ค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของเฟนิทอยน์
ในเด็กไทยที่เป็นโรคลมชัก

นางสาว ภริษา วิสุทธิวงศ์
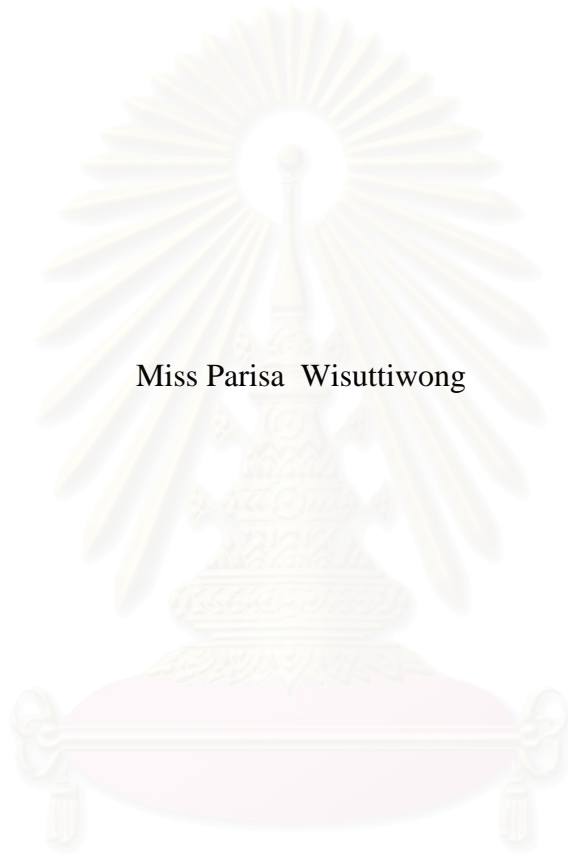
วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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PHARMACOKINETIC PARAMETERS OF
PHENYTOIN IN THAI EPILEPTIC CHILDREN

Miss Parisa Wisuttiwong

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
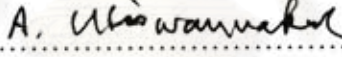Chulalongkorn University
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วัตถุประสงค์: ค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของเฟนิทอยน์ทั้งอัตราเร็วสูงสุดในการเมแทบอ
ลิซึม (V_{max}) และค่าคงที่ของมิเชลลิส-เม็นเท่น (K_m) มีความสำคัญในการกำหนดขนาดยา ทั้งนี้มีหลายปัจจัย
รวมทั้งด้านเชื้อชาติที่มีผลต่อค่าพารามิเตอร์เหล่านี้ ซึ่งการศึกษาในคนไทยยังมีน้อยโดยเฉพาะการศึกษาในเด็ก
ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อหาค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของเฟนิทอยด์ในเด็กไทยที่เป็น
โรคลมชัก รวมทั้งศึกษาปัจจัยที่มีผลต่อค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของเฟนิทอยน์

วิธีการดำเนินการวิจัย: การศึกษานี้ดำเนินการศึกษาในผู้ป่วยที่เข้ารับการรักษาในคลินิกระบบ
ประสาทเด็ก ณ โรงพยาบาลชลบุรีตั้งแต่เดือนตุลาคม พ.ศ.2550 ถึงเดือนมีนาคม พ.ศ.2551 ในการศึกษามี
จำนวนผู้ป่วยทั้งหมด 39 คน โดยผู้ป่วยทุกคนมีอายุต่ำกว่า 15 ปีและได้รับการรักษาด้วยเฟนิทอยด์เพียงอย่าง
เดียว ทั้งนี้ข้อมูลระดับยาในเลือดในสภาวะคงที่จำนวน 39 จุดที่ได้รับจากการเก็บข้อมูลแบบไปข้างหน้าใน
ผู้ป่วยจำนวน 39 คนถูกนำมาใช้วิเคราะห์ข้อมูลโดยโปรแกรม Nonlinear Mixed Effect Model (NONMEM)

ผลการศึกษา:การวิเคราะห์ข้อมูลค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของเฟนิทอยน์ในเด็กไทยที่เป็น
โรคลมชักโดยใช้ NONMEM พบอัตราเร็วสูงสุดในการเมแทบอลิซึมเท่ากับ 5.16 มิลลิกรัม/กิโลกรัม/วัน,
ค่าคงที่ของมิเชลลิส-เม็นเท่นเท่ากับ 4.5 ไมโครกรัม/มิลลิลิตร และปริมาตรการกระจายตัวเท่ากับ 0.31 ลิตร/
กิโลกรัมโดยมีค่าความแปรปรวนร้อยละ 94.33, 5.55 และ 29.86 ตามลำดับ การศึกษาปัจจัยด้านอายุ น้ำหนัก
และเพศที่มีผลต่อค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของเฟนิทอยน์ พบว่าอายุและเพศไม่มีความสัมพันธ์กับ
อัตราเร็วสูงสุดในการเมแทบอลิซึมและปริมาตรการกระจายตัว แต่พบว่าน้ำหนักตัวมีความสัมพันธ์อย่างมี
นัยสำคัญทางสถิติกับอัตราเร็วสูงสุดในการเมแทบอลิซึม(r=0.358, p=0.025) ในการพัฒนารูปแบบสมการ โดย
การเพิ่มน้ำหนักตัวในสมการพื้นฐานของอัตราเร็วในการเมแทบอลิซึมพบว่าการเปลี่ยนแปลงของค่า objective
function ไม่ลดลงอย่างมีนัยสำคัญทางสถิติ (p<0.05) ดังนั้นสมการที่ใช้ในการวิเคราะห์คือ สมการพื้นฐาน

สรุปผลการวิจัย: ค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของเฟนิทอยน์ในเด็กไทยที่เป็นโรคลมชักใน
การศึกษาครั้งนี้น่าจะเป็นประโยชน์ในการใช้คำนวณขนาดยาที่ถูกต้องและเหมาะสมแก่ผู้ป่วยเด็กไทยที่เป็น
โรคลมชัก อย่างไรก็ตามค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของเฟนิทอยน์จากการศึกษานี้ยังมีข้อจำกัด ดังนั้น
จึงควรมีการศึกษาเพิ่มเติมต่อในอนาคต

ภาควิชา............เภสัชกรรม...............ลายมือชื่อนิสิต...
สาขาวิชา.......เภสัชกรรมคลินิก.........ลายมือชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์หลัก.................
ปีการศึกษา...........2550.............ลายมือชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม...................

#4976616133: MAJOR CLINICAL PHARMACY
KEY WORD: PHARMACOKINETIC/ PARAMETERS/ PHENYTOIN/ CHILDREN/ NONMEM

PARISA WISUTTIWONG: PHARMACOKINETIC PARAMETERS OF PHENYTOIN IN THAI EPILEPTIC CHILDREN. THESIS PRINCIPAL ADVISOR: ASST. PROF. WANCHAI TREYAPRASERT, Ph.D., THESIS CO-ADVISOR: SIRIPORN PINJAROEN, M.D., 89 pp.

Objective: The pharmacokinetic parameters of phenytoin, V_{max} and K_m, are necessary for phenytoin maintenance dosage design. Many factors had influenced on these parameters. In addition, these parameters are claimed to be race dependent. Systematic study for these parameters in Thai epileptic patients are lacked especially in children. Therefore, this study was designed to determine pharmacokinetic parameters of phenytoin in Thai epileptic children and to study the factors which influence these two pharmacokinetic parameters of phenytoin

Method: The study was conducted in patients who attended at neurology clinic of children at Chonburi Hospital during October 2007 to March 2008. Total 39 patients participated throughout the study. All included patients were under 15 years old and received phenytoin monotherapy for epilepsy. There were 39 steady-state serum concentrations which collected prospectively from these 39 out-patients. Data was analysed using Nonlinear Mixed Effect Model (NONMEM), a computer program designed for pharmacokinetic analysis.

Results: For determination of pharmacokinetic parameters, a data set of steady-state serum concentrations were analysed using NONMEM. The estimated pharmacokinetic parameters of phenytoin in Thai epileptic children were V_{max}, K_m and Vd equaled to 5.16 mg/kg/d, 4.50 µg/mL and 0.31 L/Kg, respectively. The interindividual random variabilities of V_{max}, K_m and Vd were 94.33%, 5.55% and 29.86%, respectively. According to the results of exploring covariates, the influence of covariates including age, weight and gender were studied. It was found that age and gender were not significantly correlated with V_{max} and Vd while patient's body weight was statistically significant correlated with V_{max} (r=0.358, p = 0.025). For developing the model, body weight was added into the base model of V_{max}. The decrease of objective function value between base model and covariate model of V_{max} did not decreased by significantly (p<0.05). Therefore, the model that was selected and used to evaluate pharmacokinetic parameters was the base model.

Conclusion: The obtained V_{max} and K_m values in this study may be very useful for designing more accurate and appropriate dosage regimens for the treatment of Thai epileptic children. However pharmacokinetic parameters of phenytoin in this study may have limitations, the further studies should be performed in future.

Department:........Pharmacy...............Student's signature:......*Parisa Wisuttiwong*.....

Field of study:....Clinical Pharmacy...Principal advisor's signature:....*Wanchai*.....

Academic:.....…......2007...............Co-advisor's signature:......*Siriporn*.............

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

ADR	Adverse drug reaction
ALT	Alanine aminotransferase
Alb	Albumin
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
CI	Confidence interval
cm	Centrimeter
CNS	Central nervous system
conc	Concentration
C_{ss}	The average steady-state concentration
CV	Coefficient of variance
CYP450	Cytochrome P450
CYP2C9	Cytochrome P450 isoenzyme 2C9
CYP2C19	Cytochrome P450 isoenzyme 2C19
χ^2	Chi-square
d	Day
dL	Deciliter
τ	Dosing interval
EEG	Electroencephalographic
ε	Epsilon, the intraindividuall variability
η	ETA, the interindividual variability
EXP	Exponential
F	Female
FPIA	Fluorescence polarization immunoassay
g	Gram
hr	Hour
hs	Bedtime
kg	Kilogram
K_m	The Michaelis-Menten constant
L	Liter
M	Male

μg	Microgram
mg	Milligram
mL	Milliliter
No.	Number
NONMEM	Nonlinear Mixed Effect Model
OFV	Objective function value
pc	After meal
pt	Patient
r	Correlation coefficient
r^2	Coefficient of determination
SCr	Serum creatinine
SD	Standard deviation
SE	Standard error
SS	Steady-state
θ	THETA, fixed effect parameters on pharmacokinetic parameters
U/L	Unit per liter
Vd	Volume of distribution
V_{max}	The maximum rate of metabolism
WT	Weight
yr	Year

CHAPTER I

INTRODUCTION

Background and rationale

Epilepsy is an important neurological disorder that affects more than 50 million people worldwide. Many studies have found that incidence rates of epilepsy are about 20-70 cases per 100,000 per year and prevalence rates are 4-10 cases per 1,000 per year in general population *(1)*. Epidemiological studies have suggested that 4-8 children per 1,000 may be expected to experience an episode of epilepsy before the age of 15 years. Prevalence rates increase with age ranging from 2-3 cases per 1,000 in children up to 7 years of age to 4-6 cases per 1,000 at 11-15 years of age *(1-3)*.

In Thailand, the information from Chulalongkorn Comprehensive Epilepsy Program (CCEP) have reported that epilepsy is a common problem in Thailand. Approximately 1% of general population have epilepsy. With a population of over 60 millions, it is estimated that there are more than 600,000 epileptic patients in Thailand and most of them are children *(4)*. If epileptic children who don't have the appropriate treatment will have a poor and delayed development. Early treatment may consequently contribute to lower mortality and morbidity*(5)*.

Phenytoin, an effective anticonvulsant, is considered to be the drug of choice for partial and generalized tonic-clonic seizure and frequently prescribed in adults and children. Phenytoin differs from other antiepileptic drugs because it exhibits saturable metabolism and its elimination follows Michaelis-Menten model*(6)*. Because phenytoin's clearance varies with its plasma concentration, dosage adjustments result in disproportionate changes in steady-state plasma concentrations. A nonlinear relationship often exists between dose and clinically achievable serum concentrations, such that small changes in the former result in disproportionately large changes in the latter. Therefore, the nonlinear kinetics and narrow therapeutic index of phenytoin make dosing the drug is extremely difficult and important to predict *(7)*.

The pharmacokinetic calculation concerns with maximum velocity (V_{max}) and the Michaelis-Menten constant (K_m) as Michaelis-Menten pharmacokinetic parameters. V_{max} is the maximum rate of metabolism and K_m is the substrate concentration when the rate of metabolism is $V_{max}/2$. Many studies have been

demonstrated high variation of pharmacokinetic parameters. There is a large interpatient variability in pharmacokinetic parameters of phenytoin have high variations*(8)*. Based on the Michaelis-Menten pharmacokinetics, the mean V_{max} and mean K_m values for phenytoin in children patients varies from 5-20 mg/kg/d and 3-7 µg/mL, respectively

Due to the large interindividual and interethnic variability in parameter values, apparent V_{max} and K_m vary unpredictably among individuals with a coefficient of variation between 11 to 24% and approximately 50 to 73% respectively*(9, 10)*. A number of factors which influence phenytoin kinetic parameters include sex, age, weight, race, co-adminitered drugs, alcohol intake and smoking. Therefore, these factors should be also taken into account when have adjusting phenytoin doses.

To adjust phenytoin dosage for individual, one must know the accurate and precise estimates of phenytoin pharmacokinetic parameters. Population pharmacokinetic parameters play an important role in therapeutic drug monitoring. Mostly, pharmacokinetic calculation of phenytoin, the population pharmacokinetic parameter values were reported from the study of Michael E. Winter: V_{max} 7 mg/kg/d and K_m 4 µg/mL*(11)*. Since these values were estimated from data of Caucasians. Therefore, these values may be different from the values that was estimated from Thai patients *(12)*.

In addition, the differences in pharmacokinetics between adults and children make the differences of pharmacokinetic parameter values. At present, data on population-based pharmacokinetics of phenytoin monotherapy in ethnic Thai were limited while the study about the pharmacokinetics of phenytoin especially in children had never reported before. As described, pharmacokinetic calculation of phenytoin for designing dosage regimen and prediction of the serum concentration are based on derivation values for V_{max} and K_m. Consequently, using not the specific pharmacokinetic parameters of phenytoin children will not enable the prediction of the serum concentration and dose more accurately.

Following the Michaelis–Menten kinetics and close relationship between serum concentration and clinical effects, rational prescribing requires an understanding of the factors affecting its pharmacokinetics. The use of specific pharmacokinetic parameters of the population group will enable the prediction of the serum concentration and dose more accurately than using general population

pharmacokinetic parameters*(13, 14)*. Therefore, the pharmacokinetic parameters of phenytoin in Thai children will be useful for designing dosage regimens in Thai epileptic children*(15, 16)*. Accordingly, optimal seizure control and less or even no adverse or toxic effect of phenytoin therapy will eventually be achieved. Increasing of epileptic patients' quality of life should then be the benefit.

        This study was therefore designed to 1) determine pharmacokinetic parameters of phenytoin in Thai epileptic children. 2) study the factors those influence pharmacokinetic parameters of phenytoin.

Objective

1. To determine pharmacokinetic parameters of phenytoin in Thai epileptic children.
2. To study the factors which influence pharmacokinetic parameters of phenytoin

Significances of the study

1. This study will establish pharmacokinetic parameters value of phenytoin in Thai epileptic children.
2. This study will investigate the influence of factors on pharmacokinetic parameters of phenytoin.
3. Specific pharmacokinetic parameters of phenytoin in Thai children epileptic patients will be useful for designing accurate dosage regimens and predicting serum drug concentrations in Thai epileptic children.

CHAPTER II

REVIEW OF LITERATURE

1. Review of epilepsy

Definition

Epilepsy is a neurological disorder. It is characterized by a tendency to recurrent seizures and it defined by two or more unprovoked seizures. Seizures are the result of sudden, usually brief, excessive electrical discharges in a group of brain cells (neurones) and that different parts of the brain can be the site of such discharges. The clinical manifestations of seizures will vary and depend on where in the brain the disturbance first starts and how far it spreads. Transient symptoms can occur, such as loss of awareness or consciousness and disturbances of movement, sensation (including vision, hearing and taste), mood or mental function[4].

Epidemiology of epilepsy

Epilepsy is a common disorder. From many studies around the world it has been estimated that the mean prevalence of active epilepsy is approximately 8.2 per 1,000 of the general population [4]. Data from the World Health Organization (WHO) indicate there are over 50 million sufferers of the epilepsy in the world of whom 85% live in developing countries[17]. Globally, there are an estimated 2.4 million new cases each year and at least 50% of these cases begin in children or adolescence. The average annual incidence in developed countries is between 40-70 per 100,000 of general population and in developing countries is higher at around 100 -190 per 100,000 of general population per year[3, 18].

