การเปรียบเทียบค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของวาลโปรอิกแอซิดในผู้ป่วยโรคลมชัก
ที่คุมอาการชักได้และคุมอาการชักไม่ได้

นางสาวลักขณา บุญมาก

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COMPARISON OF PHARMACOKINETIC PARAMETERS OF VALPROIC ACID IN SEIZURE- CONTROLLED AND UNCONTROLLED EPILEPTIC PATIENTS

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ลักขณา บุญมาก : การเปรียบเทียบค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของวาลโปรอิกแอซิดในผู้ป่วยโรคลมชักที่คุมอาการชักได้และคุมอาการชักไม่ได้. (COMPARISON OF PHARMACOKINETIC PARAMETERS OF VALPROIC ACID IN SEIZURE-CONTROLLED AND UNCONTROLLED EPILEPTIC PATIENTS) อ.ที่ปรึกษา: รศ.ดร.ดวงจิต พนมวัน ณ อยุธยา, อ.ที่ปรึกษาร่วม : นพ. สมชาย โตวณะบุตร, 103 หน้า ISBN 974-53 -2453-1

การศึกษานี้มีวัตถุประสงค์เพื่อหาค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของวาลโปรอิกแอซิดในผู้ป่วยที่คุมอาการชักได้และคุมอาการชักไม่ได้ โดยประมาณจากความเข้มข้นของยาทั้งหมดและยารูปแบบอิสระในซีรัมของผู้ป่วย ทำการศึกษาในผู้ป่วยผู้ใหญ่โรคลมชัก 40 ราย ที่สถาบันประสาทวิทยา กรุงเทพมหานคร โดยแบ่งเป็นกลุ่มที่คุมอาการชักไม่ได้ 15 คน และกลุ่มที่คุมอาการชักได้ 25 คน

ค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ที่ประมาณจากความเข้มข้นของยาทั้งหมด พบว่าค่าครึ่งชีวิตของการขจัดยาของกลุ่มที่คุมอาการชักไม่ได้ (19.1088±5.4373 ชั่วโมง) มีค่าน้อยกว่ากลุ่มที่คุมอาการชักได้ (27.7787±16.2272 ชั่วโมง) (p=0.142) และค่าปริมาตรการกระจายยาของกลุ่มที่คุมอาการชักไม่ได้ (0.2546±0.0880 ลิตรต่อกิโลกรัม) มีค่าน้อยกว่ากลุ่มที่คุมอาการชักได้ (0.3450±0.1288 ลิตรต่อกิโลกรัม) (p=0.032) ค่าคงที่การขจัดยาของกลุ่มที่คุมอาการชักไม่ได้ (0.0394±0.0123 ต่อชั่วโมง) มีค่ามากกว่ากลุ่มที่คุมอาการชักได้ (0.0319+0.0151 ต่อชั่วโมง) (p=0.142) ส่วนค่าการขจัดยาของกลุ่มที่คุมอาการชักไม่ได้ (0.0096±0.0035 ลิตรต่อชั่วโมงต่อกิโลกรัม) ไม่แตกต่างกับกลุ่มที่คุมอาการชักได้ (0.0095±0.0030 ลิตรต่อชั่วโมงต่อกิโลกรัม) (p=0.905)

ค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ที่ประมาณจากความเข้มข้นของยารูปแบบอิสระ ในกลุ่มที่คุมอาการชักไม่ได้และคุมอาการชักได้นั้นไม่แตกต่างอย่างมีนัยสำคัญที่ $\alpha < 0.05$ เนื่องจากมีความแปรปรวนของค่าพารามิเตอร์ระหว่างผู้ป่วยคนละรายค่อนข้างสูง ทำให้ต้องการผู้ป่วยเข้าร่วมการวิจัยในจำนวนที่สูงกว่านี้ อย่างไรก็ตามมีแนวโน้มว่าเภสัชจลนศาสตร์ของยารูปแบบอิสระ จะเป็นไปในทิศทางเดียวกับเภสัชจลนศาสตร์ของยาทั้งหมดกล่าวคือ ค่าครึ่งชีวิตของการขจัดยาของกลุ่มที่คุมอาการชักไม่ได้ (11.2232±4.8796 ชั่วโมง) จะสั้นกว่ากลุ่มที่คุมอาการชักได้ (13.3632±5.8947 ชั่วโมง) (p=0.234) ค่าปริมาตรการกระจายยาของกลุ่มที่คุมอาการชักไม่ได้ (1.3105±1.1081 ลิตรต่อกิโลกรัม) น้อยกว่ากลุ่มที่คุมอาการชักได้ (1.8414±2.6351 ลิตรต่อกิโลกรัม) (p=0.383) และค่าคงที่การขจัดยากลุ่มที่คุมอาการชักไม่ได้ (0.0703 ±0.0235 ต่อชั่วโมง) จะสูงกว่ากลุ่มที่คุมอาการชักได้ (0.0616±0.0275 ต่อชั่วโมง) (p=0.234) ส่วนค่าการขจัดยาของกลุ่มที่คุมอาการชักไม่ได้ (0.0796±0.0621 ลิตรต่อชั่วโมงต่อกิโลกรัม) แตกต่างจากกลุ่มที่คุมอาการชักได้ (0.0834±0.0630 ลิตรต่อชั่วโมงต่อกิโลกรัม) น้อยมาก (p=0.578)

ผลการวิจัยนี้ทำให้เข้าใจเภสัชจลนศาสตร์ของวาลโปรอิกแอซิดได้ชัดเจนขึ้น ซึ่งสามารถนำไปประยุกต์เกี่ยวกับการใช้ยาวาลโปรอิกแอซิดให้ได้ประสิทธิผลยิ่งขึ้นต่อไป

ภาควิชา.....เภสัชกรรม.....ลายมือชื่อนิสิต.....ลักขณา บุญมาก.....

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

ปีการศึกษา.....2548.....ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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KEY WORD : PHARMACOKINETIC / VALPROIC ACID / SEIZURE-CONTROLLED PATIENT/ SEIZURE-UNCONTROLLED PATIENT / EPILEPSY

LAKKANA BOONMARK : COMPARISON OF PHARMACOKINETIC PARAMETERS OF VALPROIC ACID IN SEIZURE-CONTROLLED AND UNCONTROLLED EPILEPTIC PATIENTS.

THESIS ADVISOR: ASSOC. PROF. Duangchit Panomvana Na Ayudhaya, Ph.D.,

THESIS CO-ADVISOR: Somchai Towanabut, M.D. 103 PP. ISBN 974-53-2453-1

The purpose of this study was to determine and compare the pharmacokinetic (PK) parameters of valproic acid (VPA) in seizure-controlled and uncontrolled epileptic patients. Pharmacokinetic parameters were estimated for both total and unbound VPA. Forty adults patients were recruited from neurological clinic, Prasat Neurology Institute, Bangkok, 15 patients were in seizure-uncontrolled group and 25 patients were in seizure-controlled group.

Pharmacokinetic parameters calculated from total VPA concentrations, demonstrated that the half-life ($t_{1/2}$) of seizure-uncontrolled group (19.1088$\pm$5.4373 hr) was shorter than $t_{1/2}$ of seizure-controlled group (27.7787$\pm$16.2272 hr) (p=0.142) while the volume of distribution (V_d) of seizure-uncontrolled group (0.2546$\pm$0.0880 L/kg) was less extensive when compared to V_d of the seizure-controlled group (0.3450$\pm$0.1288 L/kg) (p=0.032). Elimination rate constant (k) of seizure-uncontrolled group (0.0394$\pm$0.0123 hr^{-1}) was higher than k of seizure-controlled group (0.0319+0.0151 hr^{-1}) (p=0.142). Clearance (Cl) of seizure-uncontrolled group (0.0096$\pm$0.0035 L/hr/kg) was not significantly different from Cl of seizure-controlled group (0.0095$\pm$0.0030 L/hr/kg) (p=0.905).

Pharmacokinetic parameters obtained from unbound concentrations showed no statistically significant at $\alpha < 0.05$ between the seizure-uncontrolled and the seizure-controlled groups due to high variations in PK parameters among patients, higher numbers of subjects were required. However, the same direction as PK of total VPA could be observed, i.e., $t_{1/2}$ of the seizure-uncontrolled group (11.2232$\pm$4.8796 hr) was less than $t_{1/2}$ of the seizure-controlled group (13.3632$\pm$5.8947 hr) (p=0.234) while the V_d of seizure-uncontrolled group (1.3105$\pm$1.1081 L/kg) was smaller as compared to the seizure-controlled group (1.8414$\pm$2.6351 L/kg) (p=0.383) and k of seizure-uncontrolled group (0.0703 $\pm$0.0235 hr^{-1}) was higher than k of seizure-controlled group (0.0616$\pm$0.0275 hr^{-1}) (p=0.234). Clearance of seizure-uncontrolled group (0.0796$\pm$0.0621 L/hr/kg) was not different from Cl of seizure-controlled group (0.0834$\pm$0.0630 L/hr/kg) (p=0.578).

The results provided a more rational understanding of VPA pharmacokinetics in the clinical setting.

Department.....Pharmacy.....Student's signature.....Lakkana Boonmark.....

Field of study.....Clinical Pharmacy....Advisor's signature.....Duangchit Panomvana.....

Academic Year.....2005.....Co-advisor's signature.....Somchai Towanabut.....

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List of abbreviations

AEDs	antiepileptic drugs
VPA	valproic acid
EEG	electroencephalogram
hr	hour
kg	kilogram
mg	milligram
mL	milliliter
μg	microgram
C_5^{th}	5^{th} hour after morning dose concentration
C_{trough}	concentration just before the morning dose
C_{ss}	concentration at steady state
F	bioavailability
k	elimination rate constant
S	salt fraction
$t_{½}$	half-life
τ	interval
V_d	volume of distribution
Cl	clearance

CHAPTER I

INTRODUCTION

Epilepsy is one of the most common neurological problems, conditions with physical risks, psychological and socioeconomic consequences, which impair quality of life. Approximately 50 million people have epilepsy *(1)*. The annual incidence ranges from 20 to 70 cases per 100,000 populations and the point prevalence is 0.4 to 0.8 percent *(2)*. The incidence rates are highest in children, plateau from the 15-65 years, and rise again among the elderly. In Thailand, the prevalence of total epilepsy in the general population who age more than 5 years in the years 1991-1992 was 29.2 cases per 1,000 populations and prevalence of active epilepsy was 5.9 cases per 1,000 populations *(3)*.

Treatment with an antiepileptic drugs (AEDs) is usually begun when the patient has had more than one unprovoked seizure, whatever the type. The selection of AEDs is based mainly on its efficacy for specific types of seizures, tolerability, and safety. The best drug for the particular type of seizure is selected and administered in a dose high enough to bring the plasma drug concentration into a therapeutic range without unacceptable side effects *(4)*. Epileptic seizures are controlled with antiepileptic drug, which might be withdrawn when the patient has been without seizures for two years.

Most people who develop epilepsy have a relatively short-lasting susceptibility to seizures and enter remission shortly after starting treatment on small doses of AEDs. *(2, 4)* However, some patients do not become completely free of seizure. Epilepsy is refractory when seizures are so frequent or severe that they limit the patient's ability. Twenty to thirty percent of people who develop epilepsy will have chronic epilepsy that responds incompletely to AED therapy, despite the choice of an adequate AED and carefully monitored treatment *(5)*. In spite of medical therapy, seizures persist in approximately 20 % of patients with primary generalized epilepsy and 35 % of those with partial epilepsy. Factors associated with seizure control were symptomatic etiology, combination of symptomatic etiology with mental retardation, long duration of epilepsy, partial epilepsy and cognitive deficits *(6)*. The management of refractory epilepsy

focuses on the optimal use of antiepileptic drugs. Knowledge of pharmacokinetics (PK) of each antiepileptic drug will lead to better antiepileptic drug concentration monitoring and will improve outcome.

