Z_{\alpha 0.05} = 1.645$

$Z_{\beta 0.20}$ = 0.84

r = correlation coefficient

Since no previous study has investigated the correlation between maximum rate of metabolism of phenytoin and carbamazepine clearance, this study will set the target correlation coefficient to be 0.60.

$$Z_0^2 = \frac{1}{2}\log_e \frac{1+(0.6)}{1-(0.6)} = 0.693$$

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

2) The sample size for the second objective was calculated from the following equation:

$$N = \frac{2\sigma_d^{\,2}(z_\alpha + z_\beta)^2}{d^2}$$

When $\sigma_d^{\,2} = \sigma_1^{\,2} + \sigma_2^{\,2} - 2r\sigma_1\sigma_2$

$\sigma_1^{\,2}$, $\sigma_2^{\,2}$ = the variance of baseline and follow up values

r = correlation coefficient between baseline and follow up values

d = the mean difference between baseline and follow up values

$Z_{\alpha 0.05}$ = 1.645

$Z_{\beta 0.20}$ = 0.84

The previous study *(70)* reported that the population value of carbamazepine clearance of patients receiving carbamazepine monotherapy and polytherapy was 0.069 ± 0.020 and 0.106 ± 0.037 L/kg/hr respectively, but there was no report about correlation coefficient. Thus, this study will set the correlation coefficient at the average value (0.50) and set the mean difference at 50% of carbamazepine clearance in monotherapy group.

σ_1 = 0.020; σ_1^2 = 0.0004

σ_2 = 0.037; σ_2^2 = 0.0014

d = 0.5(0.069) = 0.0345

d^2 = 0.0012

r = 0.50

$$\sigma_d^2 = 0.0004 + 0.0014 - 2(0.5)(0.020)(0.037)$$

$$= 0.00106$$

$$n = \frac{2(0.00106)(1.645 + 0.84)^2}{0.0012}$$

$$= 10.92 \approx 11$$

2 Definition

2.1 **Maximum rate of metabolism** (V_{max}) is the rate of metabolism which is calculated from the following equation

$$V_{max} = \frac{[(SFD/\tau)(K_m + C_{SS})]}{C_{SS}}$$

When K_m is the population Michaelis constant which is 4 μg/ml

2.2 **Michaelis constant** is the substrate concentration at which V will be one half of V_{max}

2.3 **Clearance** is the intrinsic ability of the body or its organ of elimination to remove drug from the blood or plasma.

2.4 **Clinical response**

Clinical response in this study is classified as follow:

- Controlled seizure : No seizure occurs at the dosage patients receive
- Uncontrolled seizure : There are seizures occurring at the dosage patients receive

2.5 Adverse drug reaction

This study evaluates adverse drug reactions that correlate with plasma drug concentration such as nystagmus, diplopia, or ataxia.

3 Study design

This study uses a prospective observational method to investigate the relationship between maximum rate of metabolism of phenytoin (V_{max}) and carbamazepine clearance (Cl_{CBZ}) and a retrospective observational method to determine the difference between V_{max} and Cl_{CBZ} in patients receiving drug as a monotherapy or in combination therapy. Correlation between plasma blood level, clinical response, and adverse drug reaction of phenytoin and carbamazepine will also be observed. The protocol was reviewed and approved by the ethic committee of Prasart Neurological Institute. The flow chart of the study is shown in figure 4.

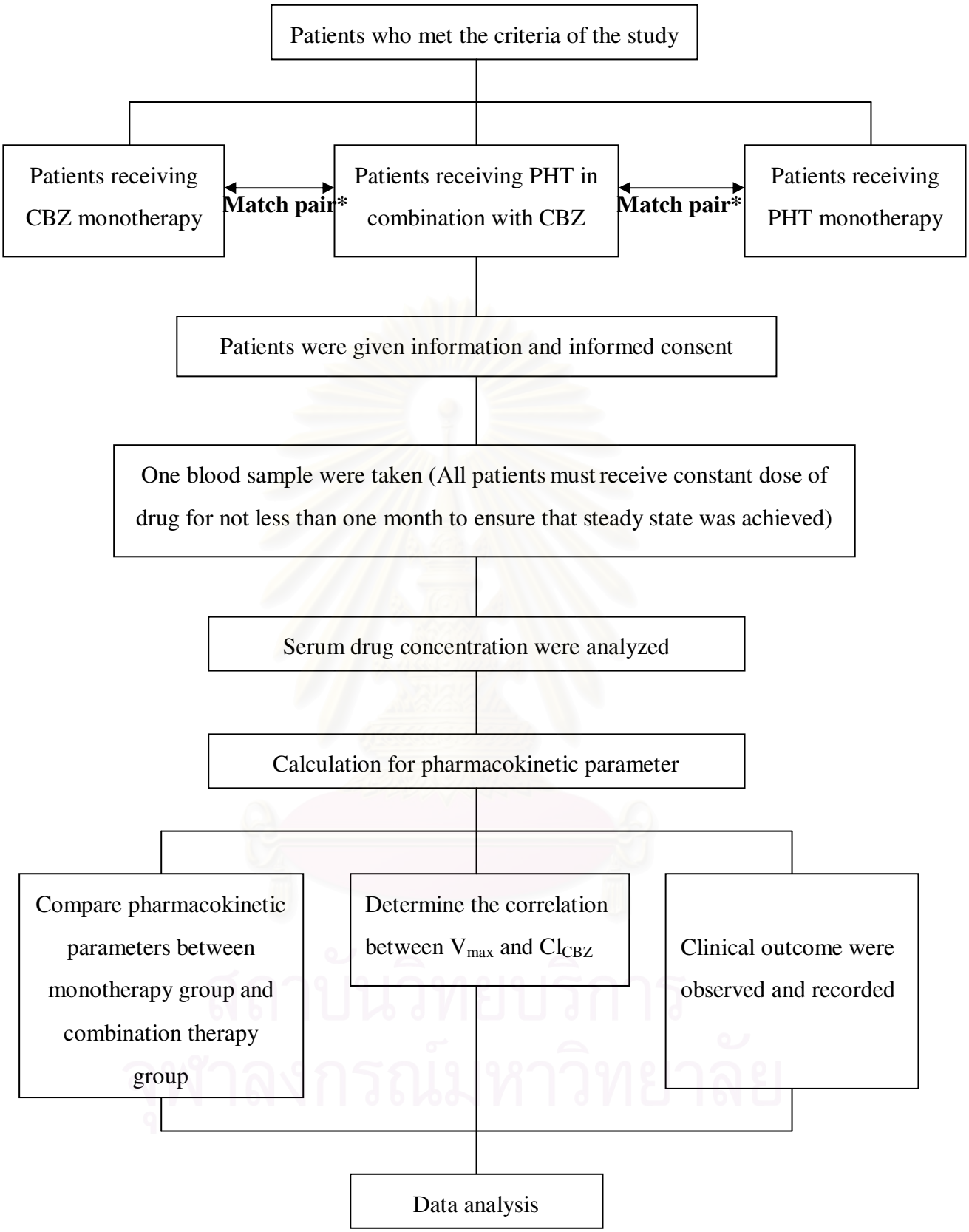
4 Drug administration and blood sampling

The usual dosage regimen of phenytoin and carbamazepine were prescribed by physician. Blood samples were obtained after patients had received constant dose for not less than one month to ensure that steady state was achieved.

Trough concentrations are generally recommended for routine monitoring of phenytoin; however, due to its long half-life, the time of sampling within the dosing interval is not critical. For carbamazepine, trough blood sample was drawn not over one hour before the administration of the next dose. Six-milliliter blood samples were collected in red-top tube. The serum portion were separated and stored at -20 ºC until analysis. The samples were analyzed within one month for phenytoin and three months for carbamazepine.

5 Clinical follow up

The important information of each patient including medical history, Physical examination, adverse drug reaction, laboratory value, and clinical response were followed and recorded.



PHT = phenytoin, CBZ = carbamazepine

* Patients were matched for age (≤ 12 yrs, 13-49 yrs, ≥ 50 yrs) and weight (±30%)

Figure 4 Flow chart of the study

6 Bioanalysis of phenytoin and carbamazepine

Quantitative analyses of phenytoin and carbamazepine in serum were performed by Fluorescense Polarization Technique (TDx®Analyzer System) and Turbidimetric Inhibition Immunoassay Technique (SYNCHRON® CX System) for prospective and retrospective data respectively. (Appendix C and Appendix D)

7 Pharmacokinetic parameter calculation

7.1 Maximum rate of metabolism (V_{max}) calculation

$$V_{max} = \frac{(SFD/\tau)(K_m + C_{ss})}{(C_{ss})} \qquad \text{(equation 1)}$$

When K_m is the population Michaelis constant which is 4 μg/ml

7.2 Carbamazepine elimination parameter calculation

$$Cl = \frac{(S)(F)(D)}{(\tau)(c_{ss\,ave})} \qquad \text{(equation 2)}$$

8 Statistical analyses

8.1 General characteristics of subjects such as gender, weight, height, age, adverse drug reaction and laboratory data were determined by descriptive statistics.

8.2 The distributions of maximum rate of metabolism of phenytoin and carbamazepine clearance were determined by Shapiro-Wilk Test.

8.3 The relationships between pharmacokinetic parameters of phenytoin and carbamazepine were determined by simple linear regression.

8.4 The differences of pharmacokinetic parameters between phenytoin or carbamazepine monotherapy and phenytoin incombination with carbamazepine were determined by unpaired t-test.

CHAPTER IV

RESULTS

1. Study population

Thirteen prospective patients (combination therapy group) and thirty-five retrospective patients (3 patients in combination therapy group and 32 patients in monotherapy group) who met the criteria of the research were recruited from epileptic patients at Prasart neurological institute during January to June 2007. The patients in monotherapy group were possibly matched in age and weight with recruited patients in the combination therapy group. All patients were studied during steady-state conditions with no change in either phenytoin or carbamazepine dosage for at least 1 month. Moreover, all the patients had no report of smoking and alcohol consuming. The study was approved by the Ethical Review Committee of the institute.

Demographic data

There were sixteen patients in each study group (phenytoin – carbamazepine combination therapy group, phenytoin monotherapy group, and carbamazepine monotherapy group). Most of the patients in the combination therapy group were recruited prospectively (13 out of the 16 patients), while all of the patients in the monotherapy groups (both for phenytoin and carbamazepine were recruited retrospectively).

The patients ranged in age from 8 to 62 years with an average of 32.25 years. Their body weights ranged from 24 to 71 kg. with an average of 52.57 kg. The dosages of phenytoin ranged from 75 to 300 mg/d while the dosages of carbamazepine ranged from 400 to 1400 mg/d. Age and weight of the patients among each group were not statistical different.

Of the 48 epileptic patients, 26 patients had a generalized tonic-clonic seizure, 16 patients had a complex partial seizure, and 6 patients had a partial with 2^{nd} generalize seizure. Types of epilepsy were presented in figure 4. Some patients had concomitant disease such as hypertension (2 patients), migraine (1 patient), and muscle pain (1 patient).