The incidence of recurrent seizures is highest in the first year of life and decline thereafter throughout childhood and adolescence. Incidence decreases from 150 per 100,000 in the first year of life to 60 per 100,000 at ages 5-9 years and 45-50 per 100,000 in older children. Rates tend to be slightly higher in boys than in girls[1].

Generalized seizures are common in field studies, especially in developing countries. Partial and generalized seizures have the highest incidence in the first year of life. The incidence rates peak in 5-10 years, the incidence of generalized seizures the highest incidence in the first year of life[5].

Classification of seizures and epilepsy

To determine the type of seizure that has occurred is essential for focusing the diagnostic approach on particular etiologies, selecting the appropriate therapy, and providing potentially vital information regarding prognosis. Failure to identify and, if possible, correct the etiology could result in continued seizures. Classification of seizure type is required for appropriate drug therapy *(16, 19, 20)*.

In 1981, the International League Against Epilepsy (ILAE) published a modified version of the International Classification of Epileptic Seizures that has continued to be a useful classification system. The classification based simply upon the visible manifestation of seizure and its electroencephalography (EEG) correlation. In this scheme seizures are divided into three groups: generalized, partial, and unclassifiable. Subdivisions of the classification are showed in Table 1 *(21, 22)*.

Table 1 The Commission   on Classification and Terminology of the ILAE 1981: Criteria used for the classification of seizures *(22)*

I. Partial seizures

 A. *Simple partial seizures*

      1. With motor sign : focal motor with or without march, versive, postural,phonatory

      2. With somatosensory or special sensory symptoms : somatosensory, visual, auditory, olfactory, gustatory, vertiginous, simple hallucinations (e.g. tingling, light flashing, buzzing)

      3. With autonomic symptoms or signs , including epigastric aura

      4. With psychic symptoms (disturbances of higher mental function) : dysphasic, dysmnestic, cognitive, affective, illusion, structured hallucinations

B. *Complex partial seizures*

      1. Simple partial onset followed by impairment of consciousness

          a. With simple partial features(A1toA4) followed by impaired of consciousness

          b. With automatisms

      2. With impairment of consciousness at onset

          a. With impairment of consciousness only

          b. With automatisms

C. *Partial seizures 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 (convulsive and non-convulsive)

 A. *Absence seizuresment of conciousness only, mild clonic components, atonic components*

      1. Absence with impairment of consciousness only, mild clonic components, atonic components, tonic components, automatism, autonomic components

      2. Atypical absence seizures with changes in tone more pronounced than in A1 and with onset and/or cessation that is not abrupt

 B. *Myoclonic seizures*

 C. *Clonic seizures*

 D. *Tonic seizures*

 E. *Toni clonic seizures*

 F. *Atonic seizures*

    (Combination may occur, such as B and F or B and D)

III. Unclassified epileptic seizures

Pharmacotherapy of epilepsy

Treatment for epileptic patients includes treatment of underlying conditions that cause or contribute to the seizures, avoidance of precipitating factors, suppression of recurrent seizures. Treatment plans must be individualized because the many different types and causes of seizures as well as the differences in efficacy and toxicity for each patients.

Antiepileptic drug therapy is the main of treatment for epileptic patients. The overall goal is to completely prevent seizures without causing any untoward side effects, preferably with a single medication and dosing schedule that is easy for the patient to follow. Classical medications such as phenytoin, valproic acid, phenobarbital, carbamazepine and ethosuximide are generally used as first- line therapy because they are effective and significantly less expensive. Furthermore, most patients require medication for at least 2 years, and need to continue for their life time. The principles of drug therapy in epilepsy are recommend :*(23)*

a. A single drug or monotherapy should be introduced to minimize risk of acute idiosyncratic and dose related toxicity. Monitoring a potential unacceptable side effect is recommended. Laboratory monitoring should be repeated at one and three months, then annually.

b. If seizures are continued, the dose should be slowly titrated to the maximum tolerated dose before switching to alternative monotherapy. Serum drug concentration may be used as a guide for titration. Seizure-free patient who is experiencing side effects will be decided to taper off drug therapy, if the benefits of remission outweigh the symptoms.

c. If seizure control is not achieved despite adequate trials of two single appropriate drugs, the combination therapy should be employed. However, there are little evidences that treatment with two drugs is superior to optimal treatment with one drug.

Although, drug of choice for individual based on a determination of the type of seizures, pharmacokinetics, drug interactions, side-effect, patient acceptability and compliance. Appropriate antiepileptic drug use according to seizure type classified by the commission of ILAE in 1981 are shown in Table 2 and antiepileptic drugs (AEDs) for seizure types in Thailand are shown in Table 3.

Table 2 The appropriate antiepileptic drugs used to treat epilepsy according to seizure type *(24)*

Seizure type	First-line drug	Alternative drug
Primary generalized	Carbamazepine Phenytoin Valproic acid	Phenobarbital Lamotrigine Topiramate
Partial[a]	Carbamazepine Phenytoin Valproic acid	Phenobarbital Lamotrigine Gabapentin[b] Topiramate[b] Tiagabine[b]
Absence	Ethosuximide Valproic acid	Lamotrigine Clonazepam
Atypical absence, Myoclonic, Atonic	Valproic acid	Lamotrigine Topiramate[b] Clonazepam

[a] Includes simple partial, complex partial, and secondarily generalized seizures.

[b] As adjunctive therapy.

Table 3 Antiepileptic drug of choice in Thailand *(25)*

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

Therapeutic drug monitoring of antiepileptic drugs

According to pharmacokinetic principles of antiepileptic drugs, serum concentrations of antiepileptic drugs have correlation with efficacy and/or toxic effects. The serum concentration of phenytoin is a better predictor of antiepileptic effect than the administered dosage*(26)*. Concentrations at therapeutic range of drugs usually cover the range of concentrations in which most patients are expected to receive therapeutic effect for control seizures without toxicity. Monitoring of clinical sings and symptoms should be performed. Moreover, determining the optimal dosage for each patients, the serum concentrations of antiepileptic drugs should be used for the pharmacokinetic calculation*(27)*. Antiepileptic drugs are drugs which therapeutic monitoring have been proved to be valuable and the indications for the monitoring are suggested as follows :*(28)*

Routine monitoring: the following conditions have been suggested for all patients who receive antiepileptic drugs:

a. After initiate antiepileptic drugs, serum concentration monitoring at steady state should be carried out to confirm baseline value.

b. One or twice yearly to verify compliance.

c. After each changing in antiepileptic drug regimen (antiepileptic drugs, dosage).

Specific monitoring: the following conditions have been recommended:

a. Patient who complains or has toxic signs which are possibly doses related, is insidious deterioration and it is not clear whether the condition is disease-or drug- related.

b. To verify compliance when could not control seizures despite an adequate prescription.

c. Drug interactions are suspected.

d. Changing renal or hepatic functions and during pregnancy.

Timing of specimen collection for therapeutic drug monitoring is important. The specimen should be collected after the absorption and distribution phases are complete and steady-state has been achieved. Drug levels obtained before steady-state has been achieved could be interpreted improperly as being subtherapeutic and prompt an increase in dosage. This could result in toxicity(29). For routine serum monitoring of drugs such as phenytoin. Trough levels are collected just prior to the next dose. Half-lives may be helpful in establishing possible toxicity and the need for therapeutic intervention. Time to steady-state serum concentration and the therapeutic range of the antiepileptic drugs are showed in Table 4 *(30).*

Table 4 Time to steady-state serum concentration and the therapeutic range of the antiepileptic drugs

Antiepileptic drug	Time to steady state (day)	Therapeutic concentration (mg/L)
Carbamazepine	3-4	4-12
Phenytoin	7-14	10-20
Phenobarbital	14-21	10-40
Valproic acid	2-3	50-120
Ethosuximide	7-10	40-120

2. Review of phenytoin

Chemistry

Phenytoin, a hydantoin compound related to barbiturates The chemical name is 5, 5-diphenyl-2, 4-imidazolidinedione (acid form).The chemical structure is shown in Figure 1. It has a molecule weight of 252.26 and pKa value of 8.06-8.33. Practical insoluble in water but slightly soluble in cold alcohol or ether. As phenytoin sodium which its salt, it has a molecular weight of 274.25 and is freely soluble in water. Its sodium salt contains phenytoin acid   92% so that 100 mg of it is equivalent to 92 mg of phenytoin acid. The solutions usually due to partial hydrolysis and absorption of carbondioxide *(31-33)*.
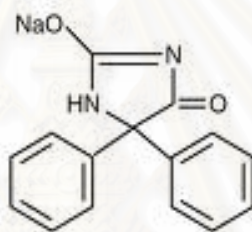
Figure 1 The structure of phenytoin

Indications

Developed in 1938, phenytoin remains a first-line medication for epilepsy. Phenytoin is indicated for use as an anticonvulsant drug in people of all ages. Evidence supporting efficacy of phenytoin as an anticonvulsant was derived from active drug-controlled studies that enrolled patients with the following seizure types: partial seizures, primary generalized tonic-clonic seizures (grand mal) *(8, 31, 32)*. Phenytoin is best used for partial-onset seizures. It generally is not effective against generalized-onset absence seizures or infantile spasms.

Phenytoin also is used in the management of arrhythmia as an antiarrhythmic agent. Furthermore, some patients who have trigerminal neuralgia, phenytoin may also used in the treatment*(8, 34, 35)*.

Mechanism of action

Phenytoin is a potent anticonvulsant drug. The primary site of action appears to be the motor cortex where spread of seizure activity is inhibited. Possibly by promoting sodium efflux from neurons, phenytoin tends to stabilize the threshold against hyperexcitability caused by excessive stimulation or environmental changes capable of reducing membrane sodium gradient*(8)*. This includes the reduction of posttetanic potentiation at synapses. Loss of posttetanic potentiation prevents cortical seizure foci from detonating adjacent cortical areas. Phenytoin reduces the maximal activity of brain stem centers responsible for the tonic phase of tonic-clonic (grand mal) seizures *(31, 32, 36)*.

These effects of phenytoin are evident at concentrations in the range of therapeutic level (1-2 mg/L) in cerebrospinal fluid of human brain. Nevertheless, the effects on sodium channels are selective at concentrations 5- to 10- fold higher. It can reduce the spontaneous activity and enhance the response of GABA and others *(36, 37)*.

Pharmacokinetics of phenytoin

a. Absorption

Phenytoin is a weak acid which is administered orally and parenterally. Following oral administration, phenytoin is slowly absorbed from the gastrointestinal tract. Absorption may be variable and sometimes incomplete. Very little of an orally administered dose of phenytoin is absorbed from the stomach because phenytoin is poorly soluble at a low pH *(38, 39)*. Absorption increases when the drug passes into the duodenum and continues at a slower rate in the jejunum and ileum. Absorption from the colon is poor. The oral absorption of phenytoin is somewhat irregular, prolonged, and is not first-order. The extent of its absorption is dependent of the rate at which it can enter the bloodstream. There is a maximal amount of gastrointestinal fluid in which the drug can be dissolved. The rate of phenytoin absorption is varied among different dosage forms *(33, 40)*.

Phenytoin acid is rapidly absorbed and time to peak of phenytoin acid is 1.5-3 hours after administration, while phenytoin sodium is more slowly absorbed and generally produces peak serum concentration in 4-12 hours after administration *(34)*. Phenytoin sodium 100 mg is approximately equivalent to phenytoin acid 92 mg. This

difference in phenytoin content should be considered when the dosage form is changed.

The percent absorbed is inversely related to the concentration or dose, at higher doses, some of the drug is undissolved, resulting in prolonged or decreased absorption. Hence, increasing dose results in a decreased rate of dissolution and absorption. Jung et al had suggested that the rate of phenytoin absorption was dose-dependent*(41)*. This study has been shown that the time to reach peak concentration had increased progressively from 8.4 hours to 13.2 hours and to 31.5 hours after a single dose of 400, 800 and 1,600 mg of phenytoin sodium, respectively.

b. Distribution

Phenytoin is rapidly and widely distributed throughout the body. The apparent volume distribution of phenytoin is about 0.5-0.8 L/kg in adults and 0.8-1.2 L/kg in children. Phenytoin distributes by passive diffusion into body fluids including cerebrospinal fluid, gastrointestinal fluid, saliva, bile, semen and breast milk. Although, phenytoin concentrations in the cerebrospinal fluid usually equal the unbound concentration in plasma, but those in saliva can be higher. Moreover, phenytoin can cross the placenta and also distribute into breast milk *(33, 42)*.

Obesity may increase its volume of distribution. Phenytoin is about 90% to 95% bound to plasma protein, mainly albumin. The free form of phenytoin passes through the blood brain barrier to the brain and provides pharmacological action. The wide range of unbound phenytoin fractions among individuals implies that reference to a therapeutic range of total phenytoin concentrations will be misleading for some patients. A decrease of albumin concentration and affinity to phenytoin reduces percentage of protein binding of phenytoin. Hypoalbuminuria is usually found in patients with chronic hepatic failure, nephritic syndrome, critical illness and burns. Althrough, in patient with uremia and severe jaundice, the affinity of phenytoin to albumin is also decreased *(8, 34, 42)*. In addition, displacement of the drug such as valproic acid, due to decreasing its protein binding sites *(43)*.

Accordingly, the increase of free phenytoin can increase its pharmacological actions. Hence, increasing the dose without evaluating the free fraction may cause the accumulation of free phenytoin that the toxic effects can appear *(30, 43)*.

c. Metabolism

The hydroxylation of phenytoin is the principle metabolic pathway of phenytoin in human. Phenytoin is metabolized by the hepatic microsomal mixed-function oxidase system (cytochrome P450 system). The cytochrome P450 isoforms of CYP2C subfamily are responsible for phenytoin hydroxylation. This is major involved by CYP2C9 (90% of the dose), while CYP2C19 contributes in a minor extent (10% of the dose)*(44)*.

Phenytoin is principally metabolized to an arene oxide intermediate via the arene oxidation. It is mainly metabolized to 5-(4-hydrophenyl)-5-phenylhydantoin (4–OH-phenytoin) which is further glucuronidated and excreted into the urine*(45)*. Phenytoin is known to exhibit nonlinear pharmacokinetics, implying that enzyme saturation must be occurred in one or more of the metabolizing enzymes. A small increase in phenytoin dose may cause unproportionally large increases of phenytoin serum levels as a complicating factor in its clinical use.

The capacity to metabolize phenytoin is highly variable among individuals because of their genetic backgrounds and environmental factors. The metabolism of phenytoin is low in neonates, increase considerably in children, adolescent and pregnancy but, decrease with advance age*(46, 47)*.

Following oral administration, phenytoin is slowly absorbed from the gastrointestinal tract. Absorption may be variable and sometimes incomplete. The drug is slowly and erratically absorbed following i.m. administration due to precipitation of the drug at the injection site. Following absorption, the drug is rapidly distributed to all tissues. Peak serum drug concentrations are achieved between 3 and 12 hours after administration of an oral dose.

Phenytoin is greater than 90% protein bound. Free fraction may increase in patients with renal or hepatic failure and/or hypoalbuminemia. These patients are predisposed to toxicity. The plasma half-life in man after oral phenytoin administration averages 22 hours, with a range of 7 to 42 hours. Time to steady state is highly variable, ranging from 1 to 5 weeks. Therapeutic drug concentrations can be obtained in 1 to 2 hours when the drug is administered intravenously*(8)*.

Phenytoin is metabolized in the liver to an inactive metabolite 5-(p-hydroxyphenyl)-5-phenylhydantoin (HPPH). This metabolite undergoes enterohepatic circulation. Approximately 60 to 75% of the daily dose of the drug is excreted in the urine as the glucuronide. Other minor metabolites also appear in the urine. In therapeutic doses, approximately 1% is excreted unchanged in the urine; in toxic doses, up to 10% of the ingested drug may be excreted unchanged by the kidneys*(33)*. Phenytoin kinetics are nonlinear and saturable, resulting in highly variable concentrations even with minor dosage changes. A small increase in dose may lead to a large increase in drug concentration as elimination becomes saturated *(48)*.

d. Elimination

The metabolism of phenytoin is described as being capacity limited. Capacity limited metabolism results in clearance values that decrease with increasing serum concentrations. This implies that as the maintenance dose is increased, the serum concentration rises disproportionately. Accordingly, disproportionate rise in the steady state serum level makes dosage adjustment difficult.

Among individuals, clearance values for phenytoin varies, range from 0.015 to 0.065 L/kg/h. Children tend to have higher values than adults. Half–life of phenytoin or $t_{50\%}$ averages 22 hours and ranges from 7 to 42 hours. Accordingly, increasing doses, a longer time is required to reach a new steady state. Thus, phenytoin half life is depending on the drug dose and the drug's plasma concentration. Moreover, the clearance of phenytoin is not a constant, it is concentration- or dose-dependent. As the dose or concentration of phenytoin increases, the clearance rate decreases *(33)*.