Valproic acid (VPA) is currently used to treat various seizure disorders, to prevent migraines, and to treat a variety of psychiatric disorders such as bipolar disorder, anxiety. VPA is available in different oral formulations such as solutions, enteric-coated tablets, and slow-released preparations. The usual dose of VPA for treatment of seizures is 15-30 mg/kg/day. VPA therapeutic range for seizure control is 50-100 mg/L *(7)*. Pharmacokinetic studies of VPA are interesting due to several reasons. First, VPA has influence on the PK of other drugs. Second, VPA is highly bound to serum albumin, and at therapeutic plasma concentrations, saturates plasma protein binding sites. VPA is largely bound to plasma proteins and has a relatively small volume of distribution (V_d) (0.1 to 0.4 L/kg). At therapeutic doses, half-life ($t_{1/2}$) of VPA varies from 10 to 20 hours in adults while it is significantly shorter (6 to 9 hours) in children *(8)*.

Previous studies have reported VPA PK parameters; Sanchez-Alcaraz A. et al. reported that the increase in clearance (Cl) as the dose of VPA increases and the decreases in Cl as plasma concentration increases depend on the age of the patient *(9)*. Wangemann M. et al. showed that there were no statistically significant differences between $t_{1/2}$ of VPA in healthy male volunteers after administration of 100, 150 and 300 mg sustained release formulation. *(10)*. Wangemann M. et al. have proposed that no statistically significant differences between $t_{1/2}$ of VPA in healthy male volunteers after administration of 300 mg sodium valproate sustained release capsule (Orfiril long) and 5 ml of valproate solution (Orfiril salt) *(11)*. Herngren L. et al. concluded that lower binding in plasma resulting in higher levels of unbound VPA and concomitant higher Cl, which is dose-dependent *(12)*.

Fernando-dongas M.C. et al. suggests that the VPA resistant group has a higher incidence of atypical characteristics including asymmetric EEG abnormalities, atypical seizure characteristics and history, and neurological and neuroimaging abnormalities *(13)*. Redenbaugh JE. et al. found that the plasma $t_{1/2}$ were characteristic of a first-order rate process and were similar in children and adult patients with intractable seizures of all types. Also reported a wide variation in the $t_{1/2}$, particularly among the children *(14)*.

Gal P. et al. stated that $t_{1/2}$ of VPA in six neonates with intractable seizures was higher than study in patients with seizure controlled *(15).* Suwanmanee J. has found that pediatric patients with uncontrolled seizure have mean V_d and $t_{1/2}$of VPA which were lower than pediatric patients with controlled seizure *(16).*

Difference in physiological characteristics among epileptic patients might affect PK of VPA in the patients, which further associate with clinical outcome of the patient to VPA treatment. Previous studies had reported PK parameters among patients with different aged groups, while some studies reported PK parameters of VPA with and without co-anticonvulsants while some studies reported PK parameters of VPA among different dosage forms. However, most of previous studies reported PK parameters of VPA in patients with controlled-seizure, PK parameters of VPA in different seizure controlled groups have never been investigated. The purpose of this study was therefore to determine and compare the PK parameters of VPA between seizure-controlled and seizure-uncontrolled patients. Outcome of this study was to use the estimated PK parameters of VPA to predict whether or not the patient should respond to VPA without further increment in the dosage.

CHAPTER II

REVIEW OF LITERATURE

EPILEPSY

1. Definition

The International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE) have come to consensus definitions for the terms epileptic seizure and epilepsy. An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain.

Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiologic, cognitive, psychological, and social consequences of this condition. The definition of epilepsy requires the occurrence of at least one epileptic seizure *(17).*

Epilepsy is as a symptom of disturbed electrical activity in the brain caused by a wide variety of disorders *(18).* Seizures occur because small numbers of neurons discharge abnormally. Anything that disrupts the normal homeostasis of the neuron and disturbs its stability may trigger abnormal activity and seizures. A genetic predisposition to seizures has been suggested. Patients with mental retardation and cerebral palsy are at increased risk for seizures. The cause of seizures in the elderly may be multifactorial and include cerebrovascular disease (both ischemic and hemorrhagic stroke), neurodegenerative disorders, tumor, head trauma, metabolic disorder, and CNS infections.

Many factors have been shown to precipitate seizures. Hyperventilation may precipitate absence seizures. Sleep deprivation, sensory stimuli and emotion stress may initiate seizures. Hormonal changes occurring around the time of menses, puberty, or pregnancy have been associated with the onset of or an increased frequency of seizures.

Epilepsy is a chronic disorder characterized by recurrent seizures. Recurrence of a first unprovoked seizure within 5 years ranges between 23% and 80% *(18)*. Almost 2 million people in the United States have epilepsy. Between 70 and 80 % of individuals are successfully treated with one of the more than 20 antiepileptic drugs (AED) now available with success rates primarily depending on the etiology of the seizure disorder *(2)*.

2. Epidemiology

Epilepsy is the most common serious neurological disorder, with a prevalence of 0.5-1% in the general population *(5)*. Prevalence of active epilepsy in each country varies from 3.0 to 14.8 cases per 1,000 populations *(19)*. On average the incidence of epilepsy is approximately 50 per 100,000 populations, but is highest at the extremes of life. There is a bimodal distribution in the occurrence of the first seizure, with one peak occurring in new bond and young children and the second peak occurring in patients older than age 65. Each year, 120 per 100,000 people in the United States come to medical attention because of a newly recognized seizure *(18)*. To estimate the incidence of unprovoked seizures and epilepsy in a general population from the southern part of the Netherlands. The overall annual incidence was 55/100,000 and 30/100,000 for unprovoked seizures and epilepsy, respectively. The age-specific annual incidence of US and epilepsy increased with age and reached 120/100,000 and 62/100,000 for the more than or equal 65 years of age group, respectively *(20)*. In Thailand, the prevalence of total epilepsy in general populations whose ages were more than 5 years in the years 1991-1992 was 29.2 cases per 1,000 populations and the prevalence of active epilepsy was 5.9 cases per 1,000 populations *(21)*.

3. Pathophysiology

Seizure activity is characterized by paroxysmal discharges occurring of cortical neurons. Initially, a small number of neurons fire abnormally. Normal membrane conductance and inhibitory synaptic currents break down, and excess excitability

spreads, either locally to produce a focal seizure or more widely to produce a generalized seizure. The clinical manifestations depend on the site of the focus, the degree of irritability of the surrounding area of the brain, and the intensity of the impulse. A relative deficiency of inhibitory neurotransmitters such as GABA or and increase in excitatory neurotransmitters such as glutamate would promote abnormal neuronal activity.

4. Seizure Type and Epilepsy Type

The International League Against Epilepsy has defined the seizure types listed in Table 1 (Commission on classification and Terminology of the International League Against Epilepsy, 1981) *(18)*

Table 1 International classification of epileptic seizures

I Partial seizure (seizures begin locally)
- A. Simple (without impairment of consciousness)
  - 1. With motor symptoms
  - 2. With special sensory or somatosensory symptoms
  - 3. With psychic symptoms
- B. Complex (with impairment of consciousness)
  - 1. Simple partial onset followed by impairment of consciousness with or without automatisms
  - 2. Impaired consciousness at onset with or with automatisms
- C. Secondarily generalized (partial onset evolving to generalized tonic- clonic seizures)

II Generalized seizures (bilaterally symmetrical and without local onset)
- A. Absence
- B. Myoclonic
- C. Clonic
- D. Tonic
- E. Tonic-clonic
- F. Atonic
- G. Infantile spasms

III Unclassified seizures

The International Classification of Epileptic Seizures combines the clinical description with certain electrophysiologic findings in order to classify epileptic seizures. Seizures are divided into two main pathophysiologic groups partial seizures and generalized seizures by EEG recordings and clinical symptomatology.

The International Classification of Epilepsies and Epilepsy Syndromes added components such as age of onset, intellectual development, findings on neurologic examination, and results of neuroimaging studies to more fully define epilepsy syndromes. Syndromes can include one or many different seizure types (e.g., Lennox Gastaut syndrome). The syndromic approach includes seizure type and possible etiologic classification (idiopathic, symptomatic, cryptogenic, or unknown). Idiopathic describes syndromes that are presumably genetic but also those in which no underlying etiology is documented or suspected. A family history of seizures is commonly present, and neurologic function is essentially normal except for the occurrence of seizures. Symptomatic cases involve evidence of brain damage or a known underlying cause. A cryptogenic syndrome is assumed to be symptomatic of an underlying condition that cannot be documented. Unknown or undetermined is used when no cause can be identified *(22)*.

TREATMENT

1. Desired Outcome

The goal of treatment for epilepsy is no seizures and no side effects with an optimal quality of life. However, a balance between efficacy and side effects must be reached, because with the older AEDs used as monotherapy, fewer than 50% of patients become seizure-free. Because therapy is extended for many years, chronic side effects must be considered. The newer AEDs offer alternatives for balancing seizure frequency and drug side effects.

2. Drug Treatment

Early control of epileptic seizure is important because it allows normalization of patients' lives and prevents acute physical harm and long-term morbidity associated with recurrent seizures *(23).* Patients who have had two or more seizures generally should be started on AEDs. To start AED therapy, monotherapy is preferred. About 65-70% of all patients with epilepsy can be maintained on one drug. However, many of these patients are not seizure free. Drugs may be combined in an attempt to help the patient become seizure-free. The percentage of patients who are seizure-free on one drug varies by seizure type. Of the 35% with unsatisfactory control 10% will be well controlled with a two-drug treatment. Of the remaining 25%, 20% of these will continue to have unsatisfactory control despite multiple-drug treatment. Early control of epileptic seizures also correlates with successful discontinuation of AED treatment after long-term seizure control.

The drug treatments of first choice depend on the type of epilepsy (Table 2) as well as on the interface between drug-specific adverse effects and patient preferences. Figure 1 is a suggested guideline for a general approach to the treatment of epilepsy *(3).*

Table 2 Drugs of choice for specific seizure disorders

Seizure type	First-line drugs	Alternative drugs
Partial seizures	Carbamazepine	Gabapentin, Falbamate
	Phenytoin, Phenobarbital	Topiramate, Primidone
	Lamotrigine, Valproic acid	Levetiracetam, Tiagabine
	Oxcarbazepine	Zonisamide
Generalized seizures		
Absence	Valproic acid	Lamotrigine
	Ethosuximide	
Myoclonic	Valproic acid	Lamotrigine, Falbamate
	Clonazepam	Topiramate
Tonic-clonic	Phenytoin	Lamotrigine
	Carbamazepine	Topiramate
	Valproic acid	Primidone
	Phenobarbital	Oxcarbazepine