The concomitant drug most frequently prescribed was folic acid. The characteristics, concomitant disease, concomitant medicine, and laboratory data of the patients in each group are shown in Table 11, 12, and 13. Comparison of patients' characteristics among phenytoin – carbamazepine combination therapy group, phenytoin monotherapy group, and carbamazepine monotherapy group are shown in Table 14.

Table 11 Characteristics of patients receiving combination therapy of phenytoin and carbamazepine

Pt no	Sex	Type of epilepsy	Concomitant disease	Concomitant drug	Age (year)	Weight (kg)	ALT	AST	ALB
com 1	M	CPS	-	-	10.6	34	20	24	3.4
com 2	M	CPS 2nd GTC	-	Folic	31	48	17	21	3.6
com 3	M	CPS	HTN	Folic, Felodipine	33	67	21	19	4.0
com 4	M	GTC (focal)	-	-	14	60	45	28	3.9
com 5	F	CPS	-	-	19	54	34	24	4.5
com 6	F	CPS	-	-	17.9	50	40	25	3.9
com 7	F	GTC (focal)	-	-	11	47	15	17	4.0
com 8	F	CPS 2nd GTC	-	Folic	48	46	-	-	-
com 9	M	GTC (TLE)	-	Folic	30.7	66	-	-	-
com 10	F	GTC	-	Folic	40	45	36	38	3.9
com 11	F	GTC	-	Folic	51.7	55	13	19	4.0
com 12	M	GTC (TLE)	-	-	52	55	-	-	-
com 13	M	GTC	-	Folic	62	62	16	24	3.7
com 14	M	GTC	-	Folic	35.3	48	12	18	4.3
com 15	M	CPS	-	Folic	42.2	58	21	38	5.2
com 16	F	GTC	-	Folic	27.8	50	26	21	4.2
Mean ± SD					32.89 ± 15.75	52.81 ± 8.59			
Range					10.6-62	34-67			

Com = Patients receiving phenytoin-carbamazepine in combination

CPS = Complex partial seizure

GTC = Generalized tonic-clonic

TLE = Temporal lobe epilepsy

CPS 2nd GTC = partial seizure with 2nd generalize seizure

Table 12 Characteristics of patients receiving phenytoin monotherapy

Pt no	Sex	Type of epilepsy	Concomitant disease	Concomitant drug	Age (year)	Weight (kg)	ALT	AST	ALB
mo.p 1	F	GTC	-	-	50	65	-	-	-
mo.p 2	M	GTC	Migraine	Mefenamic	44	71	-	-	-
mo.p 3	M	GTC (TLE)	-	Folic, MTV	55	45	25	15	5.0
mo.p 4	F	GTC	-	Folic	18	60	-	-	-
mo.p 5	F	GTC	-	Folic	44	45	-	-	-
mo.p 6	M	GTC	-	-	41	65	-	-	-
mo.p 7	M	CPS	-	-	22	67	36	23	5.3
mo.p 8	F	CPS	-	$B_{1\text{-}6\text{-}12}$	11	48	-	-	-
mo.p 9	F	2^{nd} GTC	-	-	8	28	24	17	40
mo.p 10	M	CPS	-	-	18	50	20	28	3.6
mo.p 11	F	CPS	-	-	17	48	32	25	3.9
mo.p 12	F	GTC (focal)	-	-	24	37	15	20	4.0
mo.p 13	M	GTC	-	-	16	60	-	-	-
mo.p 14	M	GTC (focal)	-	Folic	58	56.4	-	-	-
mo.p 15	F	2^{nd} GTC	-	-	14	44	-	-	-
mo.p 16	M	GTC	-	Folic	29.6	65	24	34	4.6
Mean ± SD					29.35 ± 16.67	53.15 ± 12.72			
Range					8-58	24-71			

Mo.p = Patients receiving phenytoin monotherapy

CPS = Complex partial seizure

GTC = Generalized tonic-clonic

TLE = Temporal lobe epilepsy

CPS 2^{nd} GTC = partial seizure with 2^{nd} generalize seizure

Table 13 Characteristics of patients receiving carbamazepine monotherapy

Pt no	Sex	Type of epilepsy	Concomitant disease	Concomitant drug	Age (year)	Weight (kg)	ALT	AST	ALB
mo.c 1	M	GTC	-	-	50	71	15	-	-
mo.c 2	F	GTC	-	-	57	50	16	29	-
mo.c 3	M	CPS (TLE)	HTN	Manidipine, Folic	28	68	-	-	-
mo.c 4	M	GTC	-	-	36	70	12	27	5.1
mo.c 5	F	CPS	-	-	9	30	-	-	-
mo.c 6	M	2^{nd}GTC	-	-	13	45	-	-	-
mo.c 7	F	CPS	-	Folic	36	51.3	-	-	-
mo.c 8	F	CPS (TLE)	Muscle pain	Meloxicam	52	53.9	-	-	-
mo.c 9	F	GTC	-	-	49	31	-	-	-
mo.c 10	M	GTC	-	Folic	43	58	-	-	-
mo.c 11	F	CPS	-	-	17	51	34	20	4.4
mo.c 12	M	CPS	-	-	11	37	22	24	3.6
mo.c 13	F	GTC	-	Folic	42	49	-	-	-
mo.c 14	M	2^{nd} GTC	-	B complex	49	67	38	19	3.9
mo.c 15	F	CPS	-	Folic	32	45	28	18	4.6
mo.c 16	F	GTC	-	-	28	50.8	26	20	5.0
Mean ± SD					34.50 ± 15.64	51.75 ± 12.83			
Range					9-57	30-71			

Mo.c = Patients receiving carbamazepine monotherapy

CPS = Complex partial seizure

GTC = Generalized tonic-clonic

TLE = Temporal lobe epilepsy

CPS 2^{nd} GTC = partial seizure with 2^{nd} generalize seizure

Table 14 Comparisons of patients' characteristics

Characteristics	Frequency, Mean ± SD		
	PHT-CBZ	PHT	CBZ
Male	9	8	7
Female	7	8	9
Age (yrs)	32.89 ± 15.75	29.35 ± 16.67	34.50 ± 15.64
Weight (kg)	52.81 ± 8.59	53.15 ± 12.72	51.75 ± 12.83
Dosage range (mg/d)			
phenytoin	75-300	162.50-300	-
carbamazepine	400-1400	-	600-1400
Type of epilepsy			
generalized tonic-clonic	9	10	7
complex partial seizure	5	4	7
partial seizure with 2nd generalize seizure	2	2	2
Concomitant disease			
Hypertension	1		1
Muscle pain			1
Migraine		1	

2. Plasma drug concentratonions

2.1 Phenytoin plasma concentrations

All patients were studied during steady-state conditions. The ranges of phenytoin dosage in patients receiving phenytoin-carbamazepine combination therapy and phenytoin monotherapy were 2.08 to 6.67 mg/kg/d and 2.82 to 6.77 mg/kg/d, respectively. Serum phenytoin concentrations of phenytoin-carbamazepine combination therapy group and phenytoin monotherapy group ranged from 1.40 to 26.02 µg/mL and 5.40 to 19.30 µg/mL, respectively. Table 15 presents serum phenytoin concentrations of phenytoin-carbamazepine combination therapy group and phenytoin monotherapy group.

2.2 Carbamazepine plasma concentrations

Same as phenytoin, in carbamazepine groups, the subjects were studied during steady-state conditions with no change in carbamazepine dosage for at least 1 month to ensure the complete autoinduction**.** Moreover, all patients received carbamazepine control release dosage form. The ranges of carbamazepine dosage in phenytoin-carbamazepine combination therapy group and carbamazepine monotherapy group were 6.45 to 21.28 mg/kg/d and 8.57 to 26.67 mg/kg/d, respectively. Serum carbamazepine concentrations of phenytoin-carbamazepine combination therapy group and carbamazepine monotherapy group ranged from 2.82 to 10.75 µg/mL and 6.20 to 12.30 µg/mL, respectively. Table 16 presents serum carbamazepine concentrations of phenytoin-carbamzepine combination therapy group and carbamazepine monotherapy group.

Table 15 Plasma concentrations of phenytoin-carbamazepine combination therapy group and phenytoin monotherapy group.

Patient No.	Phenytoin combination therapy			Patient No.	Phenytoin monotherapy		
	Dose(mg) (mg/kg/d)	τ (day)	Level (mg/L)		Dose(mg) (mg/kg/d)	τ (day)	Level (mg/L)
com 1	37.5	1/2	1.40	mo.p 1	200	1	10.70
	(2.21)				(3.08)		
com 2	300	1	16.28	mo.p 2	200	1	5.80
	(6.25)				(2.82)		
com 3[a]	300	1	6.30	mo.p 3 [a]	250	1	17.65
	(4.48)				(5.56)		
com 4	100	1/2	6.15	mo.p 4	300	1	13.20
	(3.33)				(5.00)		
com 5	200	1	5.55	mo.p 5 [a]	300	1	8.60
	(3.70)				(6.67)		
com 6[a]	131.25	1/2	13.00	mo.p 6 [a]	200	1	7.40
	(5.25)				(3.08)		
com 7	137.5	1/2	9.82	mo.p 7 [a]	300	1	6.75
	(5.85)				(4.48)		
com 8*	300	1	12.40	mo.p 8	112.5	1/2	5.40
	(6.52)				(4.69)		
com 9*	300	1	12.00	mo.p 9	81.25	1/2	13.20
	(4.55)				(6.77)		
com 10	300	1	5.40	mo.p 10 [a]	112.5	1/2	16.00
	(6.67)				(4.50)		
com 11[a]	300	1	10.15	mo.p 11 [a]	131.25	1/2	18.50
	(5.45)				(5.47)		
com 12*	300	1	26.02	mo.p 12	100	1/2	18.35
	(5.45)				(5.41)		
com 13	250	1	6.13	mo.p 13	300	1	8.70
	(4.03)				(3.26)		
com 14[a]	100	1	1.86	mo.p 14	300	1	6.50
	(2.08)				(5.32)		
com 15	300	1	10.35	mo.p 15	125	1/2	19.30
	(5.17)				(5.68)		
com 16	300	1	11.25	mo.p 16	300	1	11.70
	(6.00)				(4.62)		
Mean±SD (Range)	4.81±1.43 (mg/kg/d) (2.08-6.67)		9.63±5.99 (1.40-26.02)	**Mean±SD (Range)**	4.88±1.51 (mg/kg/d) (2.82-6.77)		11.73±4.97 (5.40-19.30)

Com = Patients receiving phenytoin-carbamazepine in combination
Mo.p = Patients receiving phenytoin monotherapy
* Retrospective data
[a] seizure uncontrolled patient

Table 16 Plasma concentrations of carbamazepine combination therapy group and carbamazepine monotherapy group.