Drug interaction

Drugs that have pharmacokinetic interaction with phenytoin are presented in Table 5 according to the mechanism of interaction. The mechanisms include: absorption, plasma protein binding and metabolism. Majority of interaction involve phenytoin metabolism. Phenytoin is a broad-based hepatic enzyme inducer that affects most cytochrome P450 systems. Clearance of phenytoin can be affected by drugs that have been also metabolized in the liver by CYP2C9 and CYP2C19. According to phenytoin is an enzyme inducer. This action of phenytoin enhances the clearance of other drugs metabolized via this pathway *(49, 50)*.

In Addition, mechanism of absorption of phenytoin can be reduces by any of the following: antacid, calcium salts, antineoplastic agents, sucralfate and enteral feeding products. Many drugs can displace phenytoin from plasma protein binding sites, that unbound fraction of phenytoin increases. Consequently, increasing unbound phenytoin level, may lead to phenytoin toxicity *(51-53)*.

Table 5   Drugs that alter phenytoin pharmacokinetics

Drugs that decrease phenytoin absorption	
- Activated charcoal	- Antacid
- Antineoplastic drugs	- Nutrition formulae
- Sucralfate	
Drugs that alter phenytoin protein binding	
- Diazoxide	- Salicylic acid
- Valproic acid	
Drugs that increase phenytoin metabolism	
- Antineoplastic drugs	- Midazolam
- Carbamazepine	- Pnenobarbital
- Diazoxide	- Rifampicin
- Folic acid	- Clonazepam
- Chlordiazepoxide	- Nitrofurantoin
Drug that decrease phenytoin metabolism	
- Amiodarone	- Isoniazid
- Chloramphenicol	- Omeprazole
- Cimetidine	- Trimethoprim
- Disulfuram	- Ticlopidine
- Felbamate	- valproic acid
- Fluconazole	- Fluoxein

Clinical application

Loading dose

A drug that takes a long time to reach therapeutic levels, then a higher dose (the loading dose) may be given initially before dropping down to a lower maintenance dose. Drugs which may be started with an initial loading dose. Patients who after having a seizure and whose phenytoin blood concentrations were subtherapeutic. Phenytoin for acute status epilepticus should also be given with an initial loading dose, to immediately stabilize neuronal membranes and electrical activity during a seizure(11, 30).

Phenytoin intravenous infusion, may be given for rapid achievement of therapeutic level. A loading dose for adult and children patients is 15-18 mg/kg. Then follow by initiation of the maintenance dose 12-24 hours later. And in neonates, a loading dose of phenytoin is 15-20 mg/kg. In conscious patients who do not require intravenous phenytoin loading, can be given orally. In patients who can be administered an oral loading dose which can divided to four equal doses, three to four hours apart. The recommend loading dose is shown in Table 6.

Table 6 The loading dose of phenytoin *(54)*

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

Dosage of phenytoin is depending on the type and severity of seizure. At the start of treatment until the maintenance dose has been reached which is individually determined by criteria such as freedom from attacks, side effects and plasma concentration. The drug should be given in small initial doses, gradually increasing to optimum amounts *(55)*.

Adults are generally given 300 mg/day in 1-3 divided doses. Stabilization and in particular adjustment at a higher dosage should be guided by clinical requirements while checking the plasma concentration. In most adults, phenytoin may be administered as a once daily dose. Adult patients who have received no previous treatment may be started on 100 mg of phenytoin 3 times daily. For most adults a satisfactory maintenance dose will be 300-400 mg daily; however, maintenance doses of up to 600 mg may be required. Clinical studies with phenytoin have indicated that both single and divided dosage schedules demonstrate similar rates of absorption and equilibrium concentrations in adults. This means that a patient stabilized with 100 mg doses 3 times daily by mouth may respond to the more convenient single daily dose of 300 mg. There has been no reported evidence of increased drug toxicity when single daily doses of 300 mg have been administered to patients previously receiving the same quantity in divided doses *(8, 11, 56)*.

For infants, an initial oral dose of 1.5-2.5 mg/kg/dose twice a day is recommended. For children up to 12 years 5-8 mg/kg /day are recommended while checking the plasma concentration. On the 2nd day children over 6 years may be given 150-200 mg/day as a maintenance dose and for children under 6 years the dose has to be fixed according to the plasma concentration. In children initially, 5 mg/kg/day in 2 or 3 equally divided doses with subsequent dosage individualized to a maximum of 300 mg daily. The usual maintenance dose varies between 4 and 8 mg/kg. Children over 6 years old may require the minimum adult dose (300 mg/day)*(11, 54)*. The maintenance dose of phenytoin is shown in Table 7.

Table 7 The maintenance dose of phenytoin*(54)*

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

Adverse drug reactions

Optimum control without clinical signs of toxicity occurs more often with serum levels between 5 and 20 μg/mL, although some mild cases of tonic-clonic (grand mal) epilepsy may be controlled with lower serum levels of phenytoin. Adverse effects of phenytoin may be classified into two categories, dose-related and non-dose-related adverse effects*(57)*.

The most common dose-related adverse effects of phenytoin are central nervous system (CNS) effects *(57, 58)*. Acute severe phenytoin adverse effects are often seen at serum levels above the usual therapeutic concentrations (5-20 μg/mL).The severity of phenytoin toxicity is increased with the increased blood concentrations. Since the CNS effects are level-relates, reduction in dose or cessation of phenytoin therapy may eliminate theses CNS adverse effects and/or phenytoin concentrations. Adverse effects of phenytoin are also the non-dose related adverse effect. The dose related and non dose related adverse effects of phenytoin are presented in Table 8 and 9, respectively.

Table 8 Signs and symptoms of phenytoin intoxication related to phenytoin concentrations*(59)*

Phenytoin concentration	Signs and symptoms
< 10 μg/mL	None
10-20 μg/mL	Mild nystagmus may be present
20-30 μg/mL	Nystagmus on lateral gaze, slight ataxia, drowsiness
30-40 μg/mL	Nystagmus on vertical gaze, more intense ataxia and lurching gait, vomiting, slurred speech
40-50 μg/mL	Lethargy, confusion, disorientation
> 50 μg/mL	Opisthothonic posturing

Table 9 Non- dose- related adverse effects of phenytoin *(31, 60)*

Hepatic system (1-10%)*	
- hepatitis	
- hypersensitity with hepatic involvement	
Endocrine&Metabolic system (1-10%)*	
- diabetes insipidus	
- hyperglycemia	
- osteomalacia	
Hematologic system	
- magaloblastic anemia	(1-10%)*
- leucopenia	rare*
- agranulocytosis	rare*
- pancytopenia	rare*
Dermatologic system	
- hypertrichosis	} 10%*
- coarsening of the facial features	
- scarlariniform or mobilliform rash	
- Stevens-Johnson's syndrome	} < 1 %*
- Toxic epidermal necrolysis	
Gastrointestinal system	
- nausea/vomiting	
- diarrhea	
- constipation	} > 10%*
- gastrointestinal discomfort	
- dysphagia	
- loss of taste	} 1-10%*
- anorexia	
Central nervous system (1-10%)*	
- peripheral neuropathy	
- psychotic	
- impairment of cognitive function	

* Incidence rate

Pharmacokinetic model

The capacity-limited enzyme reaction and open one compartment pharmacokinetic model are described by the Michaelis-Menten equation. The deterministic model to which the data to fit is

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

Where 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 C_{ss} is the average steady-state phenytoin concentration.

The influence of factors on pharmacokinetic parameters of phenytoin

The values for V_{max} and K_m of phenytoin are varies from 100-1,000 mg/day and 1 -15 μg/mL, respectively*(8, 61)*. These variations may be the most important factors contributing to the difficulties in phenytoin dosage design for individual patient. Various factors have been demonstrated to have influences on the Michaelis - Menten parameters. These include patient characteristics such as age, weight, gender, etc *(15)*.

a. Influence of age on phenytoin metabolism

The metabolism of drug, phenytoin changes by the variation of age in patients because the growth and development of patients. Particularly, these might be differences in hepatic drug metabolism among neonates, infants, children, adults and elderly. So that changing in metabolism is important not only to select appropriate dosage for patients who are aging but also to prevent poisoning and adverse effects of drug therapy*(62)*.

In children, the more rapid elimination of phenytoin than in adults that confirmed by a positive correlation between the serum concentration and age during children. Based on the Michaelis-Menten pharmacokinetics, the mean V_{max} and mean K_m values for phenytoin in children range from 5-20 mg/kg/day and 3-7 μg/mL, resectively*(63)*. Dodson *(46)* have reported the nonlinear kinetics of phenytoin were

evaluated in children. Increasing age was associated with a reduction in the apparent V_{max}. The apparent K_m was influenced primarily by drug interactions.

Battino et al *(63)* reviewed pharmacokinetic of anticonvulsant drugs in children. It was shown that age is the factor influence pharmacokinetic parameters of phenytoin. Patients those have variation of age, so have variations of pharamacokinetic parameters. Grasela et al *(10)* reported routine phenytoin clinical pharmacokinetic data from Japan, England, and Germany were analysed to estimate population pharmacokinetic parameters. The data were analysed using NONMEM, a computer programme designed for population pharmacokinetic analysis. The K_m for patients less than 15 years old is 43% less than that of older patients. The K_m of Japanese patients appears to be 23% less than that for European patients. Later, Yukawa et al *(64)* estimated population pharmacokinetics of phenytoin from routine clinical data in Japan. The K_m for patients less than 15 years old was 16% less than that for adults. Moreover, Rui et al *(65)* analysed population pharmacokinetic parameters of phenytoin in Chinese epileptics. The data were analysed using NONMEM program. The influence of age on the Michaelis-Menten constant (K_m) were investigated. The K_m for patients less than 15 years old was 7% less than that for adults. Many studies showed those pharmacokinetic parameters both V_{max} and K_m in children are different from adults because these age-related pharmacokinetic differences.

Bauer and Blouin *(66)* determined Michaelis-Menten parameters for V_{max} and total bilirubin and albumin concentration were normal. Divided into age groups, V_{max} values for the 60- to 79-yr-old group were substantially less than those for the youngest subjects (20- to 39-yr-old, $p < 0.05$). Linear regression analysis indicated a decline in V_{max} with age ($r = 0.518$). Km values did not appear to be influenced by age. As a result of these changes, the 60- to 79-yr-old group would require, on the average, 21% less phenytoin per day than the 20- to 39-year-old to maintain a steady-state concentration of 15 µg/mL.

Additionally, Bauer and Boulin *(67)* also determined the Michaelis-Menten pharmacokinetic parameters V_{max} and K_m were calculated for 135 epileptic pediatric patients receiving phenytoin as their only anticonvulsant therapy. Mean V_{max} and K_m values were 13.95 mg/kg/day and 6.59 µg/mL for 0.5 to 3 year old patients, 10.93 mg/kg/day and 6.82 µg/mL for the 4 to 6 year age group, 10.05 mg/kg/day and 6.51

µg/mL for the 7 to 9 year olds, and 8.25 mg/kg/day and 5.69 µg/mL for the 10 to 16 year group. Using analysis of variance, the V_{max} values were significantly different (p < 0.01) but the K_m values were not. Linear regression analysis of V_{max} versus age revealed a significant decline in V_{max} with age (r = - 0.554, p< 0.001). A plot of K_m versus age showed a poor correlation (r = -0.170) and a large amount of variability. Based on this data, the youngest age group would require on average 62% more phenytoin/kg/day than the oldest age group in order to maintain a steady-state phenytoin concentration of 15 µg/mL.

Abduljabbar et al *(68)* reported significant differences in in the Michaelis-Menten parameters between Saudi children and adults. The mean V_{max} and K_m for children less than 16 years of age were significantly less than those of adults. And in this study, the pediatric cases required 30% more phenytoin per kilogram of body weight than the adults for the achievement of similar serum concentrations.

Although, the information about the Michaelis-Menten parameters of phenytoin in pediatric patients is still controversial. Some investigators have observed an age- related trend towards increasing K_m which has not been confirmed by others. Chiba et al *(69)*, Bauer and Blouin *(67)*, Suzuki et al *(70)* found no such relationship was appeared between age and K_m values in pediatric patients. They conversely found influence of age on V_{max}. Hence, a mean V_{max} was significantly higher in children than in adults and was progressively declining during children.

El-Sayed and Islam *(71)* who determined the Michaelis-Menten pharmacokinetic parameters V_{max} and K_m in epileptic Saudi patients receiving phenytoin. Linear regression analysis of V_{max} versus age revealed a significant decline in V_{max} with age (r = -0.877, p < 0.01) but a plot of K_m vs age showed a poor correlation (r = 0.302, p < 0.01). Ismail et al *(72)* reviewed data from patients taking phenytoin for the treatment of various types of epilepsy who were on phenytoin alone to calculate Michaelis-Menten pharmacokinetic parameters. The Km was independent of age but V_{max} was no relationship with age. Later, Abduljabbar et al *(68)* also revealed a negative correlation between V_{max} and age in Saudi patients (r = - 0.903) while Km did not correlate with age.

In addition, Valodia et al *(73)* determined factors these influencing the population pharmacokinetic parameters of phenytoin in adult epileptic patients in South Africa. The influence of covariates on V_{max} and K_m estimates was determined

using nonlinear mixed-effects modeling (NONMEM). The results indicated that age significantly influenced V_{max} (p < 0.05).

In Thailand, one study was reported by Chanawong (74). It was shown that The V_{max} and K_m values in this study was 7.80 mg/kg/d and 9.28 mg/L, respectively. In this study assessed the influence of body weight, age, gender and duration of phenytoin usage. The influence of age on V_{max} was obvious but the K_m value appeared to have no correlation to any factors.

b. Influence of body weight on phenytoin metabolism

Most studies reported a substantially correlation between body weight and V_{max} values. V_{max} values tend to be risen when body weight is increased. In contrast, K_m values seem to be unaffected. Previously, Grasela et al (10) have reported routine phenytoin clinical pharmacokinetic. Data were analysed to estimate population pharmacokinetic parameters. The patient group spanned pediatric and adult ages. The data were analysed using NONMEM. The parameters of a power function of height and weight were estimated to adjust V_{max} for body size. The best function adjusts V_{max} in proportion to weight to the 0.6 power.

In Japan, Yukawa et al (75) investigated population pharmacokinetic parameters. The data were analysed using nonlinear mixed effects model (NONMEM). The influence of weight on V_{max} was investigated. The parameter of a power function of weight was estimated to adjust V_{max} for body size. The best function adjusts V_{max} in proportion to weight to the 0.737 power. Odani et al (76) determined the population pharmacokinetic parameters of phenytoin were estimated using routine therapeutic drug monitoring data. Serum concentration values at steady-state were analyzed using NONMEM. The parameter of power function of weight to V_{max} was estimated to be 0.463. And Chan et al (77) examined methods of adjusting phenytoin dosage in epileptic Chinese children and adults. This study also revealed the influence of weight on V_{max}.

However, in Saudi patients, El-Sayed and Islam (71) reported the Michaelis-Menten pharmacokinetic parameters V_{max} and K_m. There is a significant increase in V_{max} with weight (r = 0.816, p < 0.01). Abduljabbar et al (68) reported that V_{max} value was positively correlated with body weight (r = 0.953, p<0.01). Although, Ismail et al.(72) estimated individual and population Michaelis-Menten pharmacokinetic

parameters. There was a moderate correlation between V_{max} and body weight (r = 0.520) but there was a weak correlation between K_m and body weight.

Valodia et al *(73)* have studied influence of various covariates (including weight, race, smoking, gender, age, mild-to-moderate alcohol intake, and body surface area) on the population pharmacokinetic parameters of phenytoin in adult epileptic patients in South Africa. The results indicated that body weight was significantly influenced V_{max} ($p < 0.05$).Although, this study indicated that the variability in K_m was accounted for   by V_{max}. Rui et al *(65)* estimated population pharmacokinetic parameters using NONMEM approach of phenytoin in Chinese epileptics. The influence of body weight on V_{max} was investigated. The best function adjusts V_{max} to the 0.57 power in proportion to body weight.

Consequently, regarding to previous reports, V_{max} but not K_m values seems to be influenced by body weight and V_{max} values tend to be increased with the increasing body weight. Both power function (non- linear) and linear regression are used to describe the relationship between V_{max} value of phenytoin and body weight. Due to the different average body weight among patients, the equations described these relationships are varied.