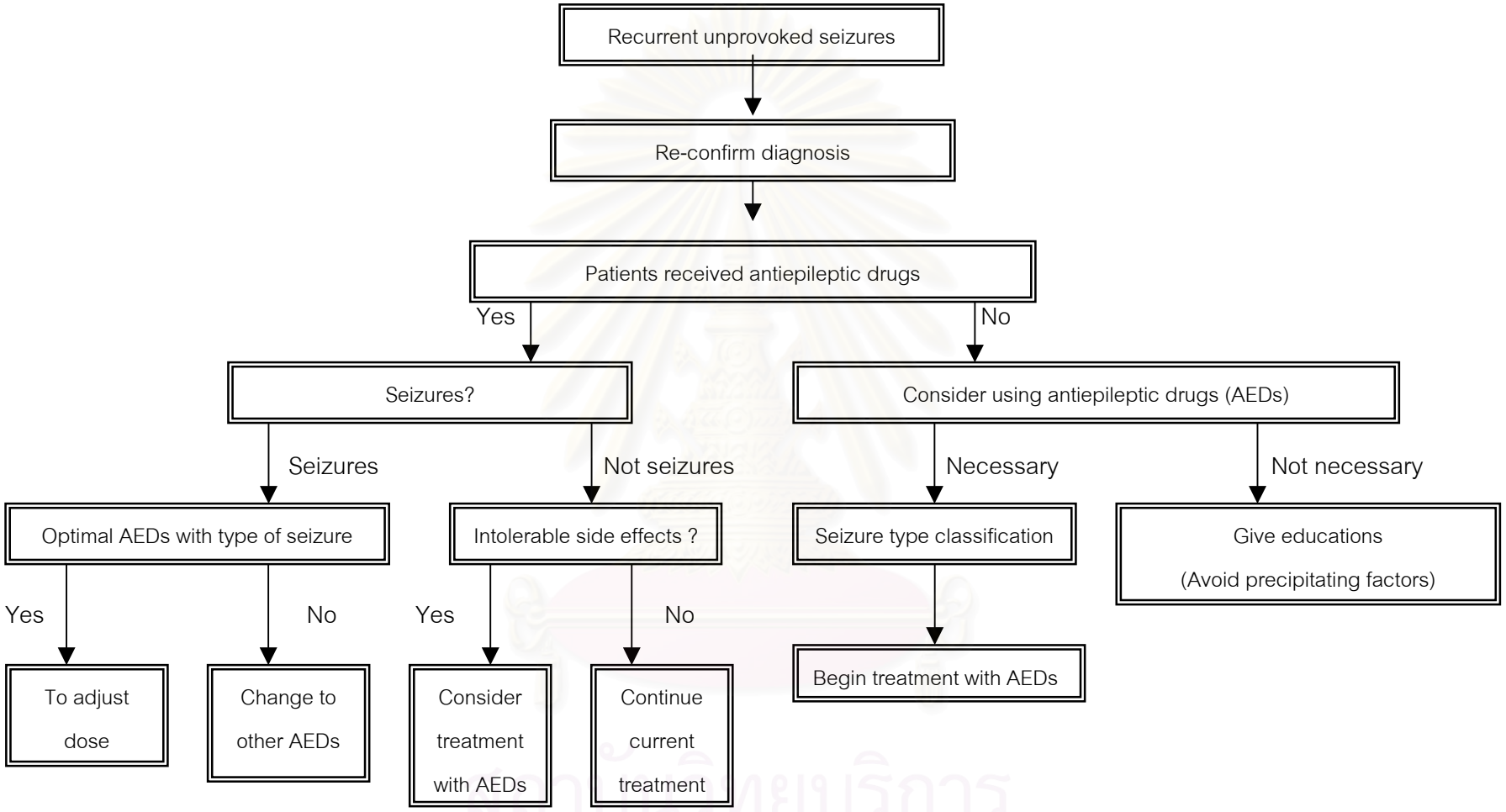


Figure 1 Guideline for recurrent unprovoked seizure

Noncompliance might be the common reason for treatment failure. It is estimated that up to 60% of patients with epilepsy are noncompliance. The rate of noncompliance is increased by the complexity of the drug regimen. Noncompliance is not influenced by age, sex, psychomotor development, seizure type, or seizure frequency.

The AEDs initially used to control seizures may not need to be given for a lifetime. Polypharmacy may be reduced, and some patients can discontinue AEDs altogether. Factors favoring successful withdrawal of AEDs include a seizure-free period of 2 to 4 years, complete seizure control within 1 year of onset, an onset of seizures after age 2 but before age 35 years, and a normal neurologic examination and EEG. The primary drugs used for epilepsy were phenobarbital, phenytoin, carbamazepine, and valproic acid. Eight newer drugs were approved for use in epilepsy; felbamate, gabapentin, lamotrigine, topiramate, tiagabine, zonisamide, levetiracetam, and oxcabazepine *(2).*

Observations from the studies lend support to the suggestion that, at population level, prognosis of newly diagnosed epilepsy may be broadly categorized into three groups, reflecting the underlying neurobiological process *(24).*

a. Excellent prognosis: In 20-30% patients, the condition enters long term remission after a variable period of time and level of activity, probably even without antiepileptic drug treatment. If treated, these patients become seizure-free on the first or second monotherapy, often requiring no more than moderate doses, which can be successfully withdrawn after a period of seizure freedom.

b. Remission with treatment only: The 20-30 % of patients comprising the second group become, and remain, seizure-free only with continuing antiepileptic drug treatment. Some may require more than one antiepileptic drug and multiple attempts may be needed to find the right combination for the individual patient. Continuing antiepileptic drug treatment is required to suppress seizure relapse.

c. Continuing seizures despite treatment: In the remaining 30-40% of patients, seizures recur in varying degrees of intensity and frequency despite antiepileptic drug treatment. Some patients have frequent debilitating seizures qualifying as having “refractory” epilepsy. Conditions in this category include many symptomatic/cryptogenic localization related epilepsies such as those associated with mesial temporal sclerosis,

cortical dysplasia, gross structural brain lesions, and the progressive myoclonic epilepsies.

3. Pharmacoresistant

Considering that epilepsy is one of the most common chronic neurologic disorders, refractory or drug-resistant epilepsy is a major public health problem. The consequences of drug-resistant epilepsy can be quite severe, including mortality rates that are 4 to 7 times higher in people with drug-resistant seizures. It is not known why and how epilepsy becomes drug resistant in some patients while others with seemingly identical seizure types and epilepsy syndromes can achieve seizure control with medication *(25).* Although the terms 'pharmacoresistant' or 'medically refractory' lack a precise definition, most clinicians would consider an epilepsy pharmacoresistant that had not been controlled by any of two to three first-line AEDs usually used for a give epilepsy syndrome *(2,4).* Definitions usually include the number of AED failures and the minimal remission or seizure frequency during a specified duration of therapy. Wirrell E. et al. stated that intractable epilepsy was defined as using three or more AEDs for seizure control during the course of epilepsy, and having more than one seizure per month over the final year of follow-up *(26).* In general, many experts would agree that whenever a patient does not become seizure free for 12 months during long-term state-of-the-art treatment with several suitable AEDs at maximal tolerated doses, the epilepsy could be broadly classified as drug-resistant, pharmacoresistant, or medically refractory.

In general, the definition of refractory partial seizure is defined as partial seizures which can not be controlled by at least one AEDs in optimal dose and time. This can be defined as "clinical" refractory partial seizure. If the seizure attacks still occurred after risk factors adjustment (concomitant medications, drug compliance, drug storage, lifestyles, avoidance of trigger factors and adjusted to the maximum tolerable dose which could be confirmed by monitoring blood levels) and treatment with at least two AEDs in optimal dose and time, the patients were classified as "true" refractory cases *(19).*

The probability of intractability largely depends on the type of seizures and epilepsy, with complex partial seizures such as those occurring in temporal lobe epilepsy having the poorest prognosis of all seizure type in adults. For symptomatic or cryptogenic generalized epilepsies, which are commonly intractable *(27)*. Patients not controlled on monotherapy with the first AED have a chance of only about 10% or lower to be controlled by other AEDs *(4)*. Wirrell E. et al. stated that initial AED was successful in 60% of children with absence epilepsy. If the first AED failed, the outcome was less favorable, with a lower rate of terminal remission and a higher rate of progression to JME and intractable epilepsy *(26)*.

Risk factors associated with seizure control were noted in only a few studies. Age at onset, if over the age of 5, combined with normal intelligence were predictive of an excellent outcome while the presence of a neurological disorder, and hence symptomatic etiology or a combination of symptomatic etiology with mental retardation, and long duration of epilepsy were predictive of poor outcome in children after a relapse. In adolescents' juvenile myoclonic epilepsy and in adults epilepsy with complex partial seizures was associated with poor seizure control. Schmidt D. seizure recurrence rate after AED discontinuation ranged between 12 to 66%. Factors associated with poor treatment outcome of treating recurrences were symptomatic etiology, partial epilepsy and cognitive deficits *(6)*.

Alterations in specific drug targets as a major cause of pharmacoresistant epilepsy, but rather points to nonspecific and possibly adaptive mechanisms, such as decreased drug uptake into the brain by seizure-induced over-expression of multidrug transporters in the blood-brain barrier. Several putative neurobiologic mechanisms underlying drug resistance in epilepsy have been identified in recently years. Two major theories have been put forward: (a) removal of AEDs from the epileptogenic tissue through excessive expression of multidrug transporters, and (b) reduced drug-target sensitivity in epileptogenic brain studied in animal models and humans.

Kwan P. et al. have suggested that the pathogenesis underlying pharmacoresistance in epilepsy is unclear. One of the candidate mechanisms that has attracted growing interest is the limitation of antiepileptic drug access to the seizure focus by a range of efflux transporters, the prototype of which is P-glycoprotein (P-gp).

P-gp is encoded by the multidrug resistance (MDR1 or ABCB1) gene. It has been hypothesized that overexpression of P-gp and other efflux transporters in the cerebrovascular endothelium, in the region of the epileptic focus, also may lead to drug resistance in epilepsy *(28).*

Loscher W. et al. have proposed that the uptake of valproate from blood to brain is facilitated by a medium-chain fatty acid transporter, which accounts for two-thirds of the barrier permeability, whereas the mechanisms governing the efflux of valproate from the brain involve a probenecid-sensitive, active transport system at the brain capillary endothelium. Recent data from Huai-Yun et al. show that valproate is a substrate for MRPs in brain capillary endothelial cells, which raises the possibility that MRPs may serve as the efflux transporters of valproate and explains the previously described effects of probenecid on brain and CSF levels of valproate, because probenecid is an inhibitor of MRP1 and MRP2 *(4).*

4. Epidemiology of Pharmacoresistant Epilepsy

In a population-based study of 176 Finnish children with epilepsy treated with AEDs, seen first between 1961 and 1964 and followed up until 1992. Figure 2 showed epidemiology of drug-resistant epilepsies. The figure from a long-term prospective study shows that, in many cases, the underlying epilepsy syndrome may largely determine drug resistance. The majority of surviving patients (49%, 46 of 93) with symptomatic partial (SPE) or symptomatic generalized epilepsies (SGE) (78%, seven of nine) are drug resistant, whereas only a minority of patients with idiopathic generalized epilepsies (IGE) (13%, four of 30) does not enter 5-year terminal remission, 92% of patients with no remission were continued taking AEDs. *(29)*. The prevalence of refractory partial epileptic in Thai patients at Prasat Neurological Institute was estimated as 3.3 per 1,000 cases of the epilepsy populations *(19).*

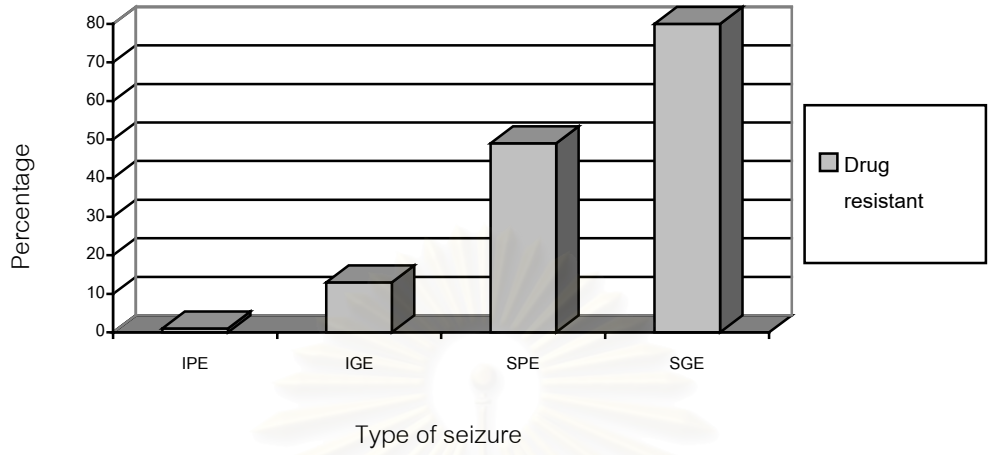


Figure 2 Epidemiology of drug-resistant epilepsies.

VALPROIC ACID

1. Structure

VPA is a branched-chain fatty acid (Figure 3). The drug is available in several forms, including the parent compound, its sodium salt, it amide derivative, and a combination of the parent compound and its sodium salt. In addition, VPA and valproate are available in enteric-coated tablet, liquid, intravenous and controlled-release formulations *(8)*. Since the drug is available in a variety of salts and formulations throughout the world, the term "VPA" is use throughout the present review.

The terms valproate, VPA, and divalproex sodium have distinct meanings that designate different forms of the same drug. When taken orally, this molecule dissociates from its acid from resulting in the appearance of the valproate ion in the gastrointestinal tract *(30)*.