Patient No.	Carbamazepine combination therapy			Patient No.	Carbamazepine monotherapy		
	Dose(mg) (mg/kg/d)	τ (day)	Level (mg/L)		Dose(mg) (mg/kg/d)	τ (day)	Level (mg/L)
com 1	300 (17.65)	1/2	7.91	mo.c 1	700 (19.72)	1/2	12.30
com 2	400 (16.67)	1/2	6.61	mo.c 2	200 (12.00)	1/3	11.06
com 3 [a]	700 (20.9)	1/2	7.62	mo.c 3	600 (17.65)	1/2	6.20
com 4	400 (13.33)	1/2	7.60	mo.c 4	300 (8.57)	1/2	7.70
com 5	400 (14.81)	1/2	10.75	mo.c 5 [a]	300 (20.00)	1/2	8.40
com 6 [a]	500 (20.0)	1/2	5.73	mo.c 6	500 (22.22)	1/2	8.20
com 7	500 (21.28)	1/2	6.84	mo.c 7 [a]	400 (15.59)	1/2	10.30
com 8*	200 (8.70)	1/2	2.90	mo.c 8	400 (14.84)	1/2	7.10
com 9*	300 (9.09)	1/2	5.30	mo.c 9	400 (25.80)	1/2	8.10
com 10	400 (17.78)	1/2	3.30	mo.c 10	400 (13.79)	1/2	7.60
com 11 [a]	400 (14.55)	1/2	7.40	mo.c 11	400 (15.69)	1/2	10.50
com 12*	400 (12.73)	1/2	6.83	mo.c 12	400 (18.92)	1/2	8.40
com 13	400 (6.45)	1	2.82	mo.c 13	400 (16.33)	1/2	10.20
com 14 [a]	400 (16.67)	1/2	8.15	mo.c 14	300 (8.96)	1/2	9.21
com 15	300 (10.34)	1/2	6.20	mo.c 15 [a]	600 (26.67)	1/2	9.85
com 16	300 (12.00)	1/2	3.60	mo.c 16	400 (15.75)	1/2	10.10
Mean±SD (Range)	14.67±4.47 (mg/kg/d) 6.45-21.28		6.22±2.20 (2.82-10.75)	**Mean±SD (Range)**	17.03±5.17 (mg/kg/d) 8.57-26.67		9.08±1.63 (6.20-12.30)

Com = Patients receiving phenytoin-carbamazepine in combination
Mo.c = Patients receiving carbamazepine monotherapy
* Retrospective data
[a] seizure uncontrolled patient

3. Pharmacokinetic parameters

To calculate a maximum rate of metabolism of phenytoin (V_{max}), the Michaelis-menten equation (equation 1) was used with an assumed population Michaelis constant (K_m) of 4 mg/L. V_{max} of a combination therapy group and a monotherapy group ranged from 289.29 - 480.44 (mean 362.80 ± 51.78) and 211.74 - 445.85 (mean 331.64 ± 71.93) mg/d, respectively. When divided by patient's weights, V_{max} ranged from 5.50-10.68 mg/kg/d (mean 6.95 ± 1.37 mg/kg/d) and 3.89-8.99 mg/kg/d (mean 6.47 ± 1.51 mg/kg/d) in combination therapy group and monotherapy group respectively.

Carbamazepine clearance (Cl_{CBZ}) was calculated by using steady-state continuous infusion equation (equation 2) with a bioavailability of 0.8 and the fraction of the administered dose that is active drug (s) is 1.0. Cl_{CBZ} of combination therapy group and monotherapy group ranged from 59.53 - 193.94 L/d (mean 105.35 ± 35.45 L/d) and 43.40 - 154.84 L/d (mean 76.45 ± 26.62 L/d), respectively.

Table 17 Pharmacokinetic parameters of phenytoin and carbamazepine in combination therapy group

	Combination therapy				
Patient No.	V_{max}^{Φ} (mg/d)	V_{max}^{Φ} (mg/kg/d)	Cl_{CBZ}^{Ψ} (L/d)	Cl_{CBZ}^{Ψ} (L/kg/d)	Cl_{CBZ}^{Ψ} (L/kg/hr)
com 1	289.29	8.51	60.680	1.78	0.074
com 2	343.81	7.16	96.82	2.02	0.084
com 3	451.24	6.73	149.98	2.24	0.093
com 4	330.08	5.50	84.21	1.40	0.058
com 5	316.61	5.86	59.53	1.10	0.046
com 6	343.27	6.87	139.62	2.79	0.116
com 7	387.02	8.23	116.96	2.49	0.104
com 8*	365.03	7.94	110.34	2.40	0.100
com 9*	368.00	5.58	90.57	1.37	0.057
com 10	480.44	10.68	193.94	4.31	0.180
com 11	384.77	7.00	86.49	1.57	0.065
com 12*	318.43	5.79	93.70	1.70	0.071
com 13	380.08	6.13	113.48	1.83	0.076
com 14	289.85	6.04	78.53	1.64	0.068
com 15	382.67	6.60	77.42	1.33	0.055
com 16	374.13	7.48	133.33	2.67	0.111
Mean±SD	362.80 ± 51.78	7.01 ± 1.35	105.35 ± 35.45	2.04 ± 0.79	0.085 ± 0.03
Range	289.29 – 480.44	5.50 – 10.68	59.53 – 193.94	1.10 – 4.31	0.05 – 0.18

Com = Patients receiving phenytoin-carbamazepine in combination
Φ maximum rate of metabolism
Ψ carbamazepine clearance
* Retrospective data

Table 18 Pharmacokinetic parameters of phenytoin and carbamazepine in monotherapy group

Monotherapy						
Patient No.	V_{max}^{Φ} (mg/d)	V_{max}^{Φ} (mg/kg/d)	Patient No.	Cl_{CBZ}^{Ψ} (L/d)	Cl_{CBZ}^{Ψ} (L/kg/d)	Cl_{CBZ}^{Ψ} (L/kg/hr)
mo.p 1	252.79	7.44	mo.c 1	91.06	2.68	0.112
mo.p 2	310.90	6.48	mo.c 2	43.40	0.90	0.038
mo.p 3	287.03	4.28	mo.c 3	154.84	2.31	0.096
mo.p 4	359.64	5.99	mo.c 4	62.34	1.04	0.043
mo.p 5	404.37	7.49	mo.c 5	57.14	1.06	0.044
mo.p 6	283.46	5.67	mo.c 6	97.56	1.95	0.081
mo.p 7	439.56	9.35	mo.c 7	62.14	1.32	0.055
mo.p 8	391.67	8.51	mo.c 8	90.14	1.96	0.082
mo.p 9	211.74	3.21	mo.c 9	79.01	1.20	0.050
mo.p 10	281.25	6.25	mo.c 10	82.21	1.83	0.076
mo.p 11	319.26	5.80	mo.c 11	60.95	1.11	0.046
mo.p 12	243.60	4.43	mo.c 12	66.67	1.21	0.050
mo.p 13	402.90	6.50	mo.c 13	62.75	1.01	0.042
mo.p 14	445.85	9.29	mo.c 14	52.12	1.09	0.045
mo.p 15	301.81	5.20	mo.c 15	97.46	1.68	0.070
mo.p 16	370.36	7.41	mo.c 16	63.37	1.27	0.053
Mean±SD	331.64 ± 71.93	6.46 ± 1.75	**Mean±SD**	76.45 ± 26.62	1.48 ± 0.53	0.061 ± 0.02
Range	211.74 – 445.85	3.21 – 9.35	**Range**	43.40 – 154.84	0.90 – 2.68	0.04 – 0.11

Mo.p = Patients receiving phenytoin monotherapy
Mo.c = Patients receiving carbamazepine monotherapy
Φ maximum rate of metabolism
Ψ carbamazepine clearance

4. Correlation between maximum rate of metabolism of phenytoin and carbamazepine clearance

Correlation between maximum rate of metabolism of phenytoin and carbamazepine clearance was determined in sixteen patients who were receiving both drugs in combination. Three serum drug concentrations were retrospective data (patient no. 8, 9, 12). Scatterplots of maximum rate of metabolism of phenytoin versus carbamazepine clearance are shown in Figure 5-6. A correlation between maximum rate of metabolism of phenytoin and carbamazepine clearance was high and significant ($r = 0.804$, $P < 0.001$). There was no extreme outliner data; however, when excluded retrospective data**,** a small increase of correlation coefficient was found ($r = 0.816$, $P = 0.001$).When divided V_{max} by patient's weight resulting in the unit of mg/kg/d, a high correlation was also found ($r = 0.828$; $P = 0.001$). There was a small decrease of correlation coefficient when excluded patient no. 8, 9, 12 ($r = 0.818$; $P = 0.001$)

Regression equations between maximum rate of metabolism of phenytoin and carbamazepine clearance are performed and shown in Table 19.

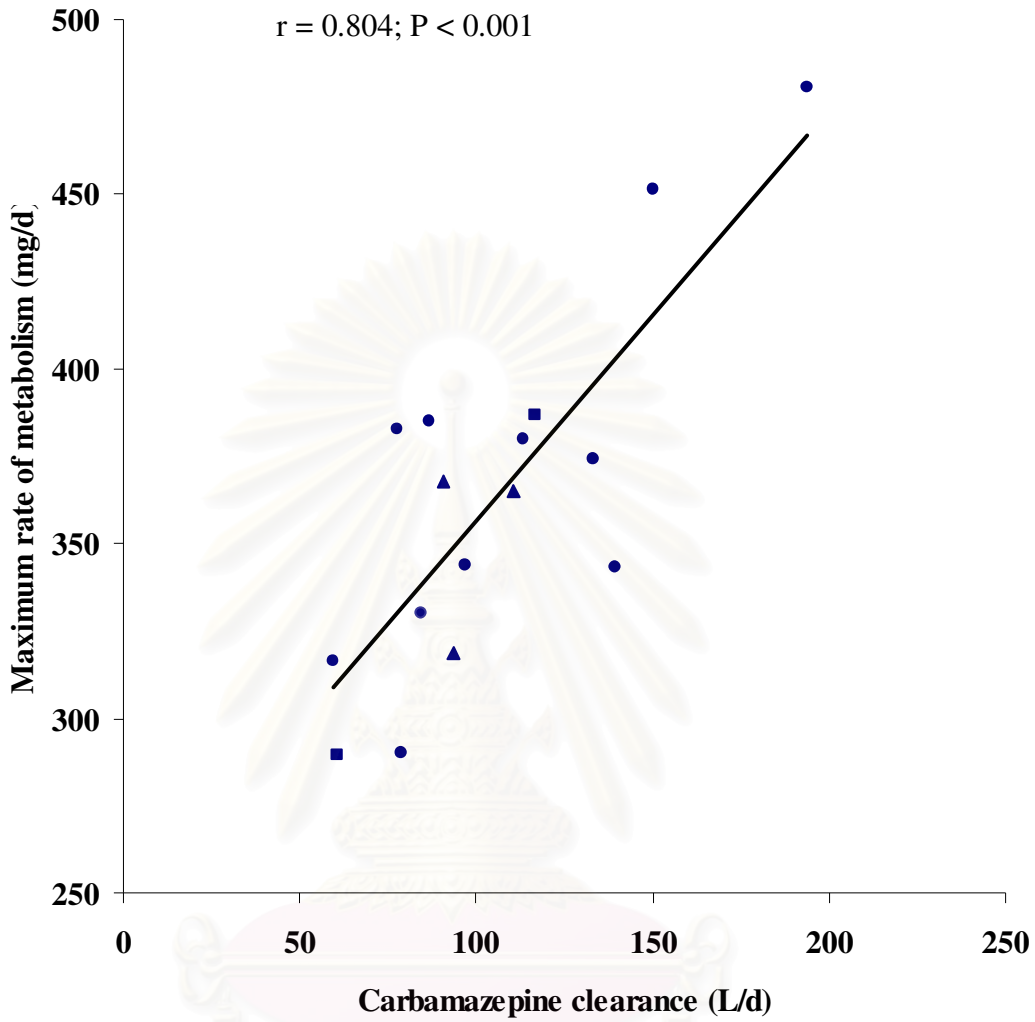


Figure 5 Scatterplots of maximum rate of metabolism of phenytoin (mg/d) versus carbamazepine clearance (L/d) (n = 16)