The study in Thailand, Kanjanasilp et al *(12)* determined population pharmacokinetics of phenytoin in Thai epileptic patients by NONMEM. V_{max} was estimated to be 12.50 mg/kg/d and K_m value was 16.10 mg/L. The results from this study indicated that body weight influenced K_m. There appears to be a linear function of weight on K_m ($K_m = 0.265$ x weight).

c. Influence of gender on phenytoin metabolism

Few studies have revealed the influence of gender on pharmacokinetic of phenytoin. For metabolism of phenytoin, most studies reported no influence of gender on pharmacokinetics parameter of phenytoin both V_{max} and K_m value. Nonetheless, Ismail et al *(78)* found a significant difference of V_{max} value in males and females($p = 0.026$) whereas K_m value was independent of gender.

Furthermore, under various states of disease and drug interactions, the pharmacokinetic parameters both V_{max} and K_m values of phenytoin may also altered. K_m value is mostly increased in the presence of a competitive inhibitor whereas in the

presence of drug that displaces serum protein binding of phenytoin, this value will be decreased.

4. Review of assay methods

Many analytical techniques have been used to determine the concentrations of phenytoin in biological fluids. These include spectrophotometry, fluorescence polarization immunoassay, enzyme-mediated immunoassay, radioimmunoassay, gas-liquid chromatography and high-performance liquid chromatography. Immunoassay techniques have the advantage of being rapid and sensitive[79].

The fluorescence polarization immunoassay (FPIA) is chosen in this study because it has rapid turn around time, calibration stability, acceptable accuracy and precision. Furthermore, the method requires a minimum of reagent and sample and is simple to perform [80].

The Abbott TDx[®] system is based on FPIA technique. This method has fundamental principle as follows:

Sample drug and tracer compete for limited number of binding sites on antibodies specific to the drug being measured. The concentration of unlabeled drug from patient sample will determine how much labeled drug can bind to the specific antibody. The label on the tracer drug is the fluorescent dye-fluorescein 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.

Although, the changes of polarization angle reflect tracer binding to 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 precise relationship between polarization and concentration of the unlabeled drug is established by measuring the polarization valves of calibrators with known concentrations of drug. Unknown are determined from this standard curve [81].

5. Review of Nonlinear Mixed Effect Model (NONMEM)

NONMEM was developed by the NONMEM Project Group at the University of California at San Francisco for analyzing population pharmacokinetic data in particular. It is a computer program designed for population pharmacokinetic analysis that allows pooling of data from many individuals*(82)*. The standard approach's objective function can describe NONMEM. The most important and useful features of NONMEM :*(83)*

- NONMEM can fit both individual and population models.
- NONMEM has a menu of pharmacokinetic models from which the one appropriate to the problem at hand can be chosen.
- The user specifies the relationship of pharmacokinetic parameters to independent variables such as weight age, using population parameters that will be estimated.
- The user also specifies which parameters vary between individuals and the form (model) for this variability, as well as the form (model) for the difference between observations from an individual and their predictions for this individual.
- NONMEM estimates parameters describing both kinds of variability.
- NONMEM provides estimates (standard errors) of the precision of its parameter estimates, including those describing variability.
- NONMEM provides a means of deciding whether one model fits the data better than another using the minimum objective function value, a good-of-fit statistic.
- NONMEM provides graphics, useful in judging the adequacy of the model currently fit to the data.

Data typically collected from clinical studies of the pharmaceutic agents, involving the administration of a drug to individuals the subsequent observation of drug levels in the plasma. Proper modeling of these data involves accounting for both unexplainable inter- and intra-subject effects (random effects), as well as measured concomitant effects (fixed effect). NONMEM allows this mixed effect modeling. Such modeling is especially useful when there are only a few pharmacokinetic measurements from each individual sampled in the population, or when the data collection design varies considerably between these individuals. However, NONMEM is a general program which can be used to fit models to a wide variety of data *(61)*.

NONMEM consists of two components, the structural model (which may or may not contain covariates) and the statistical or variance model. Development of

pharmacokinetic model is to identify the base or structural model, which is the model that best describes the data in the absence of covariates. In addition, influence of covariates may influence the pharmacokinetics of drug. Therefore, next step is to select the covariates for inclusion in the base model and build the model using covariates.

For phenytoin, NONMEM pools data from all individuals but explicitly models and handles the complicated error structure arising from a proper accounting of the interindividual and intraindividual random effects. It gives an estimate of the population mean parameters, V_{max}, K_m and the interindividual variabilities (ω^2_{Vm}, ω^2_{Km}) and also the intraindividual variability. NONMEM provides estimates of the standard errors for all the parameters, and the standard errors can be used to define confidence intervals. The statistical significance of the parameters was also evaluated for the objective function produced by NONMEM. The influence of factors on pharmacokinetic parameters were investigated.

CHAPTER III

MATERIALS AND METHODS

Materials

1. Drug

Phenytoin

The oral preparations of phenytoin prescribed to patients were phenytoin sodium as an extended release capsule (Dilantin Kapseal®, Parke-Davis) containing 100 mg of phenytoin sodium equivalent to phenytoin acid 92 mg and phenytoin acid as a chewable tablet (Dilantin Infatab®, Parke-Davis) containing 50 mg of phenytoin acid.

2. Reagents

2.1 No. 9507-01, Phenytoin calibrators (Lot.no.40012Q100)

Six vials of accurately measured amounts of phenytoin in human serum at the following concentrations:

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

Preservative: 0.1% Sodium azide

2.2   No.9507-10, Phenytoin controls (Lot.no.52639Q100)

Three levels of phenytoin in 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	13.5 - 16.5
H	30.0	27.0 - 33.0

Preservative: 0.1%Sodium azide

2.3   No.9507-60, Phenytoin reagent pack (Lot.no.48212Q100)

The phenytoin reagents consist of the following:

Vial	Components
P	Pretreatment solution. Surfactant in buffer containing protein stabilizer Preservative: 0.1% Sodium azide
S	< 1% Phenytoin Antiserum (Sheep) in buffer with protein stabilizer. Preservative: 0.1% Sodium azide
T	< 0.01% Phenytoin flurescein tracer in buffer containing surfactant and protein stabilizer. Preservative: 0.1% Sodium azide

2.4   No.9519-02, Dilution buffer (Lot.no.51106M102)

Bovine gamma globulin in phosphate buffer is used as a buffer solution and has been prepared with 1.2% sodium azide.

3. Instruments

   3.1 TDx® Analyzer System, Automated Fluorescense Polarization Analyzer (Diagnostic Division Abbott Laboratories, Inc,TDx, U.S.A.Serial No.15581-96)

   3.2 Centrifuge machine (model 30s, Hettich Zentrifugen Universal™, Germany).

   3.3 Freezer (Electrolux, Medical Refrigeration 350/35, United Kingdom)

Methods

1. Definition

   1.1 Maximum rate of metabolism (V_{max}) is the rate of metabolism

   1.2 Michealis-Menten constant (K_m) is the substate concentration at which the rate of metabolism is a half of V_{max}

   1.3 Children are patients who are less than 15 year olds.

   1.4 Adverse drug reaction
   This study evaluates adverse drug reactions that correlate with plasma drug concentration.

   1.5 Good control is no seizure attack occurs during the time of phenytoin administration.

   1.6 Poor control is one or more seizure attacks occur during the time of phenytoin administration.

2. Study design

This study is retrospective and prospective design to determine pharmacokinetic parameters of phenytoin in Thai epileptic children and investigate the influence of factors on pharmacokinetic parameters of phenytoin.

3. Patients

Thai epileptic children who were treated in out-patient at neurology clinic of children, Chonburi Hospital. The retrospective study was collected data from February 2005 to September 2007 while the prospective study was collected data from October 2007 to March 2008.

3.1 Inclusion criteria

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

    a. All patients were younger than 15 years old.

    b. The patients who received phenytoin monotherapy for epilepsy and good compliance.

    c. The patients who had history of normal serum albumin (3.5-5.0 g/dL).

    d. The patients who agreed with the study after the objectives and procedures of the study were explained and signed informed consent before commencing the study.

3.2 Exclusion criteria

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

    a. renal disease, creatinine clearance < 25 ml/min.

    b. hepatic disease with hepatic enzyme(AST and ALT) out of normal range, > three times of normal value.

    c. concurrently used drugs which were reported to have suspected, probable or established documentation of drug interaction with phenytoin (such as phenobarbital, carbamazepine, valproic acid, methylphenidate, cotrimoxazole, cimetidine, dexamethasone, folic acid, metronidazole, etc.)

    d. received oral dosage form of phenytoin pass nasograstric.

4. Sample size

The sample size was calculated from the following equation:

$$N \quad = \quad [(Z_{\alpha/2} + Z_{\beta})^2/Z_o^2] + 3$$

When $\quad Z_o \quad = \quad 1/2 \ln [(1+r)/(1-r)]$

$\qquad \qquad \alpha \quad = \quad 0.05$ (two-tailed); $Z_{\alpha/2} = 1.96$

$\qquad \qquad \beta \quad = \quad 0.10$ (one-tailed); $Z_{\beta} \ = 1.28$

$\qquad \qquad r \quad = \quad$ correlation coefficient

This study will set the target correlation coefficient (r) to be 0.518. This value was obtained from previous study that investigated the correlation between K_m and age *(66)*.

$$Z_o \quad = \quad 1/2 \ln [(1+ 0.518) / (1- 0.518)]$$

$$= \quad 0.57$$

$$N \quad = \quad [(1.96+1.28)^2/ (0.57)^2] + 3$$

$$= \quad 35.78 \ \approx \ 36$$

5. Drug administration

The usual dosage regimen of phenytoin prescribed for patients were prescribed by neurologist physician.

a. Sodium phenytoin capsule 100 mg composes of 92% phenytoin.

b. Phenytoin tablet composes of 100% phenytoin.

Oral preparation were Dilantin® capsule 100 mg and Dilantin® infatab 50 mg (Parke-Davis). Phenytoin serum concentration was considered to achieve steady state after the fixed dosage of the drug were given to the patients at least 3-5 half-lives of drug for determination of serum concentration.

6. Sample collection

        Trough concentrations are generally recommended for routine monitoring of phenytoin. Blood samples were obtained after patients had received constant dose for not less than 14 days to ensure that steady state was achieved and were collected at approximately 8 hours after dose administration and before next dose.

        All samples were allowed to clot and centrifuged immediately (5,000 rpm for 5 minutes at room temperature). The serum portion were separated and stored at -20° C until being assayed within 24-48 hours.

7. Analytical method

        Phenytoin plasma levels were determined by fluorescence polarization immunoassay method using $TDx^{®}$ Analyzer system, Abott Laboratories. Calibration and sample assay run were performed using operation manual of $TDx^{®}$ analyzer.

8. Determination of pharmacokinetic parameters of phenytoin

        Determination of pharmacokinetic parameters of phenytoin was performed with Nonlinear Mixed Effect Model (NONMEM). The data was transferred in to program and then the pharmacokinetic modeling was performed in double precision using NONMEM version VI. One compartment was employed using the PREDPP library subroutine ADVAN10.

9. Method of model building

    9.1 Fixed effects modeling

One-compartment model with the Michaelis-Menten equation was used.

Structural Model

$$R_{ij} = \frac{V_{max}ij \times C_{ss}ij}{K_m j + C_{ss}ij}$$

R_{ij} (mg/d)	=	dosing rate of phenytoin predicted
$C_{ss}ij$(mg/L)	=	plasma concentration at steady-state
$V_{max}ij$(mg/d)	=	the maximum metabolic rate
$K_m ij(\mu g/mL)$	=	Michaelis-Menten constant of phenytoin

Primary pharamacokinetic parameters, the maximum metabolic rate (V_{max}), Michaelis-Menten constant (K_m) and volume of distribution (V_d) were estimated. A primary analysis was conducted by permitting NONMEM to estimate the pararmeters of the one compartment base model with no covariates.

9.2 Random effects modeling

The interindividual variability of individual parameters value from the typical value in the patients and the intraindividual variability were estimated. The base model for estimation of interindividual and intraindividual variation was the exponential model and the additive model, respectively. The models were as follows:

Interindividual variation

V_{max}	=	$\theta 1 * EXP(\eta 1)$
K_m	=	$\theta 2 * EXP(\eta 2)$
V_d	=	$\theta 3 * EXP(\eta 3)$

$\theta 1$ for V_{max}, $\theta 2$ for K_m and $\theta 3$ for V_d are fixed effect parameters.

$\eta 1$ for V_{max}, $\eta 2$ for K_m and $\eta 3$ for V_d are random variables describing interindividual variabilities with zero mean and variances equal to $\omega^2{}_{Vmax}$, $\omega^2{}_{Km}$ and $\omega^2{}_{Vd}$, respectively.

Intraindividual variation

$$\text{Cssij}^{\,o} = \text{Cssij} + \varepsilon ij$$

$\text{Cssij}^{\,o}$ = the observed serum concentration for the ith pair in the jth patient

Cssij = the expected serum concentration for the ith pair in the jth patient

εij = a random variable describing intraindividual (residual) variability with zero mean and variance equal to σ^2

Consequently, the apparent influence of covariates on pharmacokinetic parameters were screened by using the base model and observed by changing the objective function value (OFV). Significant covariates were added cumulatively to the model in the order of their contribution to the reduction in the OFV in the preliminary analysis until there was no further reduction in the OFV. Only covariates showing a significant contribution were retained.

10. Data analysis of Nonlinear Mixed Effect Model (NONMEM)

NONMEM pools data from all individuals but explicitly models and handles the complicated error structure arising from a proper accounting of the interindividual and intraindividual random effects. It gives an estimate of the population mean parameters, V_{max}, K_m, Vd and the interindividual variability ($\omega^2_{\,Vmax}$, $\omega^2_{\,Km}$ and ω^2_{Vd}) and also the intraindividual variability (σ^2)*(61, 83)* .

NONMEM provides estimates of the standard errors for all the parameters and the standard error can be used to define confidence intervals. The statistical significance of the parameters was also evaluated for the objective function produced by NONMEM. When the difference of 2-log likelihood between two models allowing a parameter of interest freely estimated and fixed to a hypothetical value was greater than 3.84, the parameter value was considered to be statistically significant (p<0.05) *(83).*

11. Statistical analysis

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

11.2 The differences of the pharmacokinetic parameters in the different groups of age were analysed by one way analysis of variance (ANOVA).

11.3 The correlation between pharmacokinetic parameters of phenytoin and factors such as gender, age and weight were determined by simple linear regression.

CHAPTER IV

RESULTS AND DISCUSSION

From study design of this study, subject data were collected from both retrospective and prospective study. However, results from retrospective study from February 2005 to September 2007 found that the collected data from characteristics of patients were not enough to enroll these patients in retrospective study due to incomplete laboratory data of liver and renal function such as AST, ALT, serum creatinine, albumin levels and no data of sampling time that need especially for NONMEM analyzing. Therefore, thirty-nine outpatient epileptic children participated in the prospective study. All patients were recruited from neurology clinic of children at Chonburi Hospital during October 2007 to March 2008. All patients were treated with phenytoin as monotherapy and this study was approved in 25 November 2007 by the Ethical Review Committee of Chonburi Hospital.

The data of these studied patients were presented and analyzed under the following topics.

1. Demographic data of patients

Of total 39 patients, 29 (74.4%) were male and 10 (25.6%) were female. As the inclusion criteria of this study, all patients were under 15 years old. Their age was ranged from 10 months to 14 years with mean ± SD of 8.58 ± 3.82 years. Six patients (15 %) were 0 to 3 years of age, four patients (10 %) were 4 to 6 years of age, thirteen patients (33%) were 7 to 9 years of age and sixteen patients (42%) were over 10 years of age (as shown in Figure 2). The body weight was ranged from 9 to 99 kg with mean ± SD of 32.28 ± 18.04 kg. Patients' height was in range 43 to 175 cm with a mean ± SD of 127.17 ± 28.24 cm.

The most common coexisting risk for seizure of these patients was fever (13 patients, 33.3%) and others were trauma (3 patients, 7.7%) and autism spectrum (2 patients, 5.1%). Patients who had unknown risk for first seizure were twenty-one (53.9%).

All included patients had no liver and renal diseases. They had normal serum albumin level (3.5-5 g/dL). As phenytoin is high protein bound drug, a decrease of

albumin concentration and affinity to phenytoin reduce the percentage of protein binding of phenytoin. Characteristics of the patients are shown in Table10 and data of patients are summarized in Table 11.