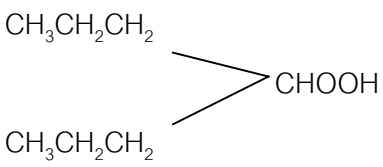


Figure 3 Chemical structure of VPA

2. Chemistry and Stability

VPA, valproate sodium, and divalproex sodium are carbolic acid-derivative anticonvulsants. VPA is structurally unrelated to other commercial available anticonvulsants; it lacks nitrogen and/or an aromatic moiety found in most anticonvulsants. VPA has a pKa of 4.56 and thus at physiological pH 7.42. VPA is highly ionized into a carboxylate moiety, valproate *(31).* Divalproex sodium is a stable coordination compound consisting of VPA and valproate sodium in a 1:1 molar ratio and is formed during partial neutralization of VPA with sodium hydroxide. Divalproex sodium is a prodrug of valproate, dissociating into valproate in the gastrointestinal tract *(7).*

2.1 Valproic acid

VPA occurs as a colorless to pale yellow, slightly viscous, clear liquid with a characteristic odor and slightly soluble in water and freely soluble in alcohol. VPA has a pKa of 4.8. USP recommends that VPA capsules be stored in tight containers at 15-30 °C; however, the manufacturer of Depakene recommends that the capsules be stored in tight containers at 15-25 °C

2.2 Valproate sodium

Valproate sodium occurs as a white, crystalline, very hygroscopic powder with a saline taste and is very soluble in water and in alcohol. Valproate sodium oral solution has a pH of 7-8. Valproate sodium oral solution should be stored in tight containers at a temperature less than 40°C, preferably between 15-30 °C; freezing should be avoided.

2.3 Divalproex sodium

Divalproex sodium occurs as a white powder with a characteristic odor and is insoluble in water and very soluble in alcohol. Divalproex sodium delayed-release tablets should be stored in tight, light-resistant containers at a temperature less than 30 °C; divalproex sodium capsules containing coated particles should be stored at a temperature less than 25 °C

3. Pharmacology

VPA and it derivatives have been available for use as antiepileptic drugs since the 1960s *(30).* VPA is currently used to treat various seizure disorders, to prevent migraines, and to treat a variety of psychiatric disorders such as bipolar disorder, anxiety depression, psychosis, substance-abuse withdrawal, and other behavioral disturbances *(32).*

4. Mechanism

VPA has multiple effects on γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain. The catabolism of GABA is inhibited, while the synaptic release of this neurotransmitter is increased. The density of $GABA_B$ receptors in the brain becomes increased from chronic exposure to valproate. A direct effect of valproate on sodium and potassium cellular transflux has been proposed as a mechanism of action *(32).*

5. Dosing

The recommended starting dose for VPA is 15 mg/kg/day, typically given as starting dose of 250 mg once or twice daily. Dosage is titrated 5-10 mg/kg/day every 3-5 day. Maintenance dosages typically range between 15 and 60 mg/kg/day. Many patients receive dose of 30 mg/kg/day, and a few cases daily doses as high as 60 mg/kg/day have used *(32).* At higher concentrations, any given increase in the VPA dose results in proportional increase in the unbound concentration but less than proportional increase in the total serum concentration. The usual dose for prevention of migraines is 500 mg daily for 7 days followed by 1000 mg daily thereafter.

6. Therapeutic and Toxic Plasma Concentrations

VPA therapeutic range for seizure control is 50 to 100 mg/L; however, VPA concentrations in excess of 100 mg/L are often required in patients with partial seizures. When used in the treatment of bipolar psychiatric disorders the usually accepted therapeutic range is 50 to 125 mg/L *(8)*. Its concentration in CSF is approximately one-tenth that in plasma and is directly correlated with the concentration found in tears.

7. Pharmacokinetics

The fatty acid structure of VPA explains a number of its characteristics, including its distribution, sites of action, and routes of elimination. In humans, the bioavailability of oral VPA is between 96 and 100%, consistent with VPA crossing the intestinal mucosal membranes and being unaffected by first-pass metabolism *(31)*. As shown in Table 3, PK parameters of VPA without co-anticonvulsants determined in steady-state conditions (mean$\pm$SD)

Table 3 Pharmacokinetic parameters of VPA (mean±SD)

No. of Subjects	Age (y)	Cl (ml/h/kg)	V_d (ml/kg)	$t_{1/2}$ (hr)	Preparation	Duration[a] (hr)	Reference
6	22-25	6.7+0.9[b]				12	Bauer et al. (1985)
6	22-25	7.4+1.0[c]				12	
6	60-88	6.6+0.5[b]				12	
6	60-88	7.3+0.7[c]				12	
16	9.4+2.9	13.0+4.7	220+50	12.3+3.1	Syrup or plain tablets	12	Chiba et al. (1985)
5	22-33	7.60+2.29	216+86	19.4+2.3	Normal capsules	168	Pollack et al. (1986)
13	0.5-19[d]	16.2+6.6	192+49	8.5+1.6	Syrup or normal	8-12	Hall et al. (1985)
6	19-31			10.13+0.94	Normal capsules (300 mg)	12	Nitsche&Mascher (1982)
				14.50+6.4	Normal capsules (450 mg)	12 12	
				13.53+5.4	Normal Capsules (500 mg)	12	

a. Duration of blood sampling in the terminal phase.
b. Morning value
c. Evening value
d.This age range refers to the whole patient population studied (n=52)

7.1 Absorption

Both sodium valproate and VPA appear to be rapidly and completely absorbed. Consequently, both the bioavailability (F) and the salt form (S) are 1.0 for the intravenous product, oral solution, and capsules, with the exception of the extended-release tablets for which the F is 80 to 90 %. The rate of absorption depends on the formulation used. Plasma valproate concentrations usually peak 0.5 to 1 hours with the syrup, 1 to 3 hours with the capsule, and 2 to 6 hours with the enteric-coated tablet after oral administration when fasting. However, appear to slow the rate of absorption of VPA, serum concentrations peak as late as 6 to 8 hours after oral administration when taken with food. Food delays but dose not decreases the amount of VPA absorbed. The intravenous formulation has a more consistent peak that occurs at the end of the 1-hour infusion. Morrot MJ. et al. have reported that Depakine chrono 500 mg is given as a single oral dose at the midpoint of a standard breakfast, an increase in the rate of absorption is observed without modifying the total amount absorbed. The onset of absorption is not delayed *(33).* Food intake had only a slight effect on the PK parameters of this formulation of VPA; thus, it would appear not to be an important consideration when deciding on the drug regimen.

Enteric-coated VPA tablets are not sustained in their release characteristics, but absorption is delayed and is significantly influenced by the timing of meals. Absorption from the enteric coated tablet dose not begin for 2-4 hours after administration, but once absorption begins VPA reaches the systematic circulation very quickly at a rate similar to that of rapid release formulations.

Recently, a slow-release formulation of valproate was developed and has now become widely used in treating epileptic patient by replacing the conventional formulation of vaproate *(34).* Slow-release formulations have been developed as a means of reducing fluctuations in serum concentrations of VPA. The bead-filled capsule (Depakote Sprinkles) does appear to have a more sustained plasma profile and more closely approximates a continuous infusion model. Might be better tolerated due to decreased peak-trough variability. Wangemann M. et al. reported that the valproate concentration of the new sustained release capsule increased smoothly and a longer

lasting plateau was observed as compared with the solution *(11).* Depakine chrono tablet a sustained release dosage from has been developed by Sanofi in order to reduce the number of daily doses, to improve patient compliance and to maintain the concentration with the optimum range over a 24 hr period with minimum fluctuations. Multiple dose studies, this decrease fluctuation is due only to the reduction in maximum plasma concentrations since the trough concentrations are identical. Similar $C_{ss\ max}/C_{ss\ min}$ fluctuations when 1 g Depakine chrono was given once a day as compared to 0.5 g. of depakine twice a day. Plasma drug levels at a steady state swinging between 40-80 mg/ml over a 24 hr dosing interval after a once a day administration of 1 g of depakine chrono *(35).*

There is a diurnal decrease in absorption of the enteric-coated preparation following an evening dose. Kondo T. et al. have found that smaller diurnal fluctuations in valproate concentrations during treatment with slow-release formulation of valproate result in decreased formations of minor metabolism including 4-en, the most toxic metabolism *(34).* Dutta S. et al. stated that diurnal variation in plasma VPA concentrations was minimal with once-daily administration of extended-release divalproex. Evening once-daily administration of extended-release divalproex was not associated with substantial differences in PK or safety compared with morning once-daily administration *(36).*

7.2 Distribution

VPA distributes wildly throughout the body *(31).* A number of factors, including dosage and age, have been postulated to influence VPA distribution *(8).* Values of V_d are generally thought to increase as the dose is increased, probably as a result of the saturation of protein binding. On the other hand, the possibility of age-dependent changes in V_d remains controversial. VPA is 90-95 % protein bound to albumin. The binding sites for VPA are saturable, and the free faction may increase as the total concentration increase. The saturable binding may indicate that the free concentration is a better monitoring than the total VPA concentration. At therapeutic plasma levels, the free fraction of VPA is on average 6%. In fact, at total concentrations over 100 mg/L the free fraction may increase by about 50%. This effect may be magnified in patients with

significant hypoalbuminemia. The free fraction fluctuates more than the total concentrations due to fluctuations in free fatty acid concentrations in the morning and diurnal differences in unbound concentrations and total Cl. Cloyd J.C. et al. found that VPA unbound fraction decreased from 15% at maximum concentration to 9% at 45 mg/l. *(37).*

Age has been show to be positively correlated with VPA free fraction. Under steady-state conditions, Bauer et al. measured average free fractions of 6.4% in young subjects and of 10.7% in elderly people, indicating that VPA binding may be altered in the elderly. Certain diurnal variations in unbound parameters were also observed, with increased unbound Cl during the night. Elderly patients may display an increased free fraction as a result of lower serum albumin concentrations. Elderly patients have been observed to have a reduced unbound Cl (40%) as compared to younger subjects

Uptake and exit of VPA within the central nervous system can occur at either the choroids plexus of the blood brain barrier. Approximately two–three of VPA uptakes occur through a saturable carrier-mediated process and a lesser degree through passive diffusion. Thus a relatively small amount of VPA is distributed by passive diffusion into tissues, as only the non-ionized, lipid soluble portion of VPA diffuses across membranes. The efflux of VPA results in a low amount of VPA in the CNS, with a brain-to-unbound plasma concentration ratio of < 0.5 at steady state. This low amount of VPA within the CNS may explain why patients receiving VPA require a relatively large amount of VPA to achieve therapeutic efficacy.

Tissue distribution of VPA has been investigated in humans. The drug achieves brain concentrations ranging from 7-28 % of the simultaneous concentration in plasma, while CSF levels are 8-25% of those in plasma *(8).* Plasma concentration ratios despite considerable differences in protein binding. This fact may indicate that both simple diffusion and active transport mechanisms are involved in the maintenance of lower concentrations in the CNS than in plasma. CSF levels were approximately 11% of the total concentration in plasma. Lindberger M. et al. pointed out that the distribution of VPA in four patients with drug-resistant partial epilepsy to subdural CFS was rapid (Tmax, 3.5 hr in two patients and 5.5 hr in one patient) and subject to a minor delay in all three patients compared with that in the subcutaneous tissue ECF (t_{max}, 2.5 hr in all three

patients) *(38).* VPA rapidly enters the subdural CSF in unbound concentrations marginally lower than those obtained in subcutaneous ECF and plasma. It is unclear to what extent VPA subdural CSF levels reflect brain tissue levels. Intracerebral transport of molecules is carried out by diffusion and bulk flow, and although calculation of both diffusion and bulk flow is possible in principle, the present data could not inform about the distribution of VPA to deeper parts of the brain. That CSF levels were lower than plasma and subcutaneous ECF levels, particularly at steady state, suggests that VPA may be a substrate for an energy-dependent carrier-mediated transport out of the CNS. VPA appears in breast milk in concentrations as low as 3% of the simultaneous values in plasma. Half-life of 1 hour while the terminal component had a $t_{½}$ of 12.2 hours *(8).*

Volume of distribution : The apparent V_d for VPA is variable and ranges from 0.1 to 0.5 L/kg. Alterations in plasma protein binding as well as the capacity -limited binding to plasma protein by this drug account for the variable V_d of VPA *(8).* VPA binding to serum albumin appears to become saturated when VPA concentrations exceed 50 mg/L. For most patients with VPA concentrations in the range of 25 to 50 mg/L, an average V_d of 0.14 L/kg is a reasonable value to use for PK calculations, assuming the patient has normal serum albumin concentrations and normal renal function. The mean (SD.) V_d was 0.21 (0.044) L/kg following rapid infusions in patients with epilepsy.