▲: retrospective data

●: prospective data

■: patients who age less than 12 years

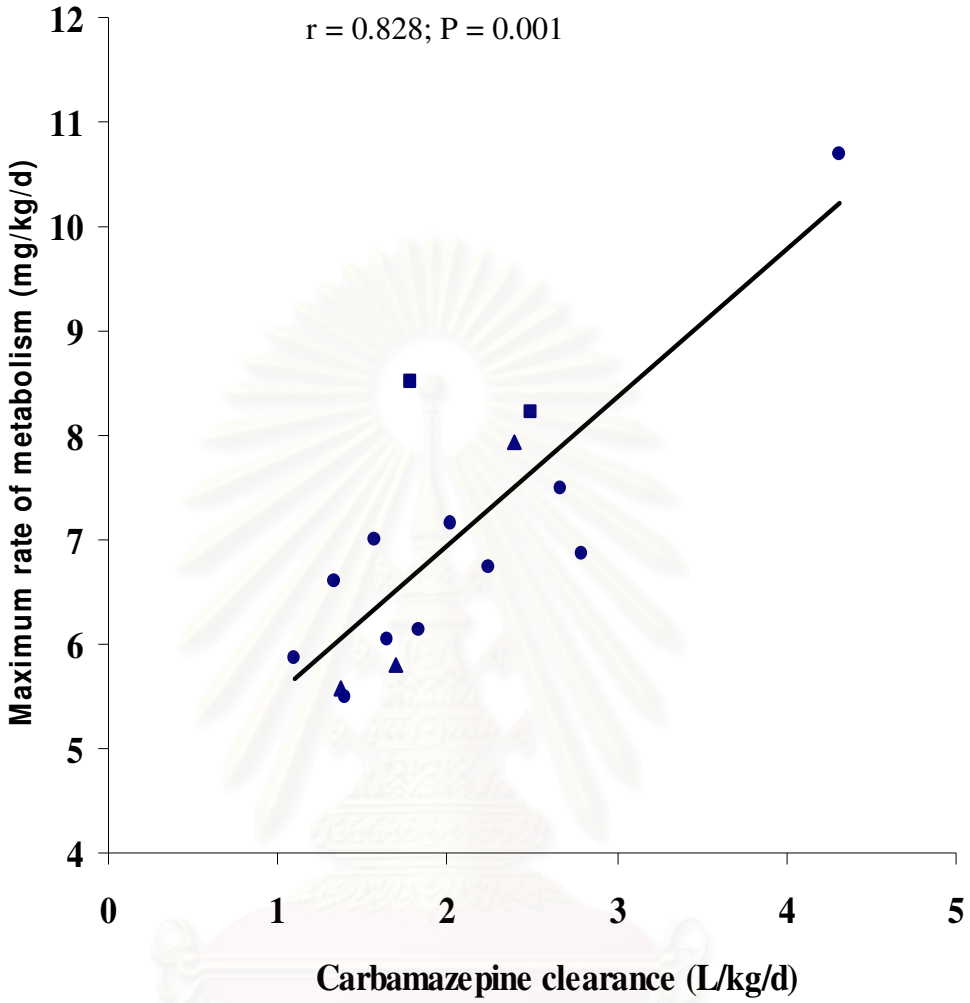


Figure 6 Scatterplots of maximum rate of metabolism of phenytoin (mg/kg/d) versus carbamazepine clearance (L/kg/d) (n = 16)

▲: retrospective data

●: prospective data

■: patients who age less than 12 years

Table 19 Relationships between maximum rate of metabolism of phenytoin and carbamazepine clearance.

Regression equation	r^{Φ}	r^2	P value*
V_{max} (mg/d) = 1.174 Cl_{CBZ} + 239.091	0.804	0.646	< 0.001
Cl_{CBZ} (L/d) = 0.55 V_{max} – 94.251			
V_{max} (mg/kg/d) = 1.421 Cl_{CBZ} + 4.107	0.828	0.686	0.001
Cl_{CBZ} (L/kg/d) = 0.483 V_{max} – 1.340			
$^{\psi}$ V_{max} (mg/d) = 1.173 Cl_{CBZ} + 240.142	0.816	0.665	0.001
$^{\psi}$ Cl_{CBZ} (L/d) = 0.567 V_{max} – 100.364			
$^{\psi}$ V_{max}(mg/kg/d)= 1.332 Cl_{CBZ} + 4.353	0.818	0.669	0.001
$^{\psi}$ Cl_{CBZ} (L/kg/d) = 0.502 V_{max} – 1.492			
$^{\S}V_{max}$ (mg/d) = 0.888 Cl_{CBZ} + 197.862	0.840	0.705	<0.001
$^{\S}Cl_{CBZ}$ (L/d) = 0.794 V_{max} – 92.886			
$^{\S}V_{max}$ (mg/kg/d) = 0.990 Cl_{CBZ} + 3.221	0.911	0.829	<0.001
$^{\S}Cl_{CBZ}$ (L/kg/d) = 0.838 V_{max} – 1.996			

Φ : from pearson correlation

* : from regression analysis

ψ : exclude patient no. 8, 9, 12 (n = 13)

§ : exclude patient no. 1, 7 (n = 14)

5. Comparison of pharmacokinetic parameters between combination therapy group and monotherapy group

Patients in the combination therapy group and monotherapy group were matched for age and weight with none of the subjects had previously been reported as having hepatic or renal disease. Furthermore, all patients were studied during steady-state conditions.

Age, weight and phenytoin dosage of the patients receiving phenytoin monotherapy group and combination therapy group were not significantly different ($p = 0.542$, 0.930, and 0.880; unpaired t-test). Also, the maximum rate of metabolism of phenytoin between monotherapy group and combination therapy group were not significantly different ($p = 0.170$, unpaired t-test). The details are shown in Table 20.

Same as phenytoin, age, weight and carbamazepine dosage of the patients receiving carbamazepine monotherapy group and combination therapy group were not significantly different ($p = 0.773$, 0.785 and 0.177; unpaired t-test). However, carbamazepine clearance between monotherapy group and combination therapy group were significantly different ($p = 0.014$, unpaired t-test). The details are shown in Table 21.

Table 20 Comparison of age, weight, dosage and maximum rate of metabolism of phenytoin between combination therapy group and monotherapy group

Parameter	n	Mean ± SD (range)		P value [a]
		Combination therapy	Monotherapy	
Age (yrs)	16	32.89 ± 15.75	29.35 ± 16.67	0.542
Weight (kgs)	16	52.81 ± 8.59	53.15 ± 12.72	0.930
Dosage (mg/kg/d)	16	4.81 ± 1.43	4.88 ± 1.51	0.880
V_{max} (mg/d)	16	362.80 ± 51.78	331.64 ± 71.93	0.170

[a] Unpaired t-test

Table 21 Comparison of age, weight, dosage and carbamazepine clearance between combination therapy group and monotherapy group

Parameter	n	Mean ± SD (range)		P value [a]
		Combination therapy	Monotherapy	
Age (yrs)	16	32.89 ± 15.75	34.50 ± 15.64	0.773
Weight (kgs)	16	52.81 ± 8.59	51.75 ± 12.83	0.785
Dosage (mg/kg/d)	16	14.67±4.47	17.03±5.17	0.177
Cl_{CBZ}(L/d)	16	105.35 ± 35.45	76.45 ± 26.62	0.014

[a] Unpaired t-test

6. Therapeutic outcome

Outcome of therapy was evaluated by physician at the dosage regimen that patients received (Table 22-24). In combination therapy group, four patients still had seizures; phenytoin dosage was increased in one patient and his seizure had been controlled later. A third drug (topiramate) had been prescribed for one patient, while two patients still received the same drugs and dosages since their seizures were due to precipitating factor (stress and sleep late). Mild dizziness was observed in one patient.

In carbamazepine monotherapy group, three patients still had seizures; the dosages of carbamazepine were increased in two patients and their seizures were improved later, one patient received valproic acid as a combination drug. Three patients had minor side effect, such as, horizontal nystagmus.

In phenytoin monotherapy group, six patients had uncontrolled seizures; phenytoin dosages were adjusted in four of them, while two patients were prescribed the second antiepileptic drug. Mild nystagmus occurred in three patients.

Table 22 Therapeutic outcome of patients receiving phenytoin-carbamazepine combination therapy

Phenytoin-carbamazepine combination therapy	n	Efficacy		Side effect
		Controlled seizure	Uncontrolled seizure	
Subtherapeutic range (PHT < 5 mg/L, CBZ < 4 mg/L)	PHT 2	1	1	-
	CBZ 4	4	-	-
Therapeutic range (PHT 5-20 mg/L, CBZ 4-12 mg/L)	9	7	3	1
Above therapeutic range (PHT > 20 mg/L, CBZ > 12 mg/L)	1	1	-	-

Table 23 Therapeutic outcome of patients receiving carbamazepine monotherapy

Carbamazepine monotherapy	n	Efficacy		Side effect
		Controlled seizure	Uncontrolled seizure	
Subtherapeutic range (<4 mg/L)	-	-	-	-
Therapeutic range (4-12 mg/L)	15	12	3	3
Above therapeutic range (>12 mg/L)	1	1	-	-

Table 24 Therapeutic outcome of patients receiving phenytoin monotherapy

Phenytoin monotherapy	n	Efficacy		Side effect
		Controlled seizure	Uncontrolled seizure	
Subtherapeutic range (<5 mg/L)	-	-	-	-
Therapeutic range (5-20 mg/L)	16	10	6	3
Above therapeutic range (>20 mg/L)	-	-	-	-

CHAPTER V

DISCUSSION

Demographic data

There were 48 epileptic patients completed this study. The patients can be categorized into three groups (phenytoin-carbamazepine in combination group, phenytoin monotherapy group, and carbamazepine monotherapy group) according to their treatment. Most patients had generalized tonic-clonic seizure followed by complex partial seizure and partial seizure with secondary generalized seizure, respectively. Types of seizure were not significantly different among group. Age and weight of patients in combination therapy group were not significantly different from monotherapy group. All patients had no report of hepatic or renal diseases. None of the patients had serum albumin out of range. Moreover, all of them were studied during steady-state condition.