Table 10   Characteristic of the patients

Patient number	Gender	Age (yr)	Weight (kg)	Height (cm)	Risk for seizure	Seizure type	AST (U/L)	ALT (U/L)	SCr (mg/dL)	Alb (g/dL)
1	M	3	19	97	unknown	CPS	48	57	0.4	4.2
2	M	8	28	118	fever	CPS	41	31	0.7	4
3	F	9	45	142	fever	CPS	39	66	0.6	4.7
4	M	7	18	108	fever	FS with 2ndGTC	30	15	0.6	4.1
5	M	12	40	157	unknown	SPS	27	18	0.8	4.2
6	M	14	54	165	unknown	GTC	29	45	0.9	4.3
7	M	8	26	134	unknown	FS with 2ndGTC	41	12	0.8	4.3
8	M	12	50	148	unknown	GTC	26	30	0.7	4
9	M	14	50	146	unknown	GTC	33	44	0.7	4.5
10	F	11	58	155	fever	FS with 2ndGTC	39	59	0.7	4.3
11	F	9	18	116	unknown	GTC	33	20	0.5	4.1
12	M	5	28	113	unknown	GTC	44	55	0.7	4.3
13	M	4	14	96	fever	FS with 2ndGTC	31	21	0.5	4

[1] CPS=Complex partial seizure, FS with 2ndGTC=Focal seizure with 2nd generalize seizure, GTC=Generalized tonic-clonic seizure, SPS=Simple partial seizure

Table 10 Characteristic of the patients (cont.)

Patient number	Gender	Age (yr)	Weight (kg)	Height (cm)	Risk for seizure	Seizure type	AST (U/L)	ALT (U/L)	SCr (mg/dL)	Alb (g/dL)
14	M	3	14	82	fever	FS with 2ndGTC	30	22	0.5	4.2
15	M	0.9	10	43	fever	GTC	36	27	0.5	4
16	M	8	24	127	unknown	FS with 2ndGTC	25	13	0.7	4.3
17	M	12	95	175	unknown	SPS	19	33	0.9	4
18	M	9	25	115	unknown	GTC	29	15	0.8	4
19	M	11	60	159	trauma	FS	25	25	0.8	4
20	M	8	29	118	unknown	GTC	40	12	0.8	4
21	M	4	17	106	unknown	FS with 2ndGTC	43	35	0.5	3.8
22	M	7	20	110	fever	GTC	30	26	0.5	3.9
23	F	10	28	122	unknown	FS with 2ndGTC	28	14	0.4	4
24	M	8	24	121	unknown	FS	66	81	0.6	4.3
25	F	12	34	140	unknown	GTC	20	15	0.7	3.6
26	M	14	46	163	unknown	GTC	26	14	0.7	4.4

[1] CPS=Complex partial seizure, FS with 2ndGTC=Focal seizure with 2nd generalize seizure, GTC=Generalized tonic-clonic seizure, SPS=Simple partial seizure

Table 10 Characteristic of the patients (cont.)

Patient number	Gender	Age (yr)	Weight (kg)	Height (cm)	Risk for seizure	Seizure type[1]	AST (U/L)	ALT (U/L)	SCr (mg/dL)	Alb (g/dL)
27	M	14	46	160	unknown	GTC	26	31	1	4.4
28	M	6	29	118	fever	GTC	30	40	0.5	3.8
29	M	11	13	124	unknown	CPS	29	20	0.7	4.3
30	F	13	34	141	autism	GTC	21	15	0.5	4
31	M	13	56	163	fever	FS	37	40	0.6	3.8
32	M	8	33	128	trauma	GTC	23	13	0.8	3.7
33	F	8	21	135	unknown	SPS	29	23	0.7	3.8
34	F	12	49	160	unknown	GTC	27	27	0.6	3.8
35	M	2	9	68	fever	GTC	50	24	0.5	4
36	M	2	12	112	fever	GTC	36	15	0.4	4.3
37	F	3	16	100	fever	GTC	42	15	0.6	4.5
38	F	8	22	130	autism	GTC	23	10	0.6	4
39	M	12	32	145	trauma	CPS	25	17	0.5	4.3

[1] CPS=Complex partial seizure, FS =Focal seizure, GTC=Generalized tonic-clonic seizure, SPS=Simple partial seizure

Table 11 Summary of all patient data*

Number of patients (M/F)	39 (29/10)
Age (yr)	8.58 ± 3.82 (0.9-14)
Weight (kg)	32.28 ± 18.04 (9-99)
Height (cm)	127.17 ± 28.24 (43-175)
AST (U/L)	32.74 ± 9.50 (19-66)
ALT (U/L)	28.07 ± 16.90 (10-81)
Serum creatinine (mg/dL)	0.64 ± 0.14 (0.4-0.9)
Albumin (g/dL)	4.10 ± 0.24 (3.6-4.7)

* Data from Table 10
Values are expressed as mean $\pm$ SD (range)
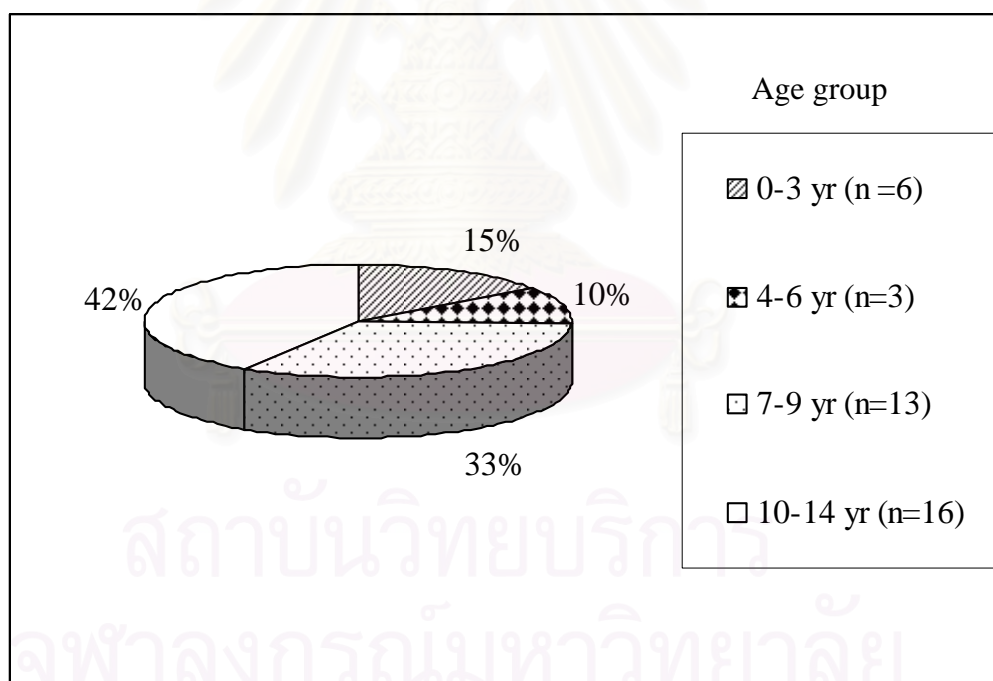
Figure 2 The distribution of age in patients

All patients were younger than 15 years of age. This study recruited patients at this age point because pharmacokinetic parameter values of phenytoin in patients younger than 15 years of age was significantly less than the older's *(10)*. As shown in Table 11, incidence rates of epileptic in children were higher in male (n=29, 74.4%). This was in agreement with report from World Health Organization (WHO) which rate of epileptic tend to be slightly higher in boys than in girls *(1)*.

Regarding to type of seizure, most of epileptic children in this study were generalized tonic-clonic seizure (n=28, 71.79%). Eighteen patients (46.2%) who were known the coexisting risk for first seizure. The most common coexisting risk for first seizure of these epileptic children was fever. But twenty-one patients (53.8%), the coexisting risk for first seizure was unknown. Some patients who had unknown coexisting risk for seizure, they were not investigated by imaging because they could not expend high cost for diagnosis. However, two of them had history of seizure in their family. All of patients completed in this study do not have any concomitant disease.

2. Phenytoin dosage regimen and serum concentrations

Phenytoin were prescribed and administered either once daily (night time) or twice a day (morning and evening). Dose of phenytoin administration was maintenance dose. All patients were studied during steady-state conditions with no change in phenytoin dosage. Phenytoin serum concentrations were considered to achieve steady-state after the fixed dosage of the drug was given to the patients for at least 14 days. Blood samples were drawn at approximately 8 hours after administration of dose and before next dose. Each patient had one measurement of the steady-state concentration of phenytoin.

Table 12 shows phenytoin dosage regimen and steady-state serum concentration of patients and Figure 3 demonstrates relationship between the different dose of phenytoin administration and steady-state serum concentrations of phenytoin.

Table 12 Phenytoin dosage regimen and steady-state serum concentration of patients

Patient number	Dosage regimen[1]	Dose (mg/d)	Dose (mg/kg/d)	Concentration (µg/mL)	Sampling time (hr)[2]
1	tab 1x2	100	5.26	20.82	13
2	tab 1.5 pc and 2 hs	175	6.25	24.94	14
3	tab 0.5 pc and 1hs, cap 1x2	275	5.75	9.96	12
4	tab 1x2	100	5.55	23.00	12.5
5	tab 1 hs, cap 2 hs	250	6.25	8.87	23.5
6	cap 3 hs	300	5.55	13.64	19.5
7	cap 1 hs	100	3.84	2.92	19
8	cap 3 hs	300	6	15.71	19
9	cap 3 hs	300	6	3.94	19
10	cap 3 hs	300	5.17	34.28	19.5
11	tab 1.5 pc and 2 hs	175	9.72	16.82	12.5
12	tab 2x2	200	7.14	15.30	13
13	tab 1x2	100	7.14	5.55	12

[1] tab = Dilantin tablet 50 mg,   cap = Dilantin capsule 100 mg
[2] Sampling time = period from last dose administration to blood sampling

Table 12 Phenytoin dosage regimen and steady-state serum concentration of patients

Patient number	Dosage regimen[1]	Dose (mg/d)	Dose (mg/kg/d)	Concentration (µg/mL)	Sampling time (hr)[2]
14	tab 1x2	100	7.14	5.41	12.5
15	tab 0.5x2	50	4.95	1.32	13
16	cap 1x2	200	8.33	16.01	12.5
17	cap 3 hs	300	2.78	1.34	18.5
18	tab 1.5x2	150	6	6.19	11
19	tab 1 hs, cap 2 hs	250	3.9	17.97	13.5
20	tab 2x2	200	6.89	3.33	12
21	tab 1x2	100	5.88	3.39	12
22	tab 1 pc and 2 hs	150	7.5	6.36	12
23	cap 1x2	200	6.57	10.95	11.5
24	tab 1 pc, cap 1 hs	150	5.91	3.35	12
25	tab 1.5x2	150	4.41	2.80	13
26	cap 3 hs	300	6	14.51	13

[1] tab = Dilantin tablet 50 mg,   cap = Dilantin capsule 100 mg
[2] Sampling time = period from last dose administration to blood sampling

Table 12 Phenytoin dosage regimen and steady-state serum concentration of patients

Patient number	Dosage regimen[1]	Dose (mg/d)	Dose (mg/kg/d)	Concentration (µg/mL)	Sampling time (hr)[2]
27	cap 3 hs	300	6.13	39.5	15
28	tab 1 pc and 2 hs	150	5.17	4.46	13
29	tab 1 hs, cap 2 hs	150	6.17	5.85	11
30	cap 2 hs	200	5.51	16.94	14
31	cap 3 hs	300	4.92	21.56	13.5
32	cap 2 hs	200	5.57	13.06	13
33	tab 1.5 pc, cap 1 hs	175	7.95	11.89	13.5
34	cap 3 hs	300	5.63	23.92	19
35	tab 0.5x2	50	5.88	1.11	13
36	tab 1x2	100	8.33	4.83	11
37	tab 1x2	100	6.25	1.26	12
38	tab 1.5x2	150	6.82	2.24	14
39	cap 2 hs	200	6.25	5.57	15.5

[1] tab = Dilantin tablet 50 mg,   cap = Dilantin capsule 100 mg
[2]Sampling time = period from last dose administration to blood sampling

Table 13 Summary of phenytoin dosage regimen and serum concentration of patients

Dose (mg/kg/d)	5.96±1.26 (range 2.78-9.72)
Dosage regimen	
twice daily	23 patients
once daily	16 patients
Dosage form	
tablet	18 patients
capsule	15 patients
combination (tablet+capsule)	6 patients
Serum concentration (µg/mL)	range 1.11 – 39.5
sub-therapeutic (<5 µg/mL)	13 patients
therapeutic (5-20 µg/mL)	19 patients
supra-therapeutic (>20 µg/mL)	7 patients

Steady-state concentration (µg/mL)

Dose (mg/kg/d)
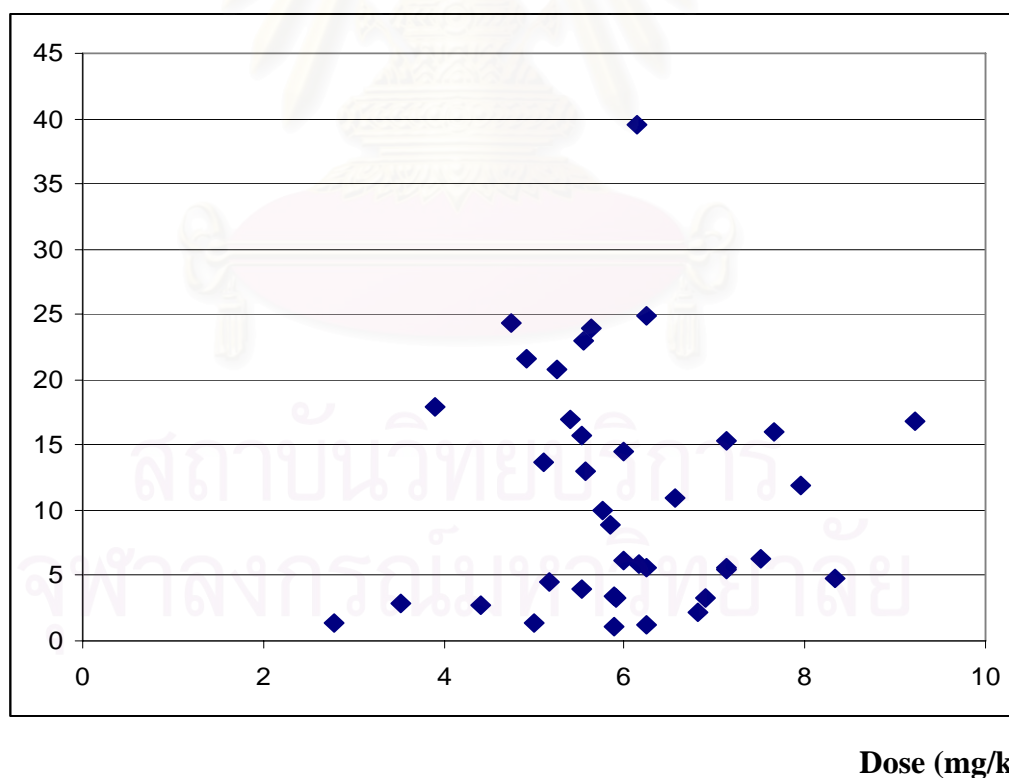
Figure 3 Relationship between the daily dose of phenytoin and steady-state serum concentration

By various reports, many drugs can alter phenytoin pharmacokinetics*(50)*. Therefore, patients with phenytoin monotherapy were selected and patients who concurrent used other drugs that affect pharmacokinetic of phenytoin were excluded. Only one brand name of phenytoin (Dilantin®) was prescribed to all patients, so that the variation in bioavailability of phenytoin according to different brand names was not existed in this study.

As seen in Table 13, the daily dose of each patient was calculated on body weight basis (as mg/kg/d). The patients received doses varied from 2.78 to 9.72 mg/kg/d with a mean ± SD of 5.96 ± 1.26 mg/kg/d. Twenty-three patients received phenytoin twice daily and sixteen patients received once daily. Neither patient received phenytoin dosage which was higher than the recommended dose of phenytoin for children(4-10 mg/kg/d)*(54)*. Although three patients received phenytoin lower than recommended dose, their seizure could be controlled.

In general, therapeutic range of phenytoin concentrations is 5 to 20 µg/mL in children *(75)*. Serum phenytoin concentrations of patient in this study ranged from 1.11 to 39.5 µg/mL. Result show that 13 patients achieved sub-therapeutic concentrations, 19 patients achieved therapeutic concentrations and 7 patients achieved supra- therapeutic concentrations.

Dosage regimen and dosage form of phenytoin in patients could affect steady-state serum concentrations. As different dosage forms, serum concentration of patients who were treated with sustained release capsule slightly lower than that of patients who were treated with tablet. Furthermore, sampling time in each patient was different. For patients who recieved once daily had longer sampling time than patients who received twice daily. Therefore, a wide variation of steady-state serum concentrations (as seen in Figure 3) that may obtained from different dosage regimens, dosage forms and sampling times.

3. Therapeutic outcome

The results of seizure control in the studied patients with varied steady-state serum concentrations of phenytoin are presented in Table 14. For the good control patients in this study, number of patients whose serum concentrations within sub-therapeutic concentrations, therapeutic concentrations and supra-therapeutic concentrations were 13(33.3%), 17(43.6%) and 7(18%), respectively. Poor seizure

control existed in two patients (5.1%) who had steady-state serum concentrations within therapeutic range. The variation in clinical outcomes related to phenytoin concentrations may explain above results.