7.3 Metabolism

The liver metabolizes VPA. There is no first-pass metabolism and the Cl is independent of hepatic blood flow. Increase in free drug results in an increase in Cl. Thus the Cl of VPA changes at higher concentrations. At least 5 parallel metabolic routes have been established for VPA metabolism in humans. Both the parent compound and several of its metabolites undergo conjugation with glucuronic acid, which is therefore the single most important metabolic pathway of the drug. Other main routes of VPA metabolism include β-oxidation and oxidation in ω, ω_1 and ω_2 positions.

a. Glucuronidation: Conjugation with glucuronic acid involves both the parent drug and a series of its metabolites. After a single dose in humans, glucuronidation of the parent drug accounts for approximately 15 to 20% of the total

amount of VPA administered, but this figure increases to 40% during long-term treatment. In humans, the extent of glucuronidation increases as the dose is increased, at the expense of the β-oxidation pathway.

b. β-oxidation: This metabolic pathway results in 2-en-valproic acid and 3-keto-valproic acid, which are found in significant concentration in plasma, and 3-OH-valproic acid, whose concentration in plasma is less.

c. Oxidation in ω, ω_1 and ω_2 positions : They account for approximately 25 to 30% of an administered dose. Products of ω-oxidation include essentially 5-OH-valproic acid and 2-propyl-glutaric acid. ω_1-oxidation gives rise to 4-OH-valproic acid. For ω_2 oxidation, in vitro observations suggest that cytochrome P450 could mediate this reaction, the pathway leading also to 3-OH-valproic acid.

7.4 Elimination

VPA is almost entirely eliminated from the body through hepatic metabolism; the renal route eliminates less than 5% of the drug. VPA-glucuronide accounts for approximately 60% of the recovered dose in the urine. Clearance depends primarily on the age of the patient and concomitant medications. In pediatric patients and in patients receiving additional antiepiletic drugs, the Cl values may be substantially higher (10 to 13 ml/kg/hr). In addition, capacity-limited plasma protein binding may result in nonlinear changes in the plasma concentration of VPA, especially when concentrations exceed 50 mg/L. Mean Cl of total VPA in plasma and whole blood was 17.8 and 28.9 ml/kg/hr, respectively.

VPA Cl may be dose dependent that only the unbound fraction of the drug undergoes metabolic transformation; since VPA binding sites can be saturated as total VPA concentration increase, an increase in the dose gives rise to increased unbound fraction and thus increased Cl of the drug *(8)*.

The usual Cl values for VPA are 6 to 10 ml/kg/hr with an average value of 8 ml/kg/hr. Dutta S. et al. have found, the mean (SD.) oral Cl values for unbound VPA were 94.3 $\pm$ 51.8 and 82.3 $\pm$ 28.2 mL/hr/kg and for total VPA were 11.2$\pm$3.77 and 9.06$\pm$2.03 mL/hr/kg in older children and adolescents, respectively *(39)*. Sanchez-Alcaraz A. et al.

reported Cl in children aged 8 months to 6 years who were receiving VPA monotherapy. The Cl values in these three age groups were 24.5±12.4 ml/kg/hr(age <2 years), 19.9±6.1 ml/kg/hr (age 2-4 years) and 12.7±3.0 ml/kg/hr (age >4 years), respectively *(40).* Park H.M. et al. documented that regression model of population-based PK parameters for intravenous VPA, and the factors influencing these parameters, in Korean adults. The typical VPA Cl predicted in a Korean patient of 60 kg with the current model was 0.24 mL/min per kg, slightly higher than Cl predicted in Japanese and Spanish patients

Diurnal variations in Cl had been reported the morning Cl appears to be lower than the evening Cl in both young and elderly. Total and unbound Cl in the young and elderly subjects were about 10% and 15% higher during the evening. These changes let to lower total and unbound concentrations. *(8).* To minimize the influence of diurnal variability, drug concentrations should be determined at the same time each day. The diurnal fluctuation in VPA concentration-time profiles is obvious. Nighttime concentrations show little peak-trough variability following Depakote administration. Morning trough concentrations can be significantly lower or higher than the trough concentrations obtained during the day. The lower apparent oral Cl of VPA at higher concentrations may reflect either saturable protein binding or partial saturation of intrinsic Cl *(41).*

Half-life: The $t_{½}$ for VPA ranges from 4 to 17 hours, with an average value of 10 to 12 hours. In children and patients receiving other antiepileptic drugs, the $t_{½}$ of VPA is reduced. Because the usual $t_{½}$ is relatively short, VPA plasma concentrations appear to plateau within 24 to 48 hours after therapy is initiated. The short $t_{½}$, coupled with the dosing interval of 8 to 12 hours, results in wide fluctuations in plasma concentrations within a dosing interval.

Polack et al. evaluated the PK of VPA and its metabolites at steady state using a prolonged washout period (1 week) after the last dose; this allowed accurate estimation of the terminal $t_{½}$ of both the drug and its metabolites. The calculated values of the terminal $t_{½}$ of VPA (mean ± SD = 19.4±2.3 hours) are distinctly longer than those generally reported after a single dose *(6).* But, this result is not sufficient to enable the conclusion to be drawn that VPA $t_{½}$ is longer at steady state than after a single dose, due

to the lack of a paired-sample study design *(8)*. To correct calculation of the elimination $t_{½}$ could only be done by using plasma concentrations obtained later than 12 hours *(8)*. Wangemann M. et al. showed that $t_{½}$ of VPA after administration of 100, 150 and 300 mg sustained release formulation were 15.9, 15.5 and 15.5 hr, respectively *(10)*. Dulac O. et al. concluded that $t_{½}$ of sodium valproate 1000 mg sustained release and modified release formulations in 24 healthy volunteers were 16.6±3.4 and 16.5±3.5 hr, respectively *(42)*. Hergren L. et al. have suggested that mean terminal $t_{½}$ of VPA in plasma and whole blood of infants are 12.5 and 15.5 hr, respectively *(12)*. In addition, terminal half lives of free VPA in plasma and whole blood are 6.4 and 6.5 hr, respectively. Suwanmanee J. has found that pediatric patients with uncontrolled seizure have V_d and $t_{½}$ of VPA, which were lower than pediatric patients with controlled seizure *(16)*.

8. Adverse Effects

One of the benefits of VPA therapy is its broad therapeutic margin of safety. VPA is associated with only rare idiosyncratic reactions and a variety of common general side effects. The most common general side effects of VPA therapy include nausea, vomiting, and heartburn, and these occur less frequently with use of enteric-coated formulations *(31)*. Less than 10% of patients complain of dose-related tremor, dermatological effects (rash or alopecia), and neurological effects, such as drowsiness, irritability, and ataxia.

In addition to these minor side effects, VPA is a potent teratogen in humans that is associated with significantly increased risk of spina bifida aperta, a fetal syndrome with defective posterior neural tube closure, as well as cardiac malformations, cleft palate, and limb defects. Metabolic disturbances including hypocarnitinemia and hyperammonemia can be observed in patients receiving VPA. Thrombocytopenia and inhibition of platelet aggregation are more common side effects, occurring in 12 % of patients receiving VPA; however, in most cases thrombocytopenia is mild, with platelet counts 100-150x10^3/mm^3. The rare and idiosyncratic, non-dosage related VPA-induced side effects include reversible idiopathic hepatitis and hemorrhagic pancreatitis. The most serious side effect reported with VPA is hepatotoxicity. Although hepatotoxicity also

has been reported in patients receiving VPA, a clear dose relationship is not established. The more common dose-related adverse effects of VPA therapy are GI (e.g. nausea, vomiting, and diarrhea).

9. Drug Interactions

In patients who are unresponsive to monotherapy, however, a combination of two or more AEDs may be needed to optimize seizure control. However, combination therapy may have adverse effects. When two or more AEDs are used, the potential for drug interactions is substantial.

Highly protein-bound drugs may displace VPA and at therapeutic plasma concentrations, saturations plasma protein binding sites. The most commonly occurring plasma-protein displacement interaction involving ADEs is the displacement of phenytoin by VPA, this interaction results in a full in total phenytoin concentration although the concentration of free phenytoin does not change.

This drug undergoes metabolism by a variety of conjugation and oxidative processes (CYP2C9, CYP2C19 and CYP2A6) *(22).* Conjugation with D-glucuronic acid has been shown to be the major route of VPA biotransformation in humans and most animals. Enzyme inducers such as rifampin, phenytoin, carbamazepine, primidone, and phenobarbital increase the elimination of VPA, often requiring higher doses. Among second generation AEDs , gabapentin, vigabatrin, levetiracetam, topiramate, tiagabine and zonisamide have no enzyme inducing effects on the metabolism of other AEDs *(43).* Drugs that induce liver enzymes may alter VPA kinetics by increasing metabolism for example, phenytoin, phenobarbital, primidone and carbamazepine all increase VPA Cl. The plasma concentration of VPA can be reduced on average by 76%, 49% and 66% in patients who are also treated with phenobarbital, phenytoin and carbamazepine, respectively *(43).* Furthermore, many of the second-generation AEDs (for example lamotrigine, levetiracetam and vigabatrin) do not induce or inhibit enzyme involved in drug metabolism *(44).*

Although stimulation of VPA metabolism by enzyme inducing AEDs typically results in decreased plasma concentrations and effectiveness of VPA, this interaction

may also lead to increased formation of hepatotoxic metabolites, which may explain why patients taking phenytoin, phenobarbital and carbamazepine are more susceptible to valproate-induced liver toxicity. One of VPA potentially hepatotoxic metabolites, 4-ene, may be produced in larger quantities in patients on concomitant enzyme inducers, leading, in part, to the higher hepatotoxicity seen in children on polytherapy. In patients taking carbamazepine, VPA can increase the concentration of the active metabolite carbamazepine-10,11-epoxide through inhibition of epoxide hydrolase, without any substantial changes in the concentration of the parent drug *(43)*. The metabolism of phenytoin also appears to be inhibited; however, this interaction is difficult to evaluate accurately because VPA also displaces phenytoin from its binding sites on serum albumin *(32)*.

VPA is an enzyme inhibitor. The concomitant administration of drugs that utilize enzyme metabolism may affect the actions of these drugs and of VPA. VPA decreases the elimination of carbamazepine epoxide (via inhibition of epoxide hydrolase) phenobarbital (30-50%) and ethosuximide. Phenytoin total concentrations can decline during co-medication with VPA, while unbound concentrations can increase secondary to inhibition of intrinsic clearance. VPA most common effect is to increase the plasma concentrations of lamotrigine. Although the increase in plasma phenobabital concentrations is mainly related to inhibition of CYP isoenzymes (probably CYP2C9 or CYP 2C19) the effect of VPA on lamotrigine metabolism involves inhibition of the UGT1A4 enzyme, which glucuronates lamotrigine *(43)*. The inhibition of lamotrigine metabolism is already maximal at VPA doses within the typical target range (> 500 mg/day in an adult) and involves a substantial lengthening of lamotrigine $t_{½}$, from 30 hr to about 60 hr. As a result of this, lamotrigine dose requirements are reduced in patients given VPA.

The combination of VPA and lamotrigine increase the risk for potentially serious rash. VPA also significantly reduces the Cl of lorazepam. Combination with falbamate can reduce VPA Cl, possibly via inhibition of β-oxidation. Kanner A.M. has reported that correlations between lamotrigine Cl and the dose and C_{ss} of VPA and comparisons of lamotrigine Cl during low and high doses of VPA demonstrated that the degree of inhibition of lamotrigine Cl is independent of the dose and C_{ss} of VPA *(45)*.