Plasma drug concentrations

The average dose of phenytoin between combination therapy group and monotherapy group were not significantly different (4.81±1.43 and 4.88±1.51 mg/kg/d; P = 0.880). There was no patient in both groups receiving phenytoin dosage higher than the recommendation dose (4-10 mg/kg/d in children, 4-8 mg/kg/d in adolescents and 4-7 mg/kg/d in adults and geriatrics)*(50)*. Same as phenytoin, the average dose of carbamazepine between combination therapy group and monotherapy group were not significantly different (14.67±4.47 and 17.03±5.17 mg/kg/d; P = 0.177). None of the patient in both groups receiving carbamazepine dosage out of recommendation dose (800-1600 mg/d in adults and children older than 12 years of age and 800-1200 mg/d in children 6-12 years of age)*(55, 57)*.

The clinical responses and side effects reported in this study were evaluated by physician. In phenytoin monotherapy group, all of the patients had serum phenytoin concentration within the range of 5-20 mg/L. Three patients having serum phenytoin concentrations lower than 10 mg/L cannot control seizure and had to increase phenytoin

dosage. This result is in agree with previous literatures which reported that the range of serum phenytoin concentrations of 10 to 20 mg/L are accepted as optimal therapeutic range; however, serum concentrations in the range of 5-10 mg/L can result in good therapeutic control for some patients *(40)*. Three patients whose serum phenytoin concentrations were within the range of 10-20 mg/L but still had seizure, were prescribed the second antiepileptic drug. Mild nystagmus occurred in three patients whose serum phenytoin concentrations were 6.75, 18.5, and 18.35 mg/L.

If the therapeutic range of carbamazepine is proposed to be 4 to 12 mg/L as in general, then, 93.75% of patients in carbamazepine monotherapy group had their plasma concentrations within the therapeutic range. Three patients whose serum carbamazepine concentrations were in the range of 8-12 mg/L had seizures. One of them was given valproic acid as a second antiepileptic drug to control seizure, the others had to increase carbamazepine dosage and their seizures were improved later. Hoppener et al. have reported that many patients will develop symptoms of toxicity when plasma concentrations exceed 9 mg/L*(71)*. In this study, three patients whose serum concentrations were in the range of 9-12 mg/L had mild horizontal nystagmus. However, one patient having serum carbamazepine concentration exceed 12 mg/L but did not develop any symptom of toxicity.

In combination therapy group, there were two patients having serum phenytoin concentrations lower than 5 mg/L. One of them could control seizure and would be given carbamazepine monotherapy. Another patient could not control seizure with phenytoin and carbamazepine, then, phenytoin was tapered off and patient was given a new antiepileptic drug. One patient had serum phenytoin exceed 20 mg/L while did not develop symptom of toxicity. Four patients had serum carbamazepine concentrations lower than therapeutic range but all of them could control seizure.

The seizure uncontrolled patients in monotherapy group were more than those in phenytoin-carbamazepine combination therapy group. This can be explained in that the patients in monotherpy group were obtained from retrospective data. Most of serum drug concentrations from retrospective data were monitored in seizure uncontrolled patients.

Pharmacokinetic parameters

Michaelis-Menten equation and steady-state continuous infusion equation were used to calculate maximum rate of metabolism of phenytoin (V_{max}) and carbamazepine clearance (Cl_{CBZ}). The average V_{max} of patient receiving phenytoin monotherapy in this study was slightly lower than most of those reported in previous studies (Table 25). While the average Cl_{CBZ} of patient receiving carbamazepine monotherapy in this study was close to those found in previous studies (Table 26).

Table 25 Overview of Maximum rate of metabolism of phenytoin obtained from different population studies.

Studies	V_{max} (mg/kg/d)
Thai (this study)	6.46
Thai (Kanjanasilp et al., 2005)*(72)*	12.50
Japan (Yukawa et al., 1989)*(73)*	6.15
Japan (Odani et al., 1996)*(74)*	9.80
Malasian (Isamail et al., 1994)*(75)*	7.32

Table 26 Overview of carbamazepine clearance obtained from different population studies.

Studies	Cl_{CBZ}(L/kg/hr)
Thai (this study)	0.061
Malasian (Isamail et al., 1993)*(76)*	0.060
Omani (Deleu et al., 2001)*(77)*	0.054
Protugese (Almeida et al., 1998)*(70)*	0.069
Singapore (Chan et al., 2001)*(78)*	0.053

Some patients had concomitant drug such as felodipine, meloxicam, mefenamic acid, manidipine, vitamin B complex, vitamin $B_{1\text{-}6\text{-}12}$, and folic acid. However, most of these drugs have not been reported to affect phenytoin and carbamazepine metabolism

except for folic acid which might affect phenytoin metabolism. Several studies have been reported that folic acid supplementation causes a decrease in serum phenytoin concentration*(79-81)*. In this study, most patients received folic acid as a comedication with phenytoin and carbamazepine, which may contribute to decrease in serum phenytoin concentration. Many researchers have indicated that folic acid is a cofactor in phenytoin metabolism; addition of folic acid causes an increase in the affinity of enzyme complex resulting in a decrease in K_m and serum phenytoin concentration; whereas, it has no effect on the V_{max} of metabolism*(79-82)*. This study used the population K_m (4mg/mL) for calculating the V_{max}, thus, the supplementation of folic acid might not affect the V_{max} value in this study.

The results of the second objective showed that carbamazepine clearance of a combination therapy group was significantly higher than that of monotherapy group (105.35 mg/mL and 76.45 mg/mL; P = 0.014). This finding is consistent with previous studies which mentioned that phenytoin induces carbamazepine clearance*(13, 15-17)*. In contrast, the V_{max} between combination therapy group and monotherapy group were not significantly different (362.80 mg/d and 331.64 mg/d; P = 0.170). This result can be supported by numerous studies which discussed that the effects of carbamazepine on phenytoin are controversial. Some researchers have reported that carbamazepine induces phenytoin metabolism*(14, 83)*, others have found that phenytoin level is elevated during comedication with carbamazepine due to the competitive metabolism of carbamazepine*(18, 20, 21)*.

Although the retrospective and prospective data used different methods to analyze serum drug levels, a study from Beckman Coulter ; Inc. *(84, 85)* indicated that the serum drug levels obtained from Synchron CX systems and Abbott TDx were strongly correlated (r = 0.992 and 0.997 for carbamazepine and phenytoin respectively). Thus, the difference between two assay methods may not affect pharmacokinetic parameters in this study.

However, this study is limited by the small number of patients, the use of retrospective data to compare the difference of Cl_{CBZ} and V_{max} between combination therapy group and monotherapy group and the use of population K_m for calculating V_{max}. This result should be confirmed by a prospective study in a large cohort of patients.

Relationship between V_{max} and Cl_{CBZ}

The relationship between maximum rate of metabolism of phenytoin (mg/kg/d) and carbamazepine clearance (L/kg/d) in this study was high and significant (r = 0.828; p = 0.001). This finding is supported by the assumption that both phenytoin and carbamazepine are largely metabolized by liver.

Phenytoin is 90% metabolized by cytochrome P450 mixed function oxidase isoenzyme CYP2C9 (major) and CYP2C19 (minor)*(8)*. While carbamazepine is about 65% metabolized via CYP3A4 (major) and CYP2C8 (minor) and 15% via conjugation with glucuronic acid and sulfate pathways*(63)*. Although they use different cytochrome P450 isoenzymes in metabolism, this study assumes that in the same patients, if their liver functions are good their whole liver enzyme would be likewise. Moreover, both phenytoin and carbamazepine metabolisms involve hydroxylation via an arene oxidase enzyme which is the rate limiting step of phenytoin metabolism*(8, 11, 86)*. Thus, it is possible that the high correlation coefficient found in this study is due to the same enzyme used in the hydroxylation process of metabolism.

Three serum drug concentrations were retrospective data (patient no. 8, 9, 12) and two serum drug concentrations were obtained from patients whose age less than 12 years (patient no. 1, 7). However, this may not affect the correlation between V_{max} and Cl_{CBZ} in this study since the correlation coefficient was still high and significant after excluded retrospective data and patients whose age less than 12 years.

CHAPTER VI

CONCLUSION

Correlation between maximum rate of metabolism of phenytoin and carbamazepine clearance was examined in sixteen epileptic patients (nine men and seven women) receiving phenytoin in combination with carbamazepine at Prasart Neurological Institute during January to June 2007. All subjects had normal hepatic and renal function and were studied during steady-state condition. None of them smoked or consumed caffeine containing substances. Most patients had a generalized tonic-clonic seizure. Their age ranged from 10.6 to 62 years (mean 32.89 ± 15.75). Their weight ranged from 34 to 67 years (mean 52.81 ± 8.59). Serum phenytoin concentrations at the steady-state ranged from 2.08-6.67 mg/L. Maximum rate of metabolism of phenytoin calculated by Michaelis-Menten equation ranged from 211.74 – 445.85 mg/d. Serum carbamazepine concentrations at steady-state ranged from 6.45-21.28 mg/L. Carbamazepine clearance calculated by steady-state continuous infusion equation ranged from 59.53 – 193.94 L/d. A high correlation coefficient between maximum rate of metabolism of phenytoin and carbamazepine clearance was found ($r = 0.804$; $p < 0.001$). When divided V_{max} and Cl_{CBZ} with patient's weight, the correlation coefficient was slightly increase ($r = 0.828$; $P = 0.001$). Regression equation indicated that the maximum rate of metabolism of phenytoin can be acculately predicted from carbamazepine clearance, and vice versa.

To compare the pharmacokinetic parameters between monotherapy group and combination therapy group, retrospective data were used. All patients had no report impaired hepatic and renal function. Two groups were matched for age and weight. Unpaired t-test was used to compare the differences between monotherapy group and combination therapy group. The average age, weight and dosage of drugs were not significantly different between monotherapy group and combination therapy group. Maximum rate of metabolism of phenytoin in monotherapy group and combination therapy group were not significantly different ($P = 0.170$; unpaired t-test). However, the maximum rated of metabolism of monotherapy group was lower than that from combination therapy group (331.64 versus 362.80 mg/d). In contrast, carbamazepine clearance of monotherapy group and combination therapy group were significantly different ($p = 0.014$; unpaired t-

test). Carbamazepine clearance of combination therapy group was higher than that of monotherapy group.

The limitations of this study include the followings:

1) Regression equations for predicting pharmacokinetic parameters of carbamazepine from phenytoin in this study were performed by using the patients who had V_{max} in the range of 211.74 to 445.85 mg/d. Thus, extrapolation to patient who has V_{max} below or above the range should be done with caution.
2) The comparison of pharmacokinetic parameters between monotherapy group and combination therapy group was done in a different group of patients. There may be some variations among individuals due to their genetic background and environmental factor; for example: duration of drug therapy and underlying diseases.
3) This study used retrospective data for comparing the pharmacokinetic parameters between monotherapy group and combination therapy group. Hence, data obtained may lack some information.
4) This study recruited only patients with normal liver function. Thus, using equations obtained from this study should be done with caution in patients with poor liver function.
5) The population K_m was used to calculate V_{max}. This may affect the value of V_{max} obtained and in turn affect the correlation between V_{max} and Cl_{CBZ}.