As previous reported, optimum control without clinical signs of toxicity occurs more often with serum levels between 5 and 20 µg/mL, however some cases of epilepsy may be controlled with lower serum levels of phenytoin and some cases could not control seizure at therapeutic concentrations(11). Hayes and Kootsikas (84) found that sub-therapeutic concentrations of phenytoin still could control seizures, while those with phenytoin serum concentrations higher than 20 µg/mL were still tolerated without any serious adverse drug reactions.

Table 14 Seizure control in patients with different steady-state serum concentrations.

Seizure control	Number of patient (%) with serum concentrations of			Total Number of patient, n = 39 (%)
	< 5 µg/mL	5-20 µg/mL	>20 µg/mL	
Good control	13 (33.3%)	17 (43.6%)	7 (18%)	37 (94.9%)
Poor control	-	2 (5.1%)	-	2 (5.1%)

As the above results, thirty-seven patients (94.9%) had good seizure control. Although two patients (pt no.12 & 30 in Table 12) had poor seizure control, they had serum concentrations within therapeutic range, 15.30 and 16.94 µg/mL, respectively. Poor seizure control may occurred from poor compliance of these patients.

The lowest steady-state serum concentration of phenytoin which resulted in good seizure control appeared to be 1.11µg/mL. For prophylaxis patients, seizure could control with low serum concentrations of phenytoin. Whereas the highest steady-state serum concentration of phenytoin which resulted in good seizure control without adverse effect appeared to be 39.5 µg/mL.

Regarding to sub-therapeutic range, low level of serum concentration may be affected from overtime of sampling which were more than 12 hours. As seen in patient number 35 (Table 12) who had lowest serum concentration (1.11 µg/mL),

blood sample was collected at 13 hours after last dose. At this time, it was over than the time of trough concentration in patients who received phenytoin tablet twice daily. Furthermore, poor compliance patient who did not received drug usually as prescribed may affect the serum concentrations. Thus, serum phenytoin concentrations were monitored as low level.

Adverse drug reactions (ADR) observed in this study were dose-related adverse effect. It was occurred in three patients who had steady-state serum concentrations within therapeutic range but all of them had good seizure control. In this study, three patients had the incidence of adverse drug reactions. Drowsiness occurred in one patient (pt no.8) whose serum concentration was 15.71 µg/mL and headache occurred in two patients (pt no.11 & 16) whose serum concentrations were 16.82 and 16.01 µg/mL, respectively. In general, the most common dose-related adverse effects of phenytoin are central nervous system (CNS) effects such as nystagmus, ataxia, drowsiness, dizziness, diplopia and headache. These incidence were increased when serum phenytoin concentrations were higher than 15µg/mL *(57, 58)*. Although three patients had adverse drug reaction, it was non-serious adverse drug reactions.

Regarding to patients who had supra-therapeutic concentrations of phenytoin such as patients number 10 and 27 had high level of phenytoin concentration (34.28 and 39.50 µg/mL, respectively) but adverse drug reaction was not occurred. Because blood samples from these patients may be collected after dose administration less than 8 hours. At this time, the level of serum concentration did not present the true trough concentrations of phenytoin.

Furthermore, non-dose related adverse drug reaction (such as gum hyperplasia) was also obvious in this study. It appeared in one patient (pt no.19) who used phenytoin for two-year, steady-state serum phenytoin concentrations was within therapeutic range (17.97 µg/mL).

4. Determination of pharmacokinetic parameters

    4.1 Base model

        To estimate pharmacokinetic parameter values of phenytoin, one-compartment model with the Michaelis-Menten equation was employed using the PREDPP library subroutine ADVAN10 in Nonlinear Mixed Effects Modeling (NONMEM) see in Appendix E. The base model consisted of structural model and statistical model. The structural model was the Michaelis-Menten equation while statistical model was interindividual and intraindividual variability model. Alternative model of statistical model was additive, proportional and exponential model. In this study, the best models for estimation of interindividual and intraindividual variation were the exponential model and the additive error model, respectively. Reason of this, the exponential model for interindividual variation and the additive error model for intraindividual variation could be used to determine residual plots. The residual plots were some of the goodness of fits of data. The acceptable range of weighted residual error was ± 3 to ± 5 μg/mL for NONMEM analyzing (83). Figure 4 shows scatterplots between population predicted concentrations (PRED) versus weighted residual error (WRES) that residual error value was within the acceptable range. Primary pharamacokinetic parameters (such as V_{max}, K_m and Vd) including the interindividual variability (ω^2_{Vmax}, ω^2_{Km} and ω^2_{Vd}) and the intraindividual variability (σ^2) were estimated.

        Steady-state blood level data after maintenance oral administration of phenytoin in 39 children patients were analysed using NONMEM. A data set for NONMEM was created for analysis (see in Appendix F). Since data of steady-state concentration of phenytoin in each patient had one measurement of phenytoin concentration (39 steady-state phenytoin concentrations-dosage pairs from 39 patients) therefore K_m must be fixed in running program. Due to previous studies of pharmacokinetic parameters in children, the results show that K_m values were ranged from 3 to 7 μg/mL *(65, 67, 68, 75)*. To determine suitable K_m value for fitting program, K_m values were fixed within range 3 to 7 μg/mL and changed by raising 0.1 in each run. The suitable K_m was the value that gave the minimum objective function value (OFV) and the covariance from NONMEM analyzing.

Each K_m value was fixed within range 3 to 7 µg/mL for NONMEM analysis. The suitable value of Km obtained from this study was 4.5 µg/mL. With this value, NONMEM could analyze the covariance that provided standard error. Standard error means precision of estimate. If K_m value was fixed with other value, NONMEM could not analyze the covariance.
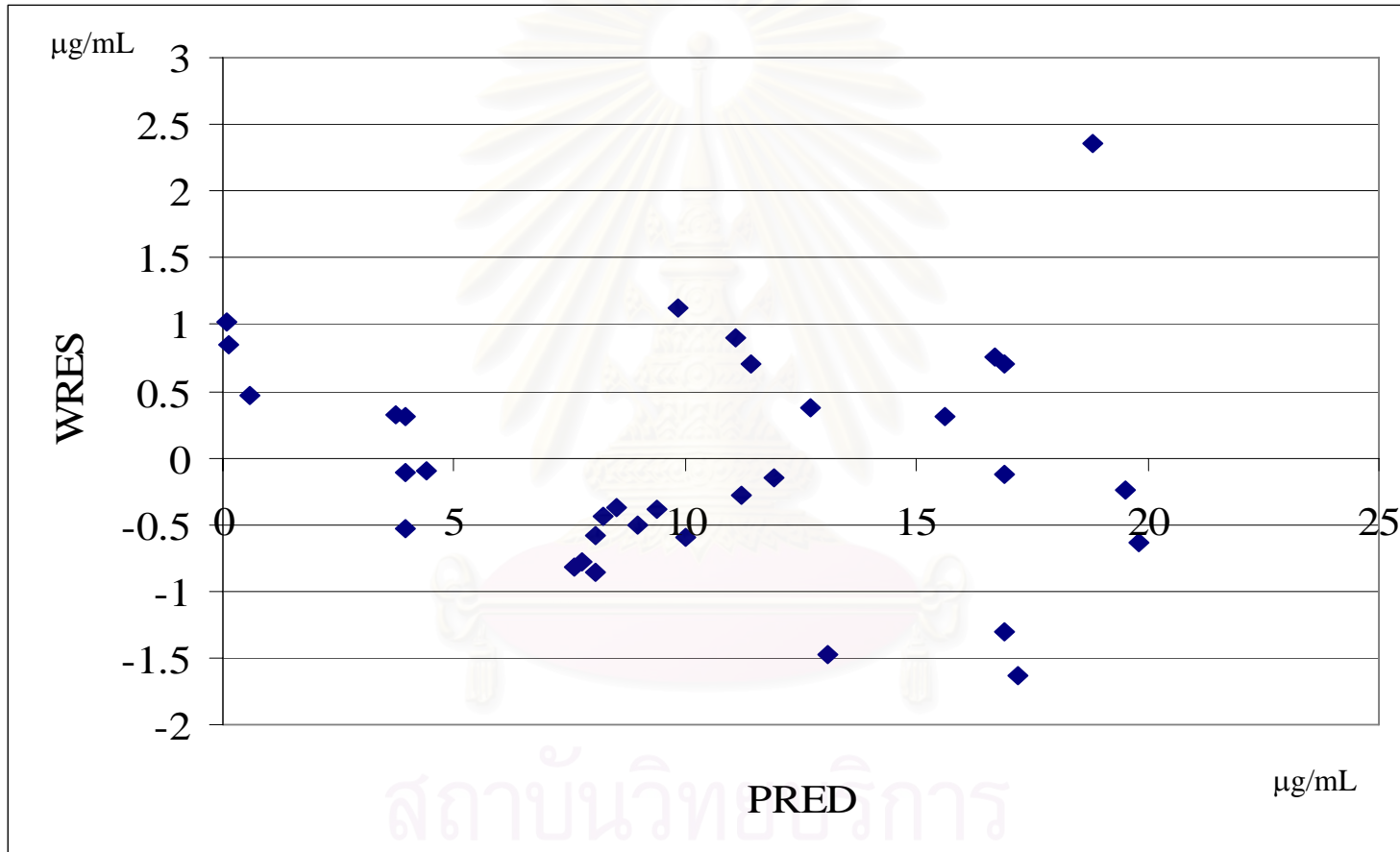
Figure 4   Scatterplots between population predicted concentrations (PRED) versus weighted residual error (WRES)

With K_m value of 4.5 μg/mL, the covariance that obtained from NONMEM analyzing provided the standard error of parameters. The 95% confidence interval (CI) values of each parameter were determined from the statistic calculation at $Z_{\alpha 0.05}$ (two-tailed). The estimates of pharmacokinetic parameters and their 95% confidence interval (CI) are shown in Table 15.

Table 15 Pharmacokinetic parameters of phenytoin (base model)

Parameters	Estimates	SE	95%CI
Objective function value	181.89	-	-
V_{max} (mg/kg/d)	5.16	0.18	4.81-5.51
K_m (μg/mL)	4.50	-	-
Vd (L/kg)	0.31	0.03	0.25-0.37
ωV_{max} (%)	94.33	0.56	93.24-95.42
ωK_m (%)	5.55	0.02	5.51-5.59
ω Vd (%)	29.86	0.14	29.59-30.13
σ (%)	7.41	0.008	7.39-7.43

ω = the interindividual variability (%CV)

σ = the intraindividual variability (%CV)

As result in Table 14, the maximum rate of metabolism (V_{max}) was estimated to be 5.16 mg/kg/d. The estimated Micheallis-Menten constant (K_m) value was 4.5 μg/mL. Volume of distribution (Vd) was estimated to be 0.31 L/kg. The interindividual variabilities of V_{max}, K_m and Vd were estimated to be 94.33%, 5.55% and 29.86%, respectively. The intraindividual (residual) random variability of serum phenytoin concentration was 7.41%.

Considering to variation of pharmacokinetic parameters of phenytoin in this study, the percentage of interindividual variability (%CV) of V_{max} was much higher than that of K_m and Vd. For reason of high variability of V_{max}, each patient had one

data of steady-state concentration therefore K_m value was fixed before estimating V_{max} value.

Table 16 The pharmacokinetic parameters of phenytoin in different age group

Parameter	Mean ± SD				p-value
	0-3 yrs (n=6)	4-6 yrs (n=4)	7-9 yrs (n=13)	10-14 yrs (n=16)	
V_{max} (mg/kg/d)	4.07±2.04	5.52±1.76	6.63±3.75	6.63±3.45	0.411
K_m (μg/mL)	4.5	4.5	4.5	4.5	-
Vd (L/kg)	0.82±0.36	0.54±0.21	0.42±0.11	0.24±0.10	0.001*

*statistically significant different between group at $p< 0.05$(two-tailed analysis)

To determine the difference of pharmacokinetic parameter values in different age groups, the result was shown in Table 16. Concerning age, 39 patients were divided into four groups; 0 to 3, 4 to 6, 7 to 9 and 10 to 14 year-old-group. Mean pharmacokinetic parameters of each group were compared using one-way ANOVA. There was no statistically significant differences in V_{max} values (p=0.411). Although there was statistically difference in Vd values among different age groups (p=0.001), Vd is not the important parameter for design the dosage of phenytoin.

4.2 Exploring covariate relationship

To assess covariate relationship on pharmacokinetic parameters, each covariate was added into the base model. In each run, change in the objective function value was noted and compared. In this study, screened covariates were age, body weight and gender. In NONMEM processing, the Michealis-Menten Constant (K_m) was fixed in the base model. Therefore, the influence of screened covariates on pharmacokinetic parameters were not assessed in the base model of K_m while the base model of the maximum rate of metabolism (V_{max}) and volume of distribution (Vd) were assessed as follows:

Influence of Age

Age of 39 patients participated throughout the study was range from 10 months to 14 years with mean $\pm$ SD of 8.58 $\pm$ 3.82 years. Results of simple regression analysis between pharmacokinetic parameters (V_{max} and Vd) and patient's age are shown in Table 17. Scatterplots of V_{max} and Vd values versus patient's age are shown in Figures 5 and 6, respectively.

Table 17 Simple regression analysis between pharmacokinetic parameters and patient's age

	V_{max} (mg/kg/d)	Vd (L)
Correlation coefficient, r	0.264	0.04
Correlation of determination, r^2	0.07	0.002
p-value	0.104	0.807
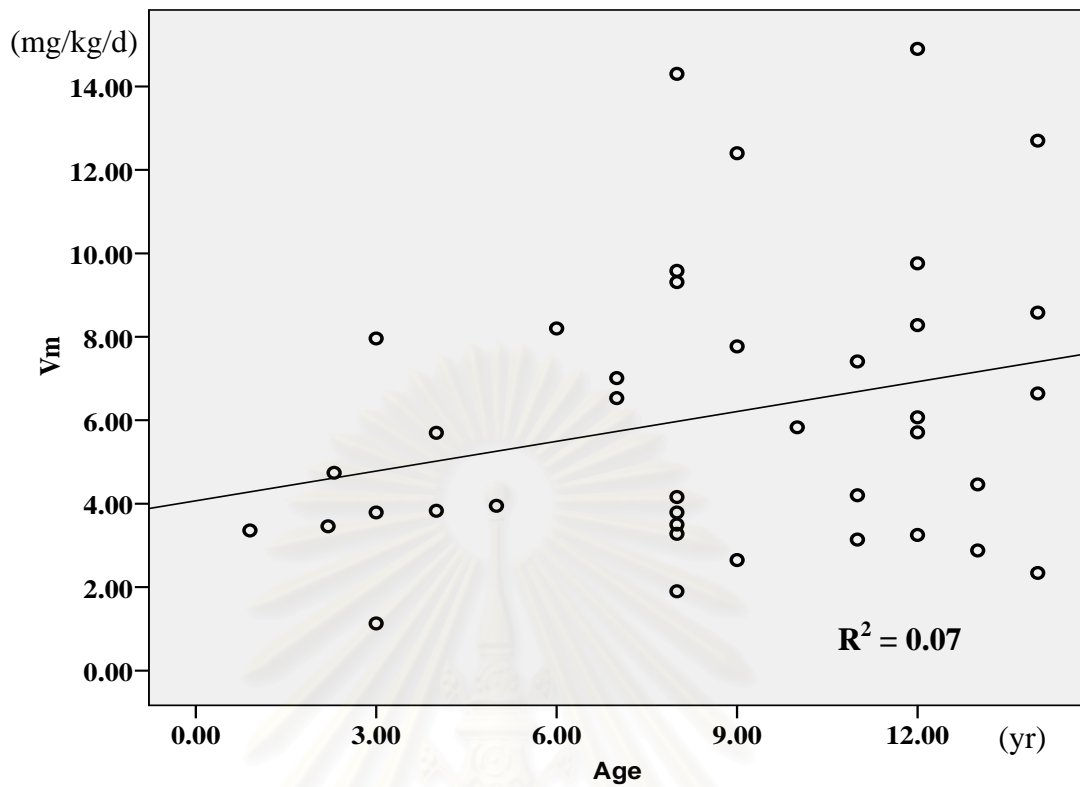
Figure 5 Scatterplots of V_{max} values versus patient's age
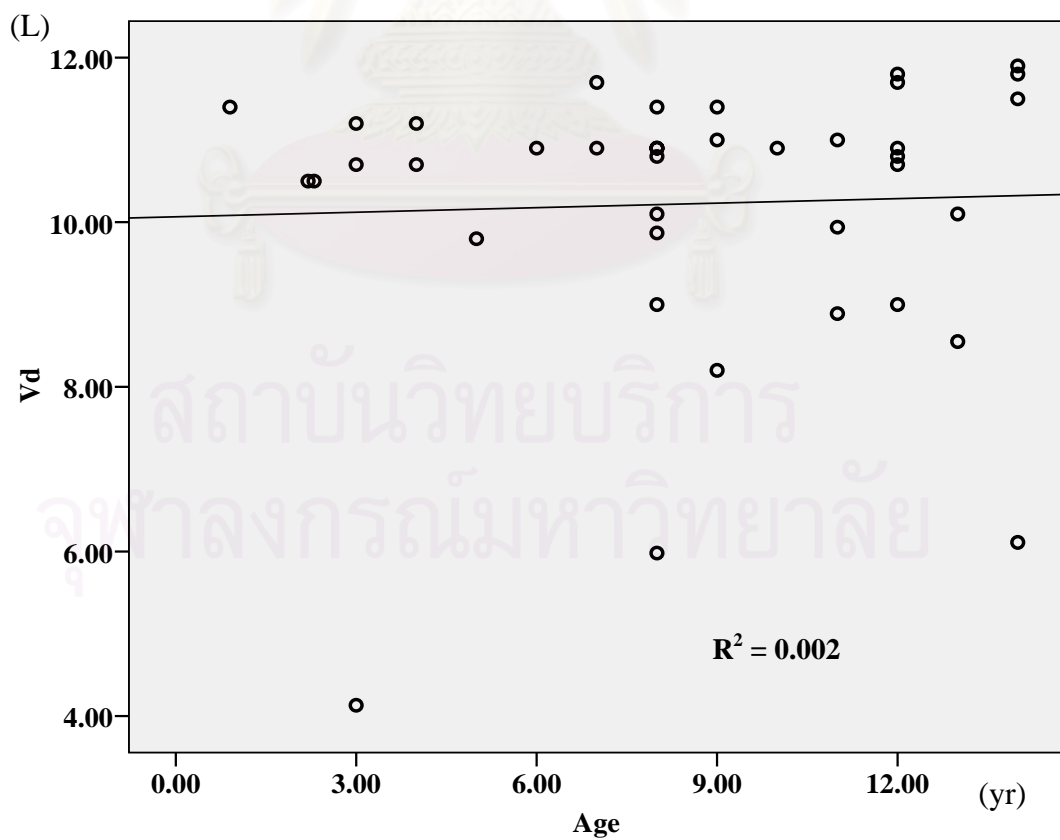
Figure 6 Scatterplots of Vd values versus patient's age