In both hepatic disease and renal impairment, an increase in the V_d and free fraction can be expected. With hepatic cirrhosis, the free fraction increases to 29%; in renal failure, the free fraction increases to 18% *(46).*

10. Valproic Acid Resistant

Clinical characteristics combined with EEG data may help in predicting which JME patients will respond favorably to VPA. Fernando-dongas M.C. et al. studies characteristics of VPA resistant juvenile myoclonic epilepsy, the VPA resistant group had a higher frequency of EEG asymmetries, atypical seizure and intellectual deficiency *(13).* In this study, atypical seizure characteristics were more frequently seen in the VPA resistant group. This raises the issue of whether the VPA resistant JME group is in fact refractory localization related epilepsy. These findings suggest that patients with VPA resistant JME have higher incidence of focal EEG abnormalities, suggestive of partial epilepsy.

The studies about intractable epilepsy were Chayasirisobhon S. et al. has reported that 52 mentally retarded patients with intractable seizures by adding VPA to other drug regimens. Sixty-one percent improved clinically. VPA significantly reduced the frequency of generalized tonic-clonic seizures, generalized myoclonus absence and atonic seizures *(47).*

There response to VPA was assessed after 6 months of therapy. The response to treatment was considered marked if seizure activity decreased by more than 50%, partial with a decrease of 25 to 50 %, and decrease and equivocal or no response if the response was less than 25%. There was a significant correlation between clinical improvement and reduction of EEG paroxysmal activity. There was no clinical improvement in the only patient with five types of seizures. VPA was less effective in complex partial seizures. Finally, in 7-23% chronic drug-resistant epilepsy emerged, mostly with complex partial seizures *(6).* Lance J.W. reported that valproate was of benefit to intractable patients with grand mal seizures and atonic attacks as well as to those with petit mal absences and myoclonus *(48).*

Previous studies PK parameters of VPA in patients with intractable seizure. Redenbaugh J.E. et al. found that $t_{½}$ of 20 patients with intractable seizures of all types ranged from 5.4 to 13.5 hours (mean, 9.5 $\pm$ 2.1 hr) for adults and from 6.0 to 18.5 hr (9.2$\pm$3.5 hr) for children. As the half-lives were similar, the V_d in the two groups must have differed, with a smaller $t_{½}$ in the children *(14).* Shen D.D. et al. studied the distribution of VPA between brain (gray matter) and serum in patients with intractable seizures who receiving chronic VPA therapy. There was a tendency for the brain-to-serum concentration ratio to be lower in tissues from the epileptic foci than in tissues from non-epileptic areas. The mean brain-to-serum ratio for non-epileptic brain specimens was 0.130$\pm$0.058 as compared with a mean of 0.100$\pm$0.044 for epileptic brain specimens *(49).* Gal P. et al. found that six neonates with prolonged, intractable seizures were treated with VPA. VPA PK measurements were as follows: for total VPA, V_d was 0.4 L/kg (range, 0.36 to 0.47 L/kg), serum Cl was14.4 mL/hr/kg (5.5 to 18.2 mL/hr/kg), $t_{½}$ was 26.4 hr (8.6 to 48.5). For unbound VPA, V_d was 2.02 L/kg (range, 1.14 to 2.44 l/kg), serum Cl was 108.9 mL/hr/kg (42.0 to 252.0 ml/hr/kg), $t_{½}$ was17.6 hr (6.7 to 34.2). Free fraction of VPA ranged from 11.3 to 31.6 % (mean, 19.2%) *(15).*

Towanabut S. et al. who studied the compliance and satisfaction consequences in epileptic patients switched from more than two times daily sodium valproate enteric-coated tablet regimen to the same total daily dose of sodium valproate slow-released form given once or twice daily. It was found that the patients were seizure free during the study period comparing sodium valproate slow-released form 76.4% to sodium valproate enteric-coated form 65.2% *(50).*

CHAPTER III

PATIENTS AND METHOD

PATIENTS AND SAMPLE COLLECTION

1. Patients

The subjects included in this study were adult patients with age more than 15 years old had been diagnosed epilepsy and come to follow up at neurological clinic, Neurology institute. Mostly were treated with VPA as a single antiepileptic drug or combined with other antiepileptic drugs that are not interfere with metabolism of VPA. The ethics committee of Neurology institute approved the study protocol. Each patient was given comprehensive verbal and written information on aims and possible risks of the study. All patient signed a consent form as evidence of consent.

Estimation of sample size with significant $p < 0.05$ is calculated from this formula

$$n = \frac{[Z_{\alpha/2} + Z_{\beta}]^2 \sigma_{\alpha}^2}{(\mu_1 - \mu_0)^2}$$

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

σ_{α}: Stand deviation of volume of distribution from related literature = 0.0641 L/kg *(51)*

Z_{β}: 20 % (two-tailed) = 0.84

$\mu_1 - \mu_0$ = 20% of mean of volume of distribution = 0.2 x 0.1868 L/kg

$$n = \frac{[1.96 + 0.84]^2 [0.0641]^2}{[0.2 \times 0.1868]^2} = \text{approximate 25 patients per groups}$$

2. Definition

2.1 Seizure-controlled patients: The patients who has had no seizures since start taking VPA or the frequency of seizure was decreased more a 50 % as compared to before start taking VPA.

2.2 Seizure-uncontrolled patients: The patients who treated with VPA monotherapy for appropriate duration and in appropriate dosage regimens or reached therapeutic concentrations the decretion in frequency of seizure was less then 50 % as compared to before start taking VPA, or other antiepileptic drug was added after treated with VPA.

2.3 Appropriate duration: Duration for observed clinical symptom of seizure after start taking VPA. Approximately 2 - 3 times of seizure cycle.

2.4 Appropriate dosage regimens: Dosage for adult is 15-30 mg/kg/day or a VPA concentration is within 50-100 mg/L.

2.5 Pharmacokinetic parameters:

- Elimination rate constant (k): It is the fraction or percentage of the total amount of drug in the body removed per unit of time.
- $t_{1/2}$: It is the time required for the total amount of drug in the body or the plasma drug concentration to decrease by one-half.
- V_d: It is the volume that accounts for the total dose administration based on the observed plasma concentration.
- Cl: It is the intrinsic ability of the body or its organs of elimination (usually the kidneys and the liver) to remove drug from the blood or plasma.

2.6 Slow-released valproic acid (Depakine Chrono 500 mg.)

2.7 Conventional valproic acid: Enteric coated valproic acid (Depakine 200 mg. or Valparin 200 mg.)

3. Inclusion Criteria

3.1 The patients with aged 15-65 years old in both sexes.

3.2. The patients have been diagnosed to all type of epilepsy which appropriated with VPA and have seizure frequency at least one time per month.

3.3 The patients who treated with monotherapy of VPA or polytherapy but did not include phenobarbital, phenytoin, carbamazepine and other drug that use same VPA metabolism enzyme such as diclofenac, celecoxib, naproxen, fluvastatin, losartan, zidovudine, amitriptyline and propranolol.

4. Exclusion Criteria

4.1 Patients who have history of VPA allergy.

4.2 Pregnancy and lactation patients.

4.3 Patients who taking phenobarbital, phenytoin, carbamazepine and other drug that use same VPA metabolism enzyme such as diclofenac, celecoxib, naproxen, fluvastatin, losartan, zidovudine, amitriptyline and propranolol.

4.4 Patients who have clinical sign of hepatic or renal disease by physician determination.

4.5 Poor or noncompliance patients by interviewing the parents and/or the patients.

4.6 Patients who have precipitating factor for seizures such as heat, loud noise, alcohol used, psychological stress and menstruation.

DATA COLLECTION

1. Patients information

All patients data related to the study; include demographic data, diagnosis, administered drug, dosage regimens, related laboratory data such as EEG and brain image, clinical response and any adverse effect were recorded. Each patient must have good compliances and not have precipitating factors for seizures, which was determined by interviewing the parents and/or patients.

2. Blood sample

Blood samples were obtained after VPA was given at the same dose for at least three months, to assure steady state condition. Patients were divided into 2 groups according to the dosage form of VPA administering, Depakine/Valparin 200 mg or Depakine chrono 500 mg. For Depakine/Valparin 200 mg dosage form, the dosage regimen of each patient was modified from either bid or tid schedules to every 12 hour or every 8 hour regimens at least 7 days prior to evaluate in order to standardize the dosing interval of the patient. Blood samples were obtained just before the morning dose of VPA (trough) and at 5^{th} hour after administration. Patients who used Depakine chrono 500 mg, the dosing intervals were modified from either bid or tid schedules to once daily regimen at least 7 days prior to blood sample collecting. The blood samples were obtained at 14^{th} and 19^{th} hours after the study dosage administration if the dosage were given in the evening, however, if the once daily dosage were given in the morning, the blood samples would be collected at 24^{th} and 29^{th} hours after the study dosage was given.

Each sample was allowed to clot and was centrifuged immediately at 5,000 rpm for 7 minutes at room temperature. The serum was then separated and frozen at -20 c^{o} until the time of assay. Serum levels of total and unbound VPA were measured by Fluorescence polarization immunoassay (TDX FLx Abbott laboratories) *(52)*; unbound VPA levels were determined after ultrafiltration (25 ^{o}C, 2000 g, 35 fixed angle x 20 min, Centrifree TM Micropartition System) *(53)*. Serum albumin was obtained from the same serum samples.

DRUG ANALYSIS

1. Analyzed Total VPA

Frozen samples were warmed to room temperature and mixed completely before assay. Serum sample were assay by utilizing fluorescence polarization immunoassay. The steps were as following.

1.1 VPA Calibration

The following VPA calibrator solutions were measured for their VPA concentrations to make a calibration curve

Vial	VPA concentration (mg/L)
A	0.0
B	12.5
C	25.0
D	50.0
E	100.0
F	150.0

Six levels of VPA calibrator solutions were used and each level of VPA was measured 2 times.

1.2 VPA Controls

Three levels of VPA control solutions (L, M and H) were measured for their VPA concentrations and compared with the standard range of VPA control concentrations.

Vial	VPA concentration (mg/L)	
	Standard	Study
L	33.75-41.25	37.11
M	67.50-82.50	71.76
H	112.5-137.50	120.58

1.3 Analyzed Total VPA

VPA reagent pack was used to measure total VPA in serum samples.

2. Analyzed Free VPA

Separation of unbound VPA in serum was done by ultrafiltration with the Centtrifree and Micropartition System. Frozen samples were warmed to room temperature and mixed completely before ultrafiltration. These were performed within 2 weeks after sampling using approximately 1 ml plasma. Centrifugation was performed at room temperature for 20 min at 25 ^{o}C in a centrifuge with fixed angle. Samples were securely capped with minimized opening prior to filtration to prevent pH changes.

2.1 Free VPA Calibration

Six levels of free VPA calibrator solutions were measured for their free VPA concentrations in order to make calibration curve. The six levels were as follow:

Vial	VPA concentration (mg/L)
A	0.0
B	2.0
C	5.0
D	9.0
E	15.0
F	25.0

Each level was measured twice.

2.2 Free VPA Controls

Three levels of free VPA control solutions (L, M and H) were measured. The concentrations of free VPA obtained were compared with the standard range of free VPA control concentrations.

Vial	VPA concentration (mg/L)	
	Standard	Study
L	3.6-4.4	3.93
M	10.8-13.2	11.88
H	18.0-22.0	19.33

2.3 Analyzed Free VPA

VPA reagent packs were used to measure free VPA in filtrate samples.