Considerations for further studies:

1) Further study to compare the difference of pharmacokinetic parameters between monotherapy group and combination therapy group should be done in the same group of patients to prevent individual's variations.
2) The predictive equations from this study should be further evaluated to determine the accuracy and precision.
3) Future study to investigate the correlation between V_{max} and Cl_{CBZ} should collect 2 serum phenytoin concentrations in a different dose to calculate individual K_m. This might result in a more accurately predictive equation.

REFERENCES

[1] Stephen, L.J., and Brodie, M.J. Seizure freedom with more than one antiepileptic drug. Seizure 11 (2002): 349-51.

[2] Czuczwar, S.J., and Borowicz, K.K. Polytherapy in epilepsy: the experimental evidence. Epilepsy Res 52 (2002): 15-23.

[3] Kwan, P., and Brodie, M.J. Epilepsy after the first drug fails: substitution or add-on? Seizure 9 (2000): 464-8.

[4] Deckers, C.L.P., et al. Selection of antiepileptic drug polytherapy based on mechanisms of action: The evidence reviewed. Epilepsia 41 (2000): 1364-74.

[5] Mattson, R.H. Combination therapy with antiepileptic drugs: potential advantages and problems. In Majkowski, J., Bourgeois, B., Patsalos, P. and Mattson, R. (eds), Antiepileptic Drugs Combination Therapy and Interactions, pp. 16-25. New York: Cambridge University Press, 2005.

[6] Froscher, W. Drug monitoring in combination therapy. In Majkowski, J., Bourgeois, B., Patsalos, P. and Mattson, R. (eds), Antiepileptic Drugs Combination Therapy and Interactions, pp. 392-402. New York: Cambridge University Press, 2005.

[7] Morselli, P.L., and Bossi, L. Carbamazepine absorption, distribution, and excretion. In: Woodbury, D.M., Penry, J.K., and Pippenger, C.E. . (eds), Antiepileptic Drugs, pp. 465-82. New York: Raven Press, 1982.

[8] Kutt, H., and Harden, C.L. Phenytoin and Congeners. In Eadie, M.J. and Vajda, F.J.E. (eds), Antiepileptic Drugs Pharmacology and Therapeutics, pp. 229-43. Berlin: Springer-Verlag, 1999.

[9] Dickinson, R.G., Eadie, M.J., and Vajda, F.J.E. Carbamazepine. In Eadie, M.J. and Vajda, F.J.E. (eds), Antiepileptic Drugs Pharmacology and Therapeutics pp. 267-317. Berlin: Springer-Verlag, 1999.

[10] Chang, T. and Glazko, A.J. Phenytoin biotransformation. In Woodbury, D.M., Penry, J.K., and Pippenger, C.E. (eds), Antiepileptic Drugs, pp. 209-26. New York: Raven Press, 1982.

[11] Faigle, J.W., and Feldmann, K.F. Carbamazepine Biotransformation. In Woodbury, D.M., Penry, J.K., and Pippenger, C.E. (eds), Antiepileptic Drugs, pp. 483-95. New York: Raven Press, 19821. .

[12] Woodbury, D.M. Phenytoin absorption, distribution, and excretion. In: Woodbury, D.M., Penry, J.K., and Pippenger, C.E. (eds), Antiepileptic Drugs, pp. 191-207. New York: Raven Press, 1982.

[13] Bertilsson, L., and Tomson, T. Clinical Pharmacokinetics and Pharmacological Effects of Carbamzepine and Carbamazepine-10,11-Epoxide. Clin Pharmacokinet 11 (1986): 177-98.

[14] Cereghino, J.J., Brock, J.T., Van Meter, J.C., Penry, J.K., Smith, L.D., and White, B.G. The efficacy of carbamazepine combination in epilepsy. Clin Pharmacol Ther 18 (1975): 733-41.

[15] Bertilsson, L. Clinical Pharmacokinetics of Carbamazepine. Clin Pharmacokinet 3 (1978): 128-43.

[16] Spina, E., Pisani, F., and Perucca, E. Clinically significant pharmacoketic drug interactions with carbamazepine. Clin Pharmacokinet 31 (1996): 198-214.

[17] Ichikou, N., Ieiri, I., Higuchi, S., Hirata, K., Yamada, H., and Aoyama, T. Analysis of the factors influencing antiepileptic drug concentrations-carbamazepine. J Clin Pharm Ther 15 (1990): 337-49.

[18] Gratz, E.S., Theodore. W.H., Newmark, M.E., Kupferberg. H.J., Porter, R.J., and QU, Z. Effect of carbamazepine on phenytoin clearance in patients with complex partial seizure. Neurology 32 (1982): A223.

[19] Lai, M.L., Lin, T.- S., and Huang, J.D. Effect of single-and multiple-dose carbamazepine on the pharmacokinetics of diphenylhydantoin. J Clin Pharmacol 43 (1992): 201-03.

[20] Browne, T.R., Szabo, G.K., Evans, J.E.,Greenblat, D.J., and Mikati, M.A. Carbamazepine increases phenytoin serum concentration and reduces phenytoin clearance. Neurology 38 (1988): 1146-50.

[21] Lakehal, F., Wurden, C.J., Kalhorn, T.F., and Levy, R.H. Carbamazepine and oxcarbazepine decrease phenytoin metabolism through inhibition of CYP2C19. Epilepsy Res 52 (2002): 79-83

[22] Wells, B.G. Neurologic disorders. In: Wells, B.G., Dipiro, J.T., Schwinghammer, T.L., and Hamilton, C.W. (eds), Pharmacotherapy Handbook, pp. 516-34. New York: McGraw-Hill, 2006.

[23] Ferrendelli, J.A. Relating pharmacology to clinical practice: The pharmacologic basis of rational polypharmacy. Neurology 45 (1995): S12-S6.

[24] French, J. The long-term therapeutic management of epilepsy. Ann Int Med 120 (1994): 411-22.

[25] Shorvon, S.D. Epidemiology, classification, natural history, and genetics of epilepsy. The Lancet 336 (1990): 93-6.

[26] Proposal for revised clinical and electroencephalographic classification of epileptic seizures. From the Commission on Classification and Terminology of the International League Against Epilepsy
Epilepsia 22 (1981): 489-501.

[27] สมาคมโรคลมชักแห่งประเทศไทย. แนวทางการบริหารยากันชัก. ใน: สมาคมโรคลมชักแห่งประเทศไทย สถาบันสุขภาพเด็กแห่งชาติมหาราชินี สำนักพัฒนาวิชาการแพทย์ กรมการแพทย์. แนวทางการรักษาโรคลมชัก. พิมพ์ครั้งที่ 1. กรุงเทพมหานคร: บริษัทสหธรรมิก จำกัด, 2547 : หน้า 29-37.

[28] Miller, J., Snow, E.K., and Litvak, K., eds. AHFS drug information 2005. pp. 2130-3. Bethesda: American Society of Health-System Pharmacists, 2004.

[29] Hunt, E.A. Phenytoin in traumatic brain injury. Arch Dis Child 86 (2002): 62-3.

[30] Chang, B.S. and Lowenstein, D.H. Practice parameter: Antiepileptic drug prophylaxis in severe traumatic brain injury. Neurology 60 (2003): 10-6.

[31] Sirven, J.I. and waterhouse, E. Management of status epilepticus. American Family Physician 68 (2003): 469-76.

[32] Merrison, A.F.A. and Fuller, G. Treatment options for trigeminal neuralgia. BMJ 327 (2003): 1361-2.

[33] Bachmann, K.A., and Belloto, R.J. Differential kinetics of phenytoin in elderly patients. Drugs Aging 15 (1999): 235-50.

[34] Battino, D., Croci, D., Rossini, A., Messina, S., Mamoli, D., and Perucca, E. Serum carbamazepine concentrations in elderly patients: A case- matched

pharmacokinetic evaluation based on therapeutic drug monitoring data. Epilepsia 44 (2003): 923-9.

[35] Birnbaum, A. et.al. Variability of total phenytoin serum concentrations within elderly nursing home residents. Neurology 60 (2003): 555-9.

[36] Bauer, L.A. and Blouin, R.A. Age and phenytoin kinetics in adult epileptics. Clin Pharmacol Ther 31 (1982): 301-4.

[37] Houghton, G.W. and Richen, A. Effect of age, height, weight and sex on serum phenytoin concentration in epileptic patients. Br J Clin Pharmacol 2 (1975): 251-6.

[38] Battino, D., Croci, D., Messina, S., Mamoli, D., and Perucca, E. Influence of aging on serum phenytoin concentrations: a pharmacokinetic analysis based on therapeutic drug monitoring data. Epilepsy Res 59 (2004): 155-65.

[39] Dodson, W.E. Nonlinear kinetics of phenytoin in children. Neurology 32 (1982): 42-8.

[40] Winter, M.E. Phenytoin. In Troy, D.B. (eds), Basic clinical pharmacokinetics, pp. 321-63. Philadelphia: Lippincot Williams & Wilkins, 2004.

[41] Martin, E., Tozer, T.N., Sheiner, L.B. and Riegelman, S. The clinical pharmacokinetics of phenytoin. J Pharmacokinet Biopharm 5 (1977): 579-96.

[42] Kutt, H. Phenytoin: Interactions with other drugs. In woodbury, D.M., Penry, J.K., and Pippenger, C.E. (eds), Antiepileptic Drugs, pp. 227-40. New York Raven Press, 1982.

[43] Nation, R.L., Evans, A.M.,and Milne, R.W. Pharmacokinetic drug interaction with phenytoin (part I). Clin Pharmacokinet 18 (1990): 131-50.

[44] Patsalos, P.N. and Perucca, E. Clinically important drug interactions in epilepsy: general features and inteactions between antiepileptic drugs. The Lancet 2 (2003): 347-56.

[45] Nation, R.L., Evans, A.M.,and Milne, R.W. Pharmacokinetic drug interaction with phenytoin (part II). Clin Pharmacokinet 18 (1990): 131-50.

[46] Perucca, E. Clinically relevant drug interactions with antiepileptic drugs. Br J Clin Pharmacol 61 (2005): 246-55.

[47] Tatro, D.S. and Carlos, S.eds. Drug Interaction Facts 2001. pp. 643, 5, 83. St. Louis: Facts and Comparisons, 2001.

[48] Glick, T.H., Workman, T.P., and Gaufberg, S.V. Preventing phenytoin intoxication: Safer use of a familiar anticonvulsant. The Journal of Family Practice 53 (2004): 197-202.

[49] Winter, M.E. and Tozer, T.N. Phenytoin. In Burton, M.E., Shaw, L.M., Schentag, J.J. and Evans, W.E. (eds), Applied Pharmacokinetics and pharmacodynamics.Principles of therapeutic drug monitoring, pp. 463-90. Philadelphia: Lippincot Williams and Wilkins, 2006.

[50] Winter, M.E. Phenytoin and Fosphenytoin. In Murphy, J.E. (eds), Clinical Pharmacokinetics Pocket Reference, pp. 285-303. Bethesda: American Sociey of Health-System Pharmacists, 2001.

[51] Schmidt, D. and Haenel, F. Therapeutic plasma levels of phenytoin, phenobarbital, and carbamazepine: Individual variation in relation to seizure frequency and type. Neurology 34 (1984): 1252-55.