Correlation between V_{max} and Vd parameters versus patient's age was determined. As from the results of simple regression analysis in Table 17, there were no significant correlation between V_{max} and Vd parameters versus patient's age (p = 0.104 and 0.807, respectively). There was no influence of age on V_{max} and Vd parameters. Thus this covariate was not added to the base model for estimate values by NONMEM.

Influence of Weight

Average body weight of the 39 patients was ranged from 9 to 99 kg with mean ± SD of 32.28 ± 18.04 kg. Simple regression analysis between pharmacokinetic parameters (V_{max} and Vd) and body weight were determined and shown in Table 18. Scatterplots of V_{max} and Vd values versus patient's weight are shown in Figures 7 and 8, respectively.

Table 18 Simple regression analysis between pharmacokinetic parameters and body weight

	V_{max} (mg/kg/d)	Vd (L)
Correlation coefficient, r	0.358	0.029
Correlation of determination, r^2	0.128	0.001
p-value	0.025*	0.859

* statistically significant correlation at p< 0.05(two-tailed analysis)
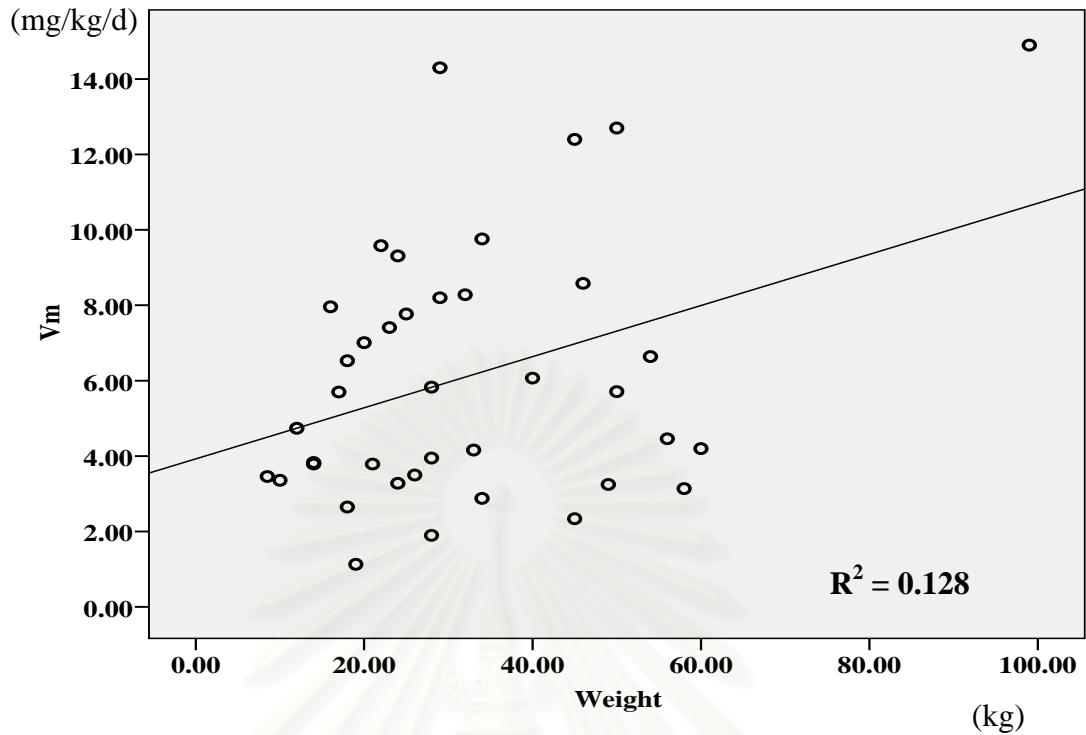
Figure 7 Scatterplots of V_{max} values versus patient's weight
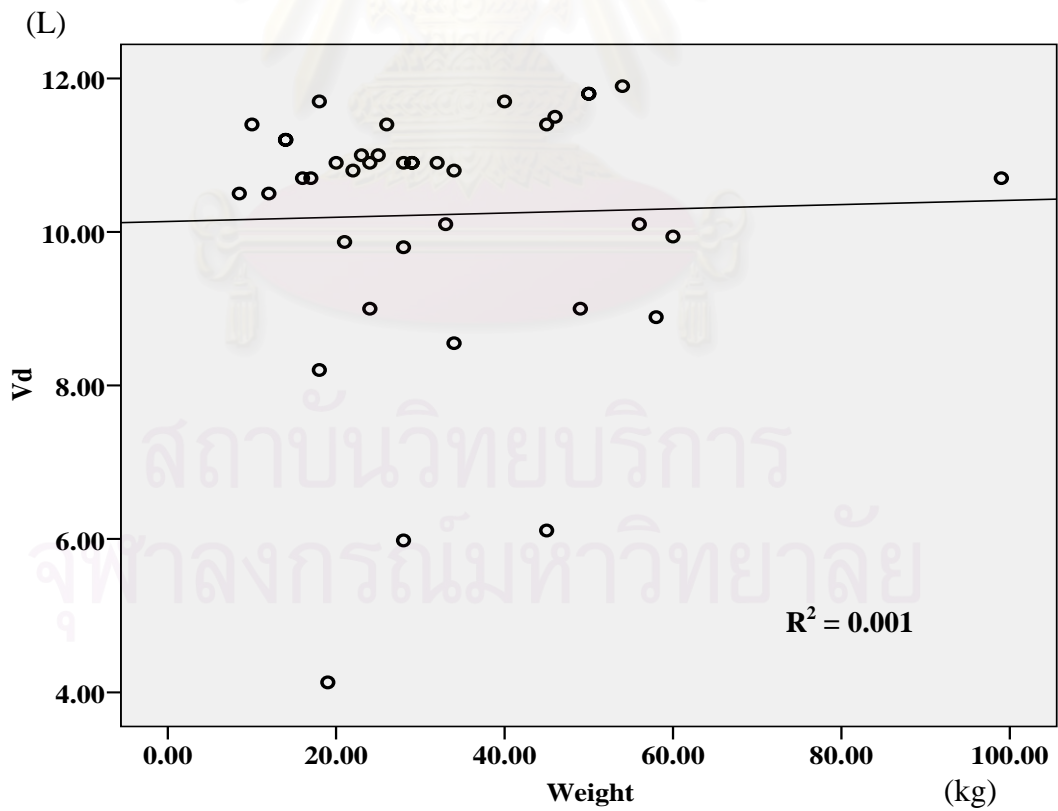
Figure 8 Scatterplots of Vd values versus patient's weight

According to the results in Table 18, there was significant correlation between V_{max} versus body weight. Body weight had positive correlation with V_{max} (r=0.358, p = 0.025) but no significant correlation with Vd (r=0.029, p = 0.859). The coefficient of determination (r^2=0.128) implies the strength of correlation that appears as a linear function of body weight on V_{max}. Results indicated that V_{max} value was influenced by body weight and increased with the increasing body weight. Thus, body weight of patient as a covariate was added into the base model of V_{max} for NONMEM running.

Influence of gender

Of total 39 patients, 29 were male (74.4%) and 10 were female (25.6%). Simple regression analysis between pharmacokinetic parameters (V_{max} and Vd) and gender were determined and shown in Table 19.

Table 19 Simple regression analysis between pharmacokinetic parameters and gender

	V_{max} (mg/kg/d)	Vd(L)
Correlation coefficient, r	0.002	0.11
Correlation of determination, r^2	0.001	0.012
p-value	0.992	0.505

As the results from Table 19, patient's gender had no statistically significant correlation with both of V_{max} and Vd parameters (r=0.002, p=0.992 and r=0.012, p=0.505, respectively). Therefore, V_{max} and Vd of female group was not significantly different from that of male group. For this aspect, the results agreed with other reports. Most studies reported no influence of gender on pharmacokinetic parameters of phenytoin *(10, 73, 75, 76)*.

Summary of influence of covariates on pharmacokinetic parameters, age and gender were no significantly correlation with V_{max} and Vd while patient's body weight had statistically significant correlation with V_{max}. It was expected that there was increase metabolic capacity of phenytoin when body weight increased. These results agreed with those reported by Valodia et al *(73)* who mentioned that body weight was the most influential factor to V_{max}. Therefore, body weight was

incorporated into V_{max} parameter model. Goodness of fit with this covariate model was examined later.

4.3 Developing the model

In developing the model, it is generally advisable to start from simplest model, and proceed toward greater complexity. An initial analysis was conducted by estimating the base model parameters without any covariates. Next step was the building of covariate model to improve the fit of the data to the model. The improved model was judged by change in objective function value (OFV). Hypothesis test was used to compare different model by using the chi-squared distribution of OFV with degree of freedom equal to the difference in number of parameter between the two models.

Results from exploring of covariates (such as age, gender and weight) indicated that weight was a potential influence on pharmacokinetic parameter. Considering to influence of weight on pharmacokinetic parameters, V_{max} had significant correlation(r=0.358) and coefficient of determination (r^2=0.128). Therefore, body weight was test as a potential factor influencing the pharmacokinetic parameter of phenytoin.

To test the significance of factors that influence pharmacokinetic parameters, the value of the minimum objective function determined using the NONMEM fitting routine. When the decrease of the objective function value was more than 3.84 (p<0.05) which approximates the chi-square distribution with 1 degree of freedom*(83)*, the influence of covariate was considered and covariate model was selected. Table 19 presents the decrease of objective function value between base model and covariate model of V_{max}. The result appeared that the decrease of objective function value was 2.05. As this value, weight was no significant covariate of V_{max}. Therefore, the model that was selected and used to evaluate pharmacokinetic parameters was the base model.

Table 20 Comparison of the minimum objective function value between the base model and the covariate model of V_{max}

	OFV	**Δ^1**	**Conclusion[2]**
Base model $V_{max} = \theta1 * EXP(\eta1)$	181.89	-	-
Covariate model $V_{max} = \theta1 *(WT/32)^{\theta2} * EXP(\eta1)$	179.84	2.05	No significant

[1] The decrease of objective function value

[2] Relative to base model

Comparison of pharmacokinetic parameters

Table 21 shows the pharmacokinetic parameter values of phenytoin in this study were different from the past which also studied in children who were under 15 years old. And also shows the difference of pharmacokinetic parameter values from many studies.

The pharmacokinetic parameters of phenytoin in 39 Thai children were estimated by using NONMEM. V_{max} was estimated to be 5.16 mg/kg/d, K_m was estimated to be 4.5 μg/mL and Vd was estimated to be 0.31 L/kg. These values were agreed with the reported V_{max} and K_m values for phenytoin in children patients that were range from 5-20 mg/kg/d and 3-7 μg/mL, respectively*(63)*. However the pharmacokinetic parameter values of Thai children in this study were different from other previous studies that also performed in children, V_{max} value was slightly low compared to the previously reported values while K_m value was higher than Caucasian and Japanese children and lower than Chinese and Saudi children. A genetic polymorphism has been evoked for the variation in the metabolism in races. Accordingly, V_{max} and K_m of phenytoin may be altered by genetic polymorphism*(85)*.

Phenytoin is metabolized in the liver principally by CYP2C9 and also to a minor extent by CYP2C19. Genetic polymorphisms of CYP2C9 are expressed at a greater frequency in Caucasians (7-10%) compared to Asians (<3%). Moreover, genetic polymorphisms of CYP2C19 is completely absent in 2-5% of Caucasians,

20% of Asians. Comparison between CYP2C9 and CYP2C19, CYP2C19 metabolized phenytoin with higher K_m *(14, 86)*.Therefore, K_m values in Asians were higher than Caucasians. This is reason why K_m value in Thai from this study was higher than Caucasians.

In case, K_m value in Thai from this study was lower than Chinese and Saudi children. Tassaneyakul et al *(87)* studied the pharmacogenomics of CYP2C19 in 107 Thai. It was revealed that the frequencies of CYP2C19 defective alleles in Thai were lower than those observed in the other Oriental populations (such as Chinese, Saudi). Because the proportion of genetic polymorphisms of CYP2C9 in Thai patients may be different from other populations and no study about CYP2C9 in Thai patients. Therefore, the effects of genetics on the population pharmacokinetic of phenytoin in Thai patients should be determined in future study.

Table 21 The pharmacokinetic parameters of phenytoin in children

Studies	Race	V_{max} (mg/kg/d)	K_m (μg/mL)
This study	Thai	5.16	4.50
Grasela et al *(10)*	Caucasian	5.92	3.80
Yukawa et al *(64)*	Japanese	5.41	3.08
Rui et al *(65)*	Chinese	7.31	5.77
Abduljabbar et al *(68)*	Saudi	10.35	4.79

For comparison with Thai adults, the obtained V_{max} and K_m values in Thai epileptic children show difference from Thai adults. Kanjanasilp et al *(12)* reported the population pharmacokinetics of phenytoin in Thai adults patient (n=167). V_{max} was estimated to be 12.5 mg/kg/d and K_m was estimated to be 16.10 μg/mL. Another study was also reported by Chanawong *(74)*. She reported V_{max} 7.80 mg/kg/d and K_m 9.28 μg/mL (n=42). This is confirming that the dosage regimens for children should be designed with pharmacokinetic parameter values which were reported from the study of children. Thus specific pharmacokinetic parameters are selected and used for dosage adjustment in specific group as children.

There were several limitations in this study. From overall of the results, a small value of correlation in this study may be due to small sample size and small variation in patients. In addition, each patient had one steady-state phenytoin concentration. Only data set of 39 patients was analyzed using Nonlinear Mixed Effects Model (NONMEM) therefore higher sample size should be required to develop more precise. Moreover, the analysis need a large number of steady-state concentrations data by each patient in the study should have more than two data of steady-state concentration-dosage pairs.

CHAPTER V

CONCLUSION

As previously reported that the pharmacokinetic parameters of phenytoin, V_{max} and K_m are necessary for phenytoin maintenance dosage design. Many factors had influenced on these parameters and these parameters are claimed to be race dependent. Systematic study for such parameters in Thai epileptic have lacked especially in children. Therefore, this study was designed to determine pharmacokinetic parameters of phenytoin in Thai epileptic children and study the factors those influence pharmacokinetic parameters of phenytoin. These results could be summarized as follows:

1. The study was conducted in patients who were attended at neurology clinic of children at Chonburi Hospital during October 2007 to March 2008. Of total 39 patients who had participated throughout the study. All included patients received phenytoin monotherapy for epilepsy. There were 39 data of steady-state serum concentrations which collected prospectively from 39 out-patients. Twenty-nine (74.4%) were male and ten (25.6%) were female. All patients were under 15 years old. Their age was range from 10 months to 14 years with mean ± SD of 8.58 ± 3.82 years. Average body weight was range from 9 to 99 kg with mean ± SD of 32.28±18.04 kg.