APPARATUS

1. Automated fluorescence polarization analyzer (TDxFLx analyzer, Diagnostic Division, Abbott Laboratories, Inc.,Irving, TX,USA)

2. Centrifuge

3. Freezer

DATA ANALYSIS

1. Pharmacokinetic Analysis

Pharmacokinetic parameters were calculated from the data using standard method. The equations used were

$$C_t = \frac{(SFD/t_{inf})}{Cl}\left(\frac{1-e^{-ktinf}}{1-e^{-k\tau}}\right) e^{-k(t-tinf)}$$

$$k = \frac{[\ln c_{t1}/c_{t2}]}{\Delta t}$$

$$t_{1/2} = \frac{0.693}{k}$$

$$V_d = \frac{(SFD/t_{inf})}{k\,C_t}\left(\frac{1-e^{-ktinf}}{1-e^{-k\tau}}\right) e^{-k(t-tinf)}$$

$$Cl = k \times V_d$$

C_t : Drug concentration at time t

F ; Bioavailability

D :Dose

V_d : Volume of distribution

t_{inf} : Infusion time equal 14 hour

S : Fraction of active drug accounting for salt from

τ : Dosing interval

k : Elimination rate constant

Cl : Clearance

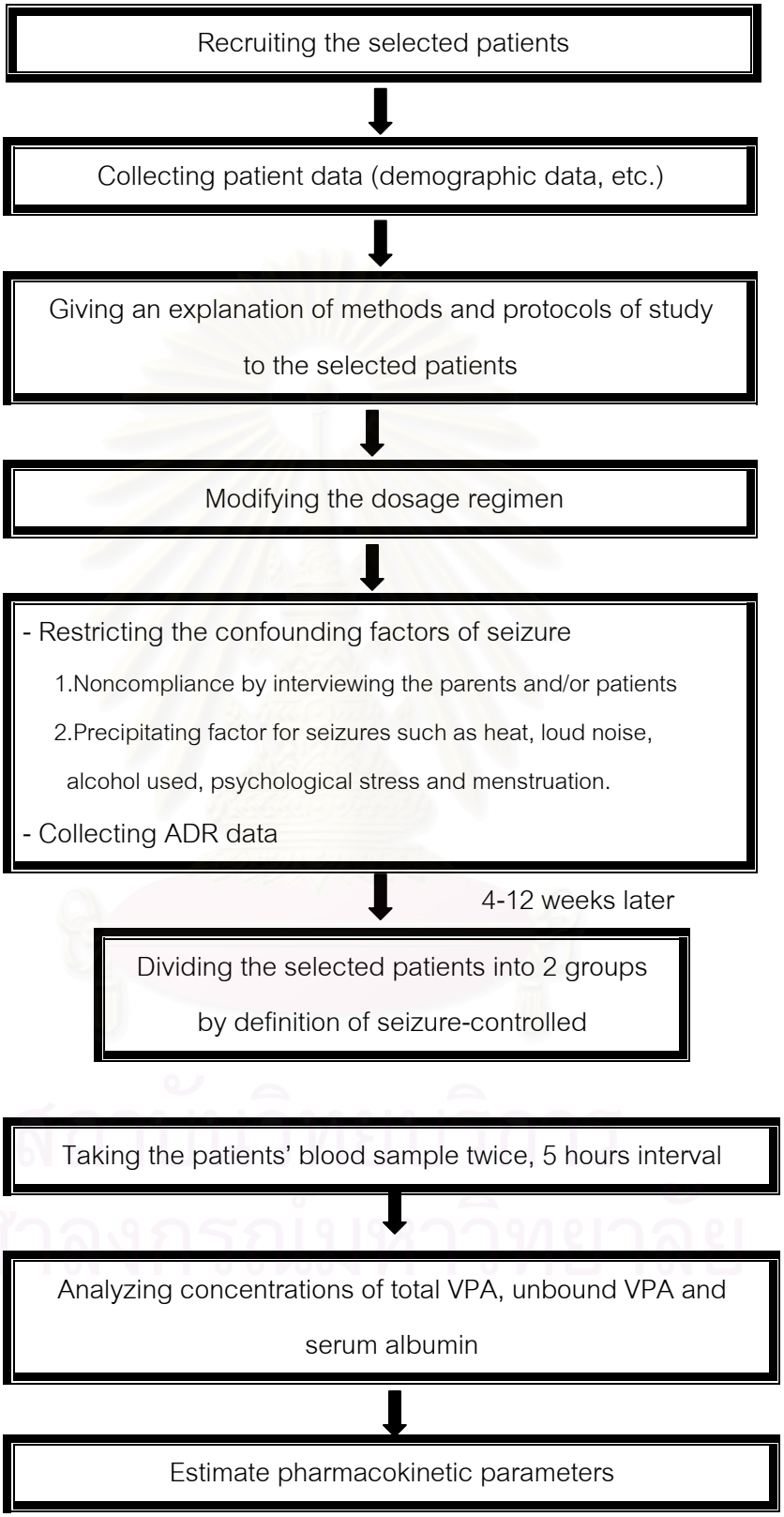
$t_{1/2}$: Half-life

2. Statistic Analysis

Demographic data were analyzed and presented as percentage and mean±SD. The PK parameters of seizure-uncontrolled group and seizure-controlled group were presented as mean±SD. PK parameters of seizure uncontrolled group and seizure controlled group were compared by Mann-Whitney U test. A significant is determined at α = 0.05 (two-sided). PK parameters were also compared between different dosage forms (Chrono versus conventional tablets) using Mann-Whitney U test.

Relationship between unbound and total concentrations was analyzed by regression. The Relationship between free fraction and total concentrations were also analyzed by regression.

Research Protocol



CHAPTER IV

RESULTS

Fifty-three patients were enrolled in this study. The treatment outcomes were classified into 2 groups that were seizure-controlled and seizure-uncontrolled groups. Of the 53 patients recruited, 40 completed the study. There were twenty-five patients (62.5%) who had no seizures with treatment of VPA and fifteen patients (37.5%) who still had seizures even though they were received the high dose of VPA.

1. Patient' s Demographic Data and Characteristics

Table 4 shows mean values of general characteristics, sex, age, weight, dosage forms of VPA, dose per day, and albumin levels of patients included in the study of both seizure-controlled and seizure-uncontrolled groups. There were no statistically significant differences of these characteristics between seizure-uncontrolled and seizure-controlled groups excepted for dose/day. Table 5 shows seizure types of seizure-uncontrolled and seizure-controlled groups. Majority of patients in the seizure-controlled group had generalized tonic clonic seizure while majority of patients in the seizure-uncontrolled group had complex partial seizure. Table 6 shows EEG, MRI and CT of seizure-controlled and seizure-uncontrolled groups. There were no statistically significant differences of these abnormal or normal of EEG, MRI and CT between both groups. Five patients in seizure-uncontrolled group had weight gain, and one patient had tremor. Nine patients in seizure-controlled group had weight gain, and two patients had tremor (as shown in appendix B).

Table 4 Demographic data

Demographic data	Number of patients (Percentage)		*p* value[#]
	Seizure-uncontrolled group (n=15)	Seizure-controlled group (n=25)	
Sex			
- Male	7 (46.7)	7 (28.0)	0.237
- Female	8 (53.3)	18 (72.0)	
Dosage form			
- Chrono	14 (93.33)	19 (76.0)	0.168
- Conventional	1 (6.67)	6 (24.0)	
Demographic data	Mean±SD (range)		*p* value[!]
	Seizure-uncontrolled group (n=15)	Seizure-controlled group (n=25)	
Age (years)	29.38±10.75 (16.02-49.03)	33.13±13.52 (15.03-65.02)	0.367
Weight (kg)	62.99±12.75 (43.50-88.00)	57.82±10.76 (44.4-88.00)	0.178
Dose/kg/day (mg/kg)	19.98±6.30 (10.07- 33.98)	15.36±4.85 (6.67-21.93)	0.013*
Albumin (g/dL)	3.85±0.44 (3.1-4.6)	3.95±0.35 (3.3-4.7)	0.456

[#] Chi-square test

[!] Student t-test

* significant different at $\alpha = 0.05$

Table 5 Seizure types of seizure-uncontrolled and seizure-controlled groups

Seizure types[+]	Number of patients (Percentage)	
	Seizure-uncontrolled group (n=15)	Seizure-controlled group (n=25)
Partial seizure		
1. Simple partial seizure	1 (6.67)	1 (4.00)
2. Complex partial seizure	8 (53.33)	3 (12.00)
3. Complex partial seizure with generalized tonic- clonic seizures	3 (20.00)	2 (8.00)
Generalized seizures		
1. Generalized tonic- clonic seizures	3 (20.00)	17 (68.00)
2. Absence	0 (0.00)	1 (4.00)
3. Atonic	0 (0.00)	1 (4.00)

[+] Based on International League Against Epilepsy 1981

Pearson Chi-square test p value = 0.028

Table 6 EEG, MRI and CT of seizure-controlled and seizure-uncontrolled groups.

Neurologic examination	Seizure-uncontrolled group		Seizure-controlled group		p value#
	Total N+	Abnormal N (%)	Total N	Abnormal N (%)	
EEG	9	7 (77.78)	14	11 (78.57)	1.000
MRI	7	6 (85.71)	5	2 (40.00)	0.222
CT	4	1 (25.00)	9	4 (44.44)	1.000

+ N=number of patient

Chi-square test

2. Total, Unbound Concentrations and Free Fraction

2.1 Therapeutic Concentration

In general, total and unbound VPA concentrations were measured from the serum taken at 14th and 19th hours or 24th and 29th hours of each patient who received chrono dosage form depended on whether the once daily dose was given as an evening or morning dose. The patients who were taking conventional dosage form (the dosing interval for conventional dosage form was every 12 hours) the serums were taken before morning dose (trough) and at 5th hour after morning dose of each patient. Serum level of total and unbound VPA were measured by fluorescence polarization immunoassay (TDxFLx Abbott Laboratories)

Table 7 shown the total and unbound VPA concentrations and free fractions in seizure-uncontrolled group. All of the total VPA concentrations measured at 24th hour after drug administration were within the proposed therapeutic range (50-100 mg/L). Three out of four patients whose blood samples were taken at 14th hour after drug administration had their VPA concentrations higher than 100 mg/L. One patient had dose-related tremor. The only one patient in this group who took conventional tablet had VPA concentrations at trough and at 5th hour after dose within the proposed therapeutic range.

Considering the unbound concentrations, all of the blood samples collected at 24th hour had their unbound VPA concentrations within 4.00-11.05 mg/L while four patients whose blood samples were take at 14th hour had their unbound VPA concentrations within the range of 12.36-35.56 mg/L

Table 8 showed seizure-controlled group. Of the seven patients whose blood samples were collected at 24th hour, only three total VPA concentrations were within the proposed therapeutic range while one was higher and the other three were lower than the proposed therapeutic range. The eight samples collected at 14th hour in this group, three were higher than 100 mg/L while the less five samples were within the therapeutic range.

The six patients who took conventional tablets, only three out of six concentrations measured at 5th hour samples were within the therapeutic range while the other three were lower than 50 mg/L. The patients who consumed conventional dosage form usually consumed in lower dosage as compared to chrono dosage form. All six concentrations measured at through were lower than 50 mg/L. For unbound concentrations majority of the concentrations measured at 14th hour and 19th hour were higher than 10 mg/L while majority of the concentrations measured at 24th hour and 29th hour were lower than 5 mg/L. Most of the unbound VPA concentrations measured from the samples collected after taken conventional tablets were lower than 5 mg/L.

Approximately 80 percent of patients in seizure-uncontrolled group were treated with the proposed therapeutic dose range (15-30 mg/kg/day). Sixty-eight percent of patients in seizure-controlled group were treated with the proposed dose range.