[52] Schmidt, D. and Seldon, L. Phenytoin. In (eds), Adverse effects of antiepileptic drugs pp. 134-40. New york: Raven Press, 1982.

[53] Pisciotta, A.V. Phenytoin: Hematological toxicity. In woodbury, D.M., Penry, J.K., and Pippenger, C.E. (eds), Antiepileptic drugs, pp. 257-68. New York: Raven Press, 1982.

[54] Kutt, H. and Kutt, H.P. Carbamazepine. Chemistry and Methods of Determination. In woodbury, D.M., Penry, J.K., and Pippenger, C.E. (eds), Antiepileptic Drugs, pp. 453-63. New York: Raven Press, 1982.

[55] Miller, J., Snow, E.K., and Litvak, K. eds AHFS drug information 2005. pp. 2410-4 Bethesda: American Society of Health-System Pharmacists, 2005.

[56] Green, M.W. and Selman, J.E. Review article: the medical management of trigeminal neuralgia. Headache 31 (1991): 588-92.

[57] Garcia-Borreguero, D., Bronisch, T., Apelt, S., Yassouridis, A. and Enwich, H.M. Treatment of benzodiazepine withdrawal symptoms with carbamazepine. Eur Arch Psychiatry Clin Neurosci 241 (1991): 145-50.

[58] Whales, J.K. Treatment of diabetes insipidus with carbamazepine. Lancet 4 (1975): 948-51.

[59] Winter, M.E. Carbamazepine. In Troy, D.B. (eds), Basic clinical pharmacokinetics, pp. 172-82. Philadelphia: Lippincot Williams & Wilkins, 2004.

[60] Bertilsson, L., Tomson, T., and Tybring, G. Pharmacokinetics: Time-Dependent Changes-Autoinduction of Carbamazepine Epoxidation. J Clin Pharmacol 26 (1986): 459-62.

[61] Levy, R.H., and Pitlick, W.H. Carbamazepine: Interactions with other drugs. In: Woodbury, D.M., Penry, J.K., and Pippenger, C.E. (eds), Antiepileptic Drugs, pp. 497-505. New York: Raven Press, 1982.

[62] Cereghino, J.J. Carbamazepine: Relation of plasma concentration to seizure control. In: Woodbury, D.M., Penry, J.K., and Pippenger, C.E. (eds), Antiepileptic Drugs, pp. 507-19. New York: Raven Press, 1982.

[63] Garnett, W.R. Carbamazepine. In Murphy, J.E. (eds), Clinical Pharmacokinetics Pocket Reference, pp. 97-117. Bethesda: American Sociey of Health-System Pharmacists, 2001.

[64] Tomson, T. Interdosage fluctuations in plasma carbamazepine concentration determine intermittent side effects. Arch Neurol 41 (1984): 830-4.

[65] Haefeli, W.E., Meyer, P.G., and Luscher, T.F. Circadian carbamazepine toxicity. Epilepsia 35 (1994): 400-2.

[66] Glazko, A.J. Phenytoin: Chemistry and methods of determination. In woodbury, D.M., Penry, J.K., and Pippenger, C.E. (eds), Antiepileptic Drugs, pp. 177-89. New York: Raven Press, 1982.

[67] Loomis, K.F., and Frye, P.M. Evaluation of the Abbott TDx^{TM} for the stat measurement of phenobarbital, phenytoin, carbamazepine, and theophylline. Am J Clin Patho 80 (1983): 686-91.

[68] Lu-Steffes, M. et.al. Fluorescence polarization immunoassay IV. Determination of phenytoin and phenobarbital in human serum and plasma. Clin chem 28 (1982): 2278-82.

[69] Coper, H., Von Herrath, U. Pharmacokinetic investigation on the combination of diphenylhydantoin and carbamazepine. Epilepsia 14 (1973): 466-7.

[70] Almeidia, A.M., Falcão, A. C., Sales, F., Leitão, F., Santos, J., Costa, I. M., Caramona, M. M. Kinetic profile of carbamazepine in an adult Portuguese outpatient population. J Clin Pharm Ther 23, 4 (August 1998): 315-21.

[71] Hoppener, R.J. et al. Correlation between the daily fluctuations of carbamazepine serum levels and intermittent side effects. epilepsia 21 (1980): 341-50.

[72] Kanjanasilp, J., Preechagoon, Y., Kaewvichit, S., and Richards, R.M.E. Population pharmacokinetics of phenytoin in Thai epileptic patients. CMU 4, 3 (2005): 287-97.

[73] Yukawa, E., Higuchi, S., and Aoyama, T. Population pharmacokinetics of phenytoin from routine clinical data in Japan [Abstract]. J Clin Pharm Ther 14 (1989): 71-7.

[74] Odani, A., Hashimoto, Y., Takayanagi, K., Otsuki, Y., Koue, T., Takano, M., and Yasuhara, M. Population pharmacokinetics of phenytoin in Japanese patients with epilepsy: Analysis with a dose-dependent clearance model. Biol Pharm Bull 19, 3 (Mar 1996): 444-8.

[75] Ismail, R., Rahman, A.F.A., and Chand, P. Pharmacokinetics of phenytoin in routine clinic patients in Malaysia. J Clin Pharm Ther 19 (1994): 245-48.

[76] Ismail, R., and Rahman, A.F.A. Estimation of population pharmacokinetics for carbamazepine in Malaysian patients using the OPT computer program. J Clin Pharm Ther 18 (1993): 55-8.

[77] Deleu, D., Arons, L., and Ahmed, I.A. Population pharmacokinetics of free carbamazepine in adult Omani epileptic patients. Eur J Clin Pharmacol 57 (2001): 243-8.

[78] Chan, E., Lee, H.S., Hue, S.S. Population pharmacokinetics of carbamazepine in Singapore epileptic patients. Br J Clin Pharmacol 51, 6 (June 2001): 567-76.

[79] Seligmann, H., Potasman, I., Weller, B., Schwartz, M., and Prokocimer, M. Phenytoin-folic acid interaction: a lesson to be learned. Clin Neurophamacol 22 (sept-oct 1999): 268-72.

[80] Rivey, M.P., Schottelius, D.D., and Berg, M.J. Phenytoin-folic acid: a review. Drug Intell Clin Pharm 18 (Apr 1984): 292-301.

[81] Berg, M.J., Fincham, R.W., Eber, B.E., and Schottelius, D.D. Phenytoin pharmacokinetics: before and after folic acid administration. epilepsia 33, 4 (1992): 712-20.

[82] Lewis, D.P., Van Dyke, D.C., Willhite, L.A., Stumbo, P.J., and Berg, M.J. Phenytoin-folic acid interaction. Ann Pharmacother 29 (1995): 726-35.

[83] Eichelbaum, M., Kothe, K.W., Hoffmann, F., and Unruth, G.E. Kinetics and metabolism of carbamazepine during combined antiepileptic drug therapy. Clin Pharmacokinet 36 (1979): 366-71.

[84] Carmazepine. Beckman Coulter™ [online]. 2002. Available from: http://www.beckmancoulter.com.

[85] phenytoin. Beckman Coulter™ [online]. 2002. Available from: http://www.beckmancoulter.com.

[86] จุฑามณี สุทธิสีสังข์. ยากันชัก. ใน: จุฑามณี สุทธิสีสังข์, รัชนี เมฆมณี, บรรณาธิการ. เภสัชวิทยา เล่ม 1. พิมพ์ครั้งที่ 2. กรุงเทพมหานคร : บริษัท นิวไทยมิตรภาพการพิมพ์ จำกัด, 2542: หน้า 230-274.

APPENDICES

APPENDIX A

แบบฟอร์มบันทึกข้อมูลผู้ป่วย

ส่วนที่ 1

ชื่อ-สกุล..... HN..... AN.....เพศ..... อายุ.....ปี
ที่อยู่ปัจจุบัน.....เบอร์โทรศัพท์.....
น้ำหนัก.....กิโลกรัม ส่วนสูง.....เมตร
การศึกษา.....อาชีพ.....รายได้ผู้ป่วยต่อเดือน.....
ประวัติโรคลมชักในครอบครัว .....
โรคประจำตัว .....
สาเหตุการชักครั้งแรก .....
เริ่มชักครั้งแรก.....ความถี่ของการชักแต่ละครั้ง.....
ระยะเวลาของการชักแต่ละครั้ง.....
ลักษณะและชนิดของโรคลมชัก .....
ประวัติการใช้ยา .....
.....
ประวัติการแพ้ยา .....
.....
สูบบุหรี่

❑ สูบ ❑ ไม่สูบ

จำนวนที่สูบ (มวน/สัปดาห์).....
ดื่มสุรา

❑ ดื่ม ❑ ไม่ดื่ม

จำนวนที่ดื่ม .....

หมายเหตุ

.....
.....
.....
.....

แบบบันทึกการใช้ยาและ การคุมอาการชัก

Medication / Date							
1.							
2.							
3.							
4.							
5.							
6.							
7.							
8.							
9.							
อาการชัก (ครั้ง) นาน (นาที)							

ผลตรวจทางห้องปฏิบัติการ

วันที่							
Phenytoin level							
Carbamazepine level							
ALT (5-40 IU/L)							
AST (5-40 IU/L)							
Serum Albumin (3.5-5.0 g/dl)							

แบบบันทึกอาการไม่พึงประสงค์จากการใช้ยา

อาการไม่พึงประสงค์	PHT	CBZ	PHT + CBZ
Nystagmus			
Ataxia			
Diplopia			
Confusion			
Dizziness			
Drowsiness			
Headache			

<u>ส่วนที่ 2</u>

การเก็บตัวอย่างเลือดและระดับยาในเลือด

ลำดับ	วันที่	เวลาที่รับประทานยา		เวลาที่เจาะเลือด		ระดับยา	
		PHT	CBZ	PHT	CBZ	PHT	CBZ
1							
2							
3							
4							

APPENDIX B

หนังสือแสดงเจตนายินยอมเข้าร่วมการวิจัย (Consent form)

ชื่อโครงการวิจัย การหาความสัมพันธ์ระหว่างพารามิเตอร์ทางเภสัชจลนศาสตร์ของยาเฟนิทอยน์และยาคาร์บามาเซปีนในผู้ป่วยโรคลมชักที่ได้รับยาทั้งสองชนิดร่วมกัน

วันที่ลงนาม.....