2. Steady-state serum phenytoin concentrations ranged from 1.11 to 39.5 µg/mL. It was found that 37 patients had good seizure control and 3 patients had poor seizure control. For the good seizure control patients, number of patients whose serum concentrations within sub-therapeutic concentrations , therapeutic concentrations and supra-therapeutic concentrations were 13(33.3%), 17(43.6%) and 7(18%), respectively. Although thirteen patients had sub-therapeutic concentrations of phenytoin, they had long time of sampling. At this time, it was over than time for trough concentration at steady-state, therefore serum concentrations were monitored as lower than normal therapeutic range. For poor seizure control patients, all of them had concentrations within therapeutic range but they may had poor compliance such as did not take phenytoin usually as prescribed. In this study, sampling time and patient's compliance could affect the level of serum concentration. Regarding to adverse drug reactions, three patients had incidences of CNS adverse drug reaction.

3. For determination of pharmacokinetic parameters, these data were analysed using Nonlinear Mixed Effect Model (NONMEM). The structural model was the Michaelis-Menten equation while the statistical base models of phenytoin for NONMEM were the exponential model and the additive error model. K_m values were fixed in analysis. Due to previous studies of pharmacokinetic parameters in children, the results show that K_m values were ranged from 3 to 7 µg/mL. Therefore, K_m values were fixed in running with range from 3 to 7 µg/mL and increased every 0.1 in each run. Pharmacokinetic parameters of phenytoin in Thai epileptic children, V_{max} was estimated to be 5.16 mg/kg/d, K_m was estimated to be 4.50 µg/mL and Vd was estimated to be 0.31 L/Kg. The interindividual variabilities of V_{max}, K_m and Vd were estimated to be 94.33%, 5.55% and 29.86%, respectively. There was no statistically significant difference in V_{max} and K_m values among different age groups (0-3, 4-6, 7-9 and 10-14 years old).

4. According to the results of exploring covariates, the influence of covariates including age, weight and gender were studied. It was found that age and gender were no significantly correlation with V_{max} and Vd while patient's body weight had statistically significant correlation with V_{max} (r=0.358, p = 0.025). For developing the model, body weight was added into the base model of V_{max}. The change of objective function value between base model and covariate model of V_{max} did not decreased by significantly (p<0.05). Therefore, the model that was selected and used to evaluate pharmacokinetic parameters was the base model.

5. The pharmacokinetic parameter values of Thai children in this study were different from other previous studies that also performed in children, V_{max} value was slightly low compared to the previously reported values while K_m value was higher than Caucasian and Japanese children and lower than Chinese and Saudi children. Genetic polymorphism could be explained for the variation in the metabolism of phenytoin. Pharmacokinetic parameters may be altered by different genetic polymorphism. Moreover, the pharmacokinetic parameters of phenytoin in Thai epileptic children were different and less than the parameters obtained from Thai adult patients in previous studies.

6. Specific pharmacokinetic parameters are appropriate to use for dosage adjustment in specific group. The obtained V_{max} and K_m values in this study may be very useful for designing dosage regimens for the treatment of Thai epileptic children.

These may help physicians and pharmacists, work together to optimize the dosage regimen. However, pharmacokinetic parameters of phenytoin in this study may have limitations because this study recruited only small number of patients and each patient had only one data of steady-state concentration of phenytoin. Therefore, the further studies should be performed in future.

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APPENDICES

APPENDIX A

<u>แบบบันทึกข้อมูลทั่วไปของผู้ป่วย</u>

HN: ....…………… AN: ……........................ วันที่เข้ารักษา…………....................

เพศ : ◯ชาย ◯ หญิง ผู้ป่วย ◯นอก ◯ ใน ward ……………. เตียง ....

วัน/เดือน/ปีเกิด.................... อายุ...................... ปี น้ำหนัก: ….........กก. ส่วนสูง: ……..........ซม.

โรคประจำตัวอื่น❑ โรคเกี่ยวกับตับ❑ โรคเกี่ยวกับไต ❑ อื่นๆ…………………………………

ผู้ปกครองปัจจุบันที่ดูแล...

ประวัติโรคลมชักในครอบครัว...

สาเหตุของการชักครั้งแรก..

ความถี่ของการชัก…………..

ระยะเวลาของการชักแต่ละครั้ง...

ลักษณะและชนิดของโรคลมชัก …………………………………………….....................

…………………………………………………………………………………………………

ประวัติการใช้ยาอื่นๆ…………………………………………………………………..

…………………………………………………………………………………………………

…………………………………………………………………………………………………

ประวัติการแพ้ยา…………………………………………………………………..…………

……………………………………………………………………………………………………

……………………………………………………………………………………………………

……………………………………………………………………………………………………

ผู้ป่วยพบแพทย์ตามกำหนดนัดหรือไม่ ❑ ใช่ ❑ ไม่ใช่ เพราะ..

ผู้ป่วยได้รับประทานยาตามแพทย์สั่งสม่ำเสมอหรือไม่ ❑ ใช่ ❑ ไม่ใช่ เพราะ...........................

ผู้ป่วยเคยหยุดยาเองหรือเพิ่มลดยาเองหรือไม่ ❑ ใช่ ❑ ไม่ใช่

<u>หมายเหตุ:</u>

……………………………………………………………………………………………………

……………………………………………………………………………………………………

……………………………………………………………………………………………………

……………………………………………………………………………………………………

……………………………………………………………………………………………………

<u>แบบบันทึกข้อมูลการใช้ยาของผู้ป่วยและการคุมอาการชัก</u>

ยาที่ได้รับ/ วันที่						
1. ……………………………..						
2. ……………………………..						
3. ……………………………..						
4. ..……………………………						
5. ………..............................						
6. …………………………..						
7. ………...…………………..						
8. ..…………………………….						
9. ………...…………………..						
10.						
อาการชัก						

<u>แบบบันทึกผลตรวจทางห้องปฏิบัติการณ์</u>

วันที่						
Liver Function Test						
ALT (7-56 U/L)						
AST (8-40 U/L)						
AlkPhos (38-126 U/L)						
Alb (3.5-5 g/dL)						
Renal Function Test						
BUN (10-20 mg/dL)						
SCr (0.5-1.6 mg/dL)						
Total Calcium (9.0-11.0mg/dL)						

<u>แบบบันทึกข้อมูลการตรวจวัดระดับยา **phenytoin** ในเลือด</u>

ครั้งที่เจาะวัด ข้อมูลระดับยา					
วันที่ได้รับยาเริ่มต้น					
เวลารับประทานยา					
วันที่เจาะเลือด					
เวลาเจาะเลือด					
ระดับยา phenytoin (µg/mL)					
หมายเหตุ					

<u>แบบบันทึกอาการไม่พึงประสงค์จากการใช้ยา</u>

อาการไม่พึงประสงค์	......./……/….	......./……/….	......./……/….	......./……/….
Nystagmus				
Diplopia				
Ataxia				
Confusion				
Dizziness				
Drowsiness				
Headache				
Nausea				
Vomitting				

APPENDIX B

หนังสือแสดงเจตนายินยอมเข้าร่วมการวิจัย (Consent form)

ชื่อ โครงการวิจัยค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของเฟนิทอยน์ในเด็กไทยที่เป็นโรคลมชัก

วันที่ .... เดือน............... พ.ศ. ....

        ข้าพเจ้าซึ่งเป็นผู้แทนโดยชอบธรรมของ(ดช./ ดญ.) ....
ได้รับทราบเกี่ยวกับ วัตถุประสงค์ของการทำวิจัย ขั้นตอนการดำเนินการวิจัย ประโยชน์ที่จะได้รับ
และผลข้างเคียงที่อาจเกิดขึ้นขณะเข้าร่วมโครงการวิจัยจากผู้ทำการวิจัยเป็นอย่างดีแล้ว โดยทราบว่า
ในขั้นตอนการเจาะเลือดจากผู้ป่วยเพื่อตรวจทางห้องปฏิบัติการณ์ ได้แก่ วิเคราะห์ระดับยาเฟนิ
ทอยน์ในเลือด การทำงานของตับไต จะทำการเจาะเลือดประมาณ 5 ซีซี หรือเท่ากับ 1 ช้อนชา
ซึ่งอาจมีความไม่สะดวกสบายจากการเจาะเลือด และอาจทำให้มีเลือดออกหรือจ้ำเลือดบริเวณแทง
เข็มได้ โดยข้าพเจ้ามีความเข้าใจดีทุกประการ

        ข้าพเจ้ามีสิทธิที่จะขอยกเลิกการเข้าร่วมโครงการวิจัยนี้ของผู้ป่วยได้ตลอดเวลาเมื่อต้องการ
และยินยอมให้ผู้ป่วยเข้าร่วมโครงการวิจัยนี้ด้วยความสมัครใจ ทั้งนี้การขอยกเลิกการเข้าร่วม
โครงการวิจัยนี้จะไม่มีผลต่อการรักษาโรค โดยผู้ป่วยยังมีสิทธิที่จะได้รับการดูแลจากแพทย์ได้
ตามปกติ

        ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับผู้ป่วยเป็นความลับ และจะเปิดเผยได้เฉพาะใน
รูปที่เป็นสรุปผลการวิจัย การเปิดเผยข้อมูลเกี่ยวกับตัวผู้ป่วยต่อหน่วยงานต่างๆที่เกี่ยวข้องกระทำได้
เฉพาะกรณีจำเป็นด้วยเหตุผลทางวิชาการเท่านั้น

        ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ และได้ลงนามในใบ
ยินยอมนี้ด้วยความเต็มใจ

                    ลงชื่อ .... ผู้ปกครอง

                        (..ชื่อ-นามสกุล ตัวบรรจง)

                    ลงชื่อ ....ผู้ดำเนินการโครงการวิจัย

                        (.......................................ชื่อ-นามสกุล ตัวบรรจง)

                    ลงชื่อ .... พยาน

                        (.......................................…...ชื่อ-นามสกุล ตัวบรรจง)

หมายเหตุ : ในกรณีที่มีปัญหาหรือข้อสงสัยสามารถติดต่อได้ที่

    พญ. ศิริพร ปิ่นเจริญ   กลุ่มงานกุมารเวชกรรม โรงพยาบาลชลบุรี   โทร 038-931388

    ภญ. ภริษา วิสุทธิวงศ์   กลุ่มงานเภสัชกรรม   โรงพยาบาลชลบุรี   โทร 08-1761-7471

APPENDIX C

Determination of serum phenytoin concentration

Serum phenytoin concentrations were determined by fluorescence polarization immunoassay (FPIA, TDx[®] Abbott Laboratories)

1. Calibration

Calibration were performed follow manual of using TDx[®] analyzer. 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 control 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 (ug/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 mg/L.

3. Precision

Precision of both within day and between day assay were tested by the manufacturer of TDx[®] with the concentrations of 7.50, 15.0, 30.0 mg/L. Results from this study typically yielded CV's of less than 5%.

4.   Accuracy of recovery

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

APPENDIX E

Control stream for model

```
$PROB  EXPONENTIAL MODEL
$INPUT ID TIME AMT DV RATE EVID AGE WT HT ISM
$DATA EXPONENTIAL.CSV IGNORE=C
$SUBR ADVAN10 TRANS1 TOL=5

$PK
 TVVM=THETA(1)
 TVKM=THETA(2)
 TVV=THETA(3)
 VM=TVVM*EXP(ETA(1))
 KM=TVKM*EXP(ETA(2))
 V=TVV*EXP(ETA(3))
 S1=V

$ERROR
 IPRED=F
 Y=F+ERR(1)

$THETA(0,10)
$THETA(4.5 FIXED)
$THETA(0,1)
$OMEGA 0.25 0.25 0.25
$SIGMA 0.1

$EST MAXEVAL=9999 PRINT=5 POSTHOC MSF=pht.MSF
$COVARIANCE
$TABLE ID TIME AMT IPRED FILE=pht.tab NOPRINT
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APPENDIX F

Data for analysis of pharmacokinetic parameters

Pharmacokinetic parameters of phenytoin
Measured conc from clinical routine (therapeutic drug monitoring)
39 Thai epileptic children
AMT=dose per day, DV= Concentration at steady state, SEX(Female=0, Male=1)

ID	TIME (hr)	AMT (mg/d)	DV (µg/mL)	RATE	EVID	AGE (yr)	BW (kg)	HT (cm)	SEX
1	0	100	0	0	1	3	19	97	1
1	13	0	20.82	0	0	3	19	97	1
2	0	175	0	0	1	8	28	118	1
2	14	0	24.94	0	0	8	28	118	1
3	0	259	0	0	1	9	45	142	0
3	12	0	9.96	0	0	9	45	142	0
4	0	100	0	0	1	7	18	108	1
4	12.5	0	2.3	0	0	7	18	108	1
5	0	234	0	0	1	12	40	157	1
5	23.5	0	8.87	0	0	12	40	157	1
6	0	276	0	0	1	14	54	165	1
6	19.5	0	13.64	0	0	14	54	165	1
7	0	92	0	0	1	8	26	134	1
7	19	0	2.92	0	0	8	26	134	1
8	0	276	0	0	1	12	50	148	1
8	19	0	15.71	0	0	12	50	148	1
9	0	276	0	0	1	14	50	146	1
9	19	0	3.94	0	0	14	50	146	1
10	0	276	0	0	1	11	58	155	0
10	19.5	0	24.28	0	0	11	58	155	0
11	0	167	0	0	1	7	18	116	0
11	12.5	0	16.82	0	0	7	18	116	0
12	0	200	0	0	1	5	28	113	1
12	13	0	15.3	0	0	5	28	113	1
13	0	100	0	0	1	4	14	96	1
13	12	0	5.55	0	0	4	14	96	1
14	0	100	0	0	1	3	14	82	1
14	12.5	0	5.41	0	0	3	14	82	1
15	0	50	0	0	1	0.9	10	43	1
15	12.5	0	1.32	0	0	0.9	10	43	1
16	0	184	0	0	1	8	24	127	1
16	12.5	0	16.01	0	0	8	24	127	1
17	0	276	0	0	1	12	99	175	1
17	18.5	0	1.34	0	0	12	99	175	1
18	0	150	0	0	1	9	25	115	1
18	11	0	6.19	0	0	9	25	115	1

19	0	234	0	0	1	11	60	159	1
19	13.5	0	17.97	0	0	11	60	159	1
20	0	200	0	0	1	8	29	118	1
20	12	0	3.33	0	0	8	29	118	1
21	0	100	0	0	1	4	17	106	1
21	12	0	3.39	0	0	4	17	106	1
22	0	150	0	0	1	7	20	110	1
22	12	0	6.36	0	0	7	20	110	1
23	0	184	0	0	1	10	28	122	0
23	11.5	0	10.95	0	0	10	28	122	0
24	0	142	0	0	1	8	24	121	1
24	12	0	3.35	0	0	8	24	121	1
25	0	150	0	0	1	12	34	140	1
25	13	0	2.8	0	0	12	34	140	1
26	0	276	0	0	1	14	46	163	1
26	13	0	14.51	0	0	14	46	163	1
27	0	276	0	0	1	14	45	160	1
27	15	0	39.5	0	0	14	45	160	1
28	0	150	0	0	1	6	29	118	1
28	13	0	4.46	0	0	6	29	118	1
29	0	142	0	0	1	11	23	124	1
29	11	0	5.85	0	0	11	23	124	1
30	0	184	0	0	1	13	34	141	1
30	14	0	16.94	0	0	13	34	141	1
31	0	276	0	0	1	13	56	163	1
31	13.5	0	21.56	0	0	13	56	163	1
32	0	184	0	0	1	8	33	128	1
32	13	0	13.06	0	0	8	33	128	1
33	0	167	0	0	1	8	21	135	0
33	13.5	0	11.89	0	0	8	21	135	0
34	0	276	0	0	1	12	49	160	0
34	19	0	23.92	0	0	12	49	160	0
35	0	50	0	0	1	2	9	68	1
35	13	0	1.11	0	0	2	9	68	1
36	0	100	0	0	1	2	12	112	1
36	11	0	4.83	0	0	2	12	112	1
37	0	100	0	0	1	3	16	100	0
37	12	0	1.26	0	0	3	16	100	0
38	0	150	0	0	1	8	22	130	0
38	14	0	2.24	0	0	8	22	130	0
39	0	184	0	0	1	12	32	145	1
39	15.5	0	5.57	0	0	12	32	145	1

Vitae

Miss Parisa Wisuttiwong was born on the 4th of June in 1981 at Chonburi. She graduated with a Bachelor Degree in Pharmacy in 2003 from the Faculty of Pharmaceutical Sciences, Chulalongkorn University. Her current position is a pharmacist in Department of Pharmacy, Chonburi Hospital, Chonburi, Thailand.