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตราย หรืออาการที่อาจเกิดขึ้นจากการวิจัย หรือจากยาที่ใช้ รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว

ผู้วิจัยรับรองว่าจะตอบคำถามต่างๆที่ข้าพเจ้าสงสัยด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ

ข้าพเจ้ามีสิทธิที่จะบอกเลิกเข้าร่วมในโครงการวิจัยเมื่อใดก็ได้ และเข้าร่วมโครงการวิจัยนี้โดยสมัครใจ และการบอกเลิกการเข้าร่วมการวิจัยนี้ จะไม่มีผลต่อการรักษาโรคที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับ และจะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่างๆที่เกี่ยวข้อง กระทำได้เฉพาะกรณีจำเป็นด้วยเหตุผลทางวิชาการเท่านั้น

ผู้วิจัยรับรองว่าหากเกิดอันตรายใดๆจากการวิจัยดังกล่าว ข้าพเจ้าจะได้รับการรักษาพยาบาลตามความเหมาะสม

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ และได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

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

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

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

ในกรณีที่ผู้เข้าร่วมโครงการวิจัยไม่สามารถลงลายมือชื่อด้วยตนเองได้ ให้ผู้แทนโดยชอบตามกฎหมายซึ่งมีส่วนเกี่ยวข้องเป็น.....ของผู้เข้าร่วมโครงการวิจัย เป็นผู้ลงนามแทน

ลงชื่อ.....ผู้แทนโดยชอบธรรม

(.....ชื่อ-นามสกุล ตัวบรรจง)

ลงชื่อ.....พยาน

(.....ชื่อ-นามสกุล ตัวบรรจง)

APPENDIX C

Bioanalysis of phenytoin

Analytical Method

Phenytoin serum levels were determined by immunoassay method using TDx Analyzer system, Abbott Laboratoories based on fluorescence polarization technique. The equipments consisted of carousel, cuvettes, sample catridges, reagent pack, calibrators and controls.

1. Calibration.

Calibration were performed follow manual of using TDx analyzer. An acceptable phenytoin assay calibration curve should meet the following criteria:

a) Polarization Error (PERR) -2.00 to +2.00 for all calibrators.

b) Root Mean Square Error (RMSE) less than or equal to 1.00.

c) All controls are within the acceptable ranges.

The following three levels of phenytoin control solution (L, M and H) were measured for their phenytoin concentration and compared with the standard range of phenytoin control concentration.

Control	Phenytoin concentration (μg/mL)	
	Standard	Study
L	6.75 – 8.25	7.76 ± 0.36
M	13.50 – 16.50	14.93 ± 0.58
H	27.00 – 33.00	29.48 ± 1.10

2. Sensitivity

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence and was determined to be 0.5 μg/mL.

3. Precision

Precision was determined as described in National Committee for Clinical Laboratory Standard (NCCLS) protocol EP5-T using human serum with 7.5, 15.0, and 30.0 μg/mL of phenytoin added. Results from these studies typically yielded CV's of less than 5%. (CV's of from this study was 4.64, 3.88, and 3.73 for low, medium, and high concentration, respectively)

4. Accuracy by recovery

Recovery was determined by adding phenytoin to human serum at clinically relevant concentrations and assaying in replicates of five. Recoveries were found to be quantitative. The average recovery is 99.3 ± 2.5%.

Chemical and reagents of phenytoin

1) No. 9507 - 10, Phenytoin calibrators (Lot.no. 40012Q100)

Six levels of accurately measured amounts of phenytoin in human serum at the following concentrations have been prepared to be calibrators.

Cal	Phenytoin concentrations (μg/ml)
A	0.0
B	2.5
C	5.0
D	10.0
E	20.0
F	40.0

Preservative: Sodium Azide

2) No. 9507 - 10, Phenytoin controls (Lot.no. 40013Q100)

Three levels of phenytoin in human serum should read within the following range.

QC	Target Conc. (μg/ml)	Range (μg/ml)
L	7.5	6.75-8.25
M	15.0	13.50-16.50
H	30.0	27.00-33.00

Preservative: Sodium Azide

3) No. 9507 - 60, Phenytoin reagent pack (Lot.no. 40472Q100)

The reagent pack consists of three vials as following:

Vials	Contents
S	< 1% Phenytoin antiserum(sheep) in buffer with protein stabilizer (4.20 mL) Preservative: Sodium azide
T	< 0.01% Phenytoin fluorescein tracer in buffer containing surfactant and protein stabilizer (3.0 mL) Preservative: Sodium azide
P	Pretreatment solution. Surfactant in buffer containing protein stabilizer (3.0 mL) Preservative: Sodium azide

4) No 9519, X SYSTEMS dilution buffer (Lot.no. 33148M202)

Bovine gamma globulin in phosphate buffer is used as a buffer solution and has been prepared with sodium azide

Instrument

Automated Fluorescense Polarization Analyzer (Diagnostic Division Abbott laboratories, Inc, TDx, U.S.A. Serial No. 18488)

APPENDIX D

Bioanalysis of carbamazepine

Analytical Method

Carbamazepine serum levels were determined by immunoassay method using TDx Analyzer system, Abbott Laboratoories based on fluorescence polarization technique. The equipments consisted of carousel, cuvettes, sample catridges, reagent pack, calibrators and controls.

1. Calibration.

Calibration were performed follow manual of using TDx analyzer. An acceptable phenytoin assay calibration curve should meet the following criteria:

a) Polarization Error (PERR) -2.00 to +2.00 for all calibrators.

b) Root Mean Square Error (RMSE) less than or equal to 1.00.

c) All controls are within the acceptable ranges.

The following three levels of carbamazepine control solution (L, M and H) were measured for their carbamazepine concentration and compared with the standard range of carbamazepine control concentration.

Control	carbamazepine concentration (μg/mL)	
	Standard	Study
L	2.55 – 3.45	2.96 ± 0.20
M	5.40 – 6.60	5.95 ± 0.34
H	14.40 – 17.60	15.90 ± 0.82

2. Sensitivity

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence and was determined to be 0.5 μg/mL.

3. Precision

Precision was determined as described in National Committee for Clinical Laboratory Standard (NCCLS) protocol EP5-T using human serum with 3.6, 6.0, and 16.0 μg/mL of carbamazepine added. Results from these studies typically yielded CV's of less than 7%. (CV's of from this study was 6.76, 5.71, and 5.16 for low, medium, and high concentration, respectively)

4. Accuracy by recovery

Recovery was determined by adding carbamazepine to human serum at clinically relevant concentrations and assaying in replicates of five. Recoveries were found to be quantitative. The average recovery is 101.1 ± 1.3%.

Chemical and reagents of carbamazepine

1) No. 9515 - 01, Carbamazepine calibrators (Lot.no. 38476Q100)

Six levels of accurately measured amounts of carbamazepine in human serum at the following concentrations have been prepared to be calibrators.

Cal	Carbamazepine concentrations (μg/ml)
A	0.0
B	2.0
C	4.0
D	8.0
E	12.0
F	20.0

Preservative: Sodium Azide

2) No. 9515 -10, Carbamazepine controls (Lot.no. 37872Q100)

Three levels of carbamazepine in human serum should read within the following range.

QC	Target Conc. (μg/ml)	Range (μg/ml)
L	3.0	2.55-3.45
M	6.0	5.40-6.60
H	16.0	14.40-17.60

Preservative: Sodium Azide

3) No. 9515 - 60, Carbamazepine reagent pack (Lot.no. 44175Q100)

The reagent pack consists of three vials as following:

Vials	Contents
S	< 1% Carbamazepine antiserum(sheep) in buffer with protein stabilizer (4.0 mL) Preservative: Sodium azide
T	< 0.01% Carbamazepine fluorescein tracer in buffer containing surfactant and protein stabilizer (3.5 mL) Preservative: Sodium azide
P	Pretreatment solution. Surfactant in buffer containing protein stabilizer (2.5 mL) Preservative: Sodium azide

4) No 9519, X SYSTEMS dilution buffer (Lot.no. 33148M202)

Bovine gamma globulin in phosphate buffer is used as a buffer solution and has been prepared with sodium azide

Instrument

Automated Fluorescense Polarization Analyzer (Diagnostic Division Abbott laboratories, Inc, TDx, U.S.A. Serial No. 18488)

APPENDIX E

Calculation of pharmacokinetic parameters

Example

Patient no.14: He received phenytoin SR. 300 mg once daily in the evening. Blood was taken in the morning, the concentration were 10.15 mg/L

1) Calculation of V_{max}

Michaelis-Menten equation was used to calculate V_{max}; when K_m is the population Michaelis constant which is 4 mg/L

$$V_{max}(mg/d) = \frac{[(SFD/\tau)(K_m + C_{SS})]}{C_{SS}}$$

$$= \frac{[(0.92)(1)(300)/1)(4+10.15)]}{10.15}$$

$$= 384.77 \text{ mg/d}$$

$$= 384.77/55$$

$$= 6.995 \approx 7 \text{ mg/kg/d}$$

Example

Patient no.11: He received carbamazepine CR. 400 mg twice daily. Blood was taken in the morning, the concentration were 7.40 mg/L

2) Calculation of Cl_{CBZ}

Steady-state continuous infusion equation was used to calculate carbamazepine clearance

$$Cl(L/d) = \frac{(S)(F)(D)}{(\tau)(c_{ss\,ave})}$$

When; F = 0.8, and S = 1.0

$$Cl(L/d) = \frac{(1)(0.8)(400)}{(1/2)(7.40)}$$

$$= 86.49 \text{ L/d}$$

$$= 86.49/55$$

$$= 1.57 \text{ L/kg/d}$$

APPENDIX F

Applied equation obtained from this study to design optimum dosage regimen of both drugs

Using pharmacokinetic parameter of one drug to predict pharmacokinetic parameter of another drug and there after to calculate the optimum dosage regimen of both drugs

Example

Patient no.11: His maximum rate of metabolism calculated from serum phenytoin concentration is 7 mg/kg/d

Using predictive equation obtained from this study to calculate Cl_{CBZ}

$$Cl_{CBZ}\ (L/kg/d) = 0.483\ V_{max} - 1.340$$

$$= 0.483(7) - 1.340$$

$$= 2.041\ L/kg/d = 2.041*55 = 112.26\ L/d$$

If the concentration of carbamazepine was proposed to be 8 mg/L; the calculated dose for carbamazepine was

$$D = \frac{(\tau)(c_{ss\ ave})(Cl)}{(S)(F)}$$

$$= \frac{(1)(8)(112.26)}{(1)(0.8)}$$

$$= 1122.6\ mg/d \approx 1200\ mg/d$$

For convenience, carbamazepine dose should be given 600 mg twice daily.

Example

Patient no.11: His carbamazepine clearance calculated from serum carbamazepine concentration is 1.57 L/kg/d

Using predictive equation obtained from this study to calculate V_{max}

$$V_{max}\ (mg/kg/d) = 1.421\ Cl_{CBZ} + 4.107$$

$$= 1.421(1.57) + 4.107$$

$$= 6.34\ mg/kg/d = 6.34*55 = 348.7\ mg/d$$

If the concentration of phenytoin was proposed to be 15 mg/L; the calculated dose for phenytoin was

$$D = \frac{(\tau)(c_{ss})(V_{max})}{(S)(F)(K_m + C_{ss})}$$

$$= \frac{(1)(15)(348.7)}{(0.92)(1)(4+15)}$$

$$= 299.22\ mg.$$

For convenience, phenytoin dose should be given 300 mg once daily.

VITAE

Miss Janthima Methaneethorn was born on the 26th of December in 1980 at Bangkok. She graduated with a Bachelor Degree in Pharmacy in 2003 from the Faculty of Pharmaceutical Sciences, Chulalongkorn University. After that, she worked at Ramathibodi Hospital for two years. Then she resigned her position to study for a Master Degree in Clinical Pharmacy, Department of Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University.