Table 7 Total and unbound VPA concentrations and free fraction in seizure-uncontrolled group

Patient No.	Dose (mg/kg)	Total VPA Conc. (mg/L)		Unbound VPA Conc. (mg/L)		Free fraction (%)	
		14^{th} hour	19^{th} hour	14^{th} hour	19^{th} hour	14^{th} hour	19^{th} hour
1	25.29	146.5	128.25	30.16	21.75	20.59	16.96
2	18.45	139.76	117.6	35.56	26.06	25.44	22.16
3	27.17	109.38	96.31	17.99	11.99	16.45	12.45
4	10.07	79.83	60.09	12.36	9.34	15.48	15.54
Patient No.	Dose (mg/kg)	Total VPA Conc. (mg/L)		Unbound VPA Conc. (mg/L)		Free fraction (%)	
		24^{th} hour	29^{th} hour	24^{th} hour	29^{th} hour	24^{th} hour	29^{th} hour
5	33.98	85.79	71.36	10.02	8.48	11.68	11.88
6	16.67	76.1	65.08	11.05	7.14	14.52	10.97
7	22.58	75.57	62.77	8.13	5.23	10.76	8.33
8	21.43	72.61	58.88	10.02	6.74	13.80	11.45
9	15.72	71.55	56.93	6.67	5.39	9.32	9.47
10	17.44	64.47	57.71	10.75	7.48	16.67	12.96
11	17.05	62.50	50.10	4.05	3.14	6.48	6.27
12	16.13	58.63	52.01	8.12	5.83	13.85	11.21
13	23.62	54.62	40.38	4.00	3.08	7.32	7.63
Patient No.	Dose (mg/kg)	Total VPA Conc. (mg/L)		Unbound VPA Conc. (mg/L)		Free fraction (%)	
		17^{th} hour	22^{nd} hour	17^{th} hour	22^{nd} hour	17^{th} hour	22^{nd} hour
14	23.44	51.55	38.23	4.29	2.38	8.32	6.23
Patient No.	Dose (mg/kg)	Total VPA Conc. (mg/L)		Unbound VPA Conc. (mg/L)		Free fraction (%)	
		5^{th} hour	12^{th} hour	5^{th} hour	12^{th} hour	5^{th} hour	12^{th} hour
15	10.71	85.82	69.15	7.90	5.37	9.21	7.77

Table 8 Total and unbound VPA concentrations and free fractions in seizure-controlled group

Patient No.	Dose (mg/kg)	Total VPA Conc. (mg/L)		Unbound VPA Conc. (mg/L)		Free fraction%	
		14th hour	19th hour	14th hour	19th hour	14th hour	19th hour
16	21.93	117.71	105.43	16.84	12.36	14.31	11.72
17	18.18	110.68	102.52	18.01	13.49	16.27	13.16
18	20.00	109.13	96.36	18.15	14.87	16.63	15.43
19	13.70	86.7	76.19	10.49	7.26	12.10	9.53
20	16.39	84.19	73.88	11.96	11.63	14.21	15.74
21	19.23	71.46	58.56	8.30	6.16	11.61	10.52
22	16.30	69.71	57.38	7.82	5.61	11.22	9.78
23	10.87	54.02	42.57	4.96	4.17	9.18	9.79
Patient No.	Dose (mg/kg)	Total VPA Conc. (mg/L)		Unbound VPA Conc. (mg/L)		Free fraction%	
		24th hour	29th hour	24th hour	29th hour	24th hour	29th hour
24	17.54	130.03	123.97	23.49	19.24	18.07	15.52
25	18.18	70.43	64.13	10.00	8.61	14.20	13.43
26	19.84	58.89	47.73	6.45	4.67	10.95	9.78
27	20.4	50.55	35.62	4.96	2.46	9.81	6.91
28	16.61	48.37	45.28	4.63	4.58	9.57	10.11
29	19.61	43.58	36.91	3.49	2.13	8.01	5.77
30	11.36	42.96	37.44	5.20	3.56	12.10	9.51
Patient No.	Dose (mg/kg)	Total VPA Conc. (mg/L)		Unbound VPA Conc. (mg/L)		Free fraction%	
		18th hour	23rd hour	18th hour	23rd hour	18th hour	23rd hour
31	20.00	77.26	63.89	9.88	7.42	12.79	11.61
Patient No.	Dose (mg/kg)	Total VPA Conc. (mg/L)		Unbound VPA Conc. (mg/L)		Free fraction%	
		36th hour	41st hour	36th hour	41st hour	36th hour	41st hour
32	17.33	90.40	84.60	17.38	12.35	18.52	13.2
33	18.18	58.75	46.63	4.48	3.43	7.63	7.35
34	17.61	29.86	25.62	1.61	1.42	5.39	5.54
Patient No.	Dose (mg/kg)	Total VPA Conc. (mg/L)		Unbound VPA Conc. (mg/L)		Free fraction%	
		5th hour	12th hour	5th hour	12th hour	5th hour	12th hour
35	13.51	64.62	39.95	10.63	8.72	16.45	21.83
36	6.45	52.95	40.13	7.19	3.58	13.58	8.92
37	8.51	50.53	39.84	3.43	2.58	6.79	6.48
38	8.00	38.59	29.06	3.12	2.03	8.08	6.99
39	6.67	35.72	27.58	2.58	1.67	7.22	6.06
40	7.55	16.93	12.49	1.10	0.79	6.50	6.33

2.2 Relationship of Total and Unbound Concentration

Figure 4 demonstrated the correlation between the total and the unbound concentrations of VPA obtained from both types of dosage form. The unbound concentrations were found significantly positive correlated to the total concentrations (R^2 =0.853). Slope of the graph indicated that the unbound concentrations increase in a higher proportion at higher concentrations when compared to those at lower concentrations. Figure 5-6 demonstrated the correlation between the total and the unbound concentrations of VPA in Chrono and conventional groups. The unbound concentrations of Chrono and conventional were correlated to their corresponding total concentrations with correlation coefficient equals to 0.879 and correlation coefficient equals to 0.490, respectively.

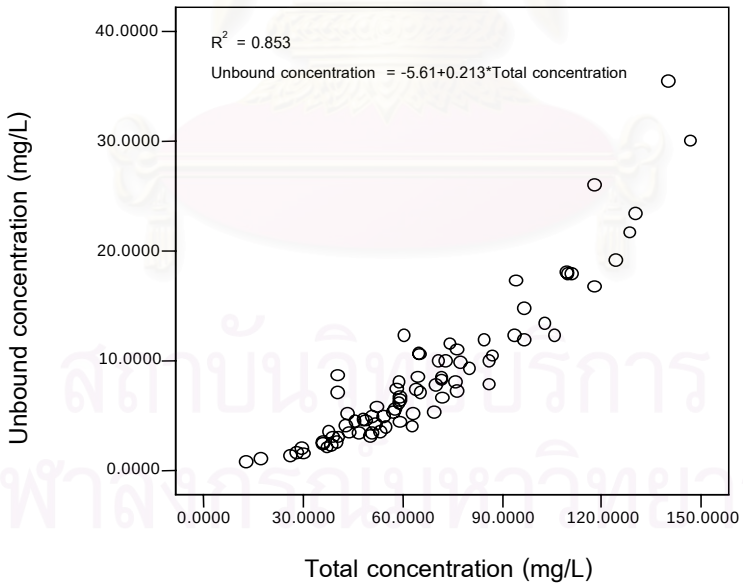


Figure 4 Correlation between total and unbound concentrations of VPA (n = 40 x 2)

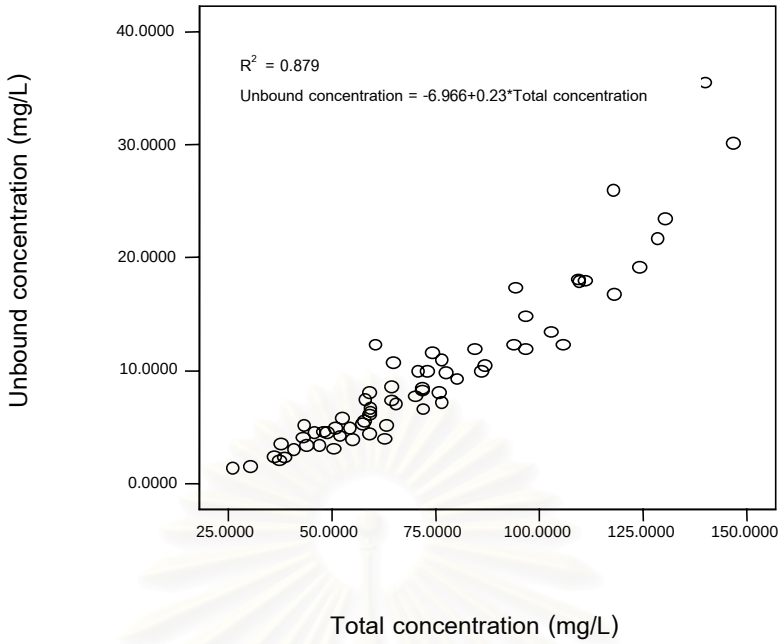


Figure 5 Correlation between total and unbound concentrations of VPA obtained from Chrono dosage form (n = 33 x 2)

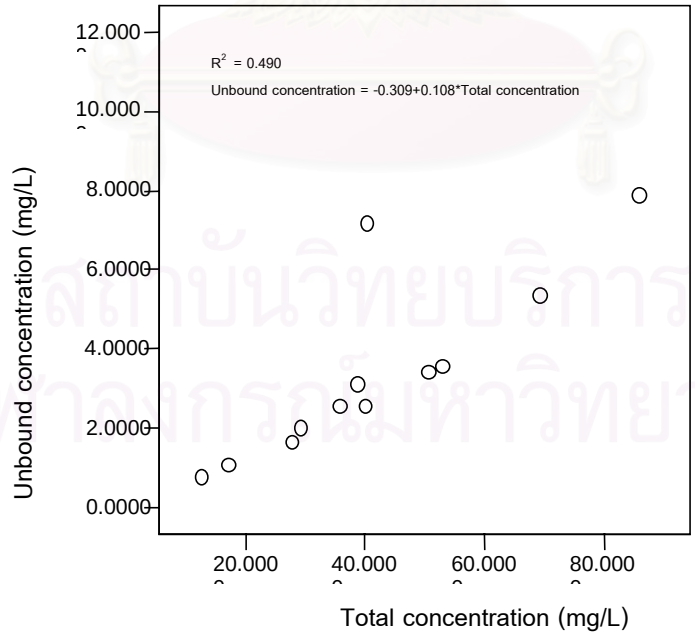


Figure 6 Correlation between total and unbound concentrations of VPA obtained from conventional dosage form (n = 7 x 2)

2.3 Free Fraction

Graphic presentations of the relationship between free fractions and total concentrations were presented in figure 7, 8 and 9.

Figure 7 showed the correlation of concentrations obtained from both types of dosage form, figure 8 showed correlation for concentrations obtained from chrono dosage form only while figure 9 showed correlation for concentrations obtained from conventional dosage form only. The equations for prediction of the free fraction based on the total concentration were also obtained from regression analysis. The correlation was better for concentrations obtained from chrono dosage form.

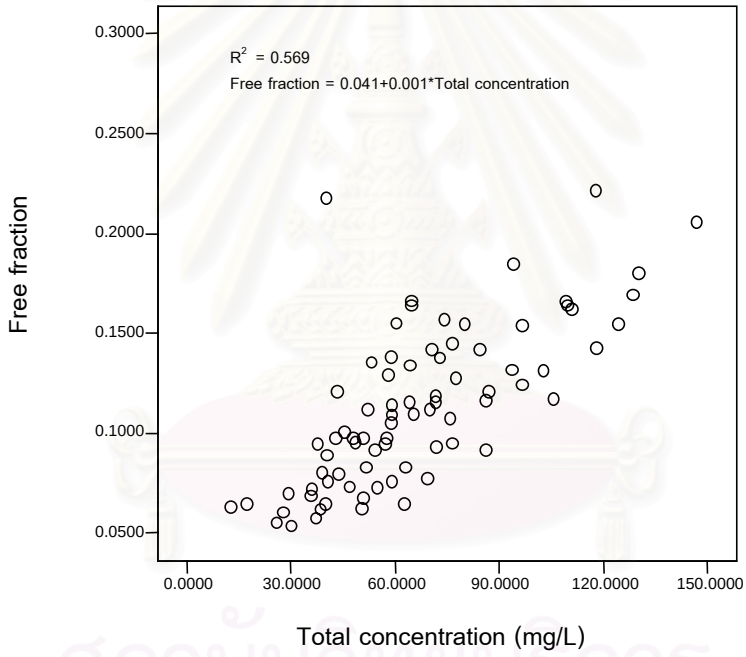


Figure 7 Correlation between total concentration and free fraction (n = 40 x 2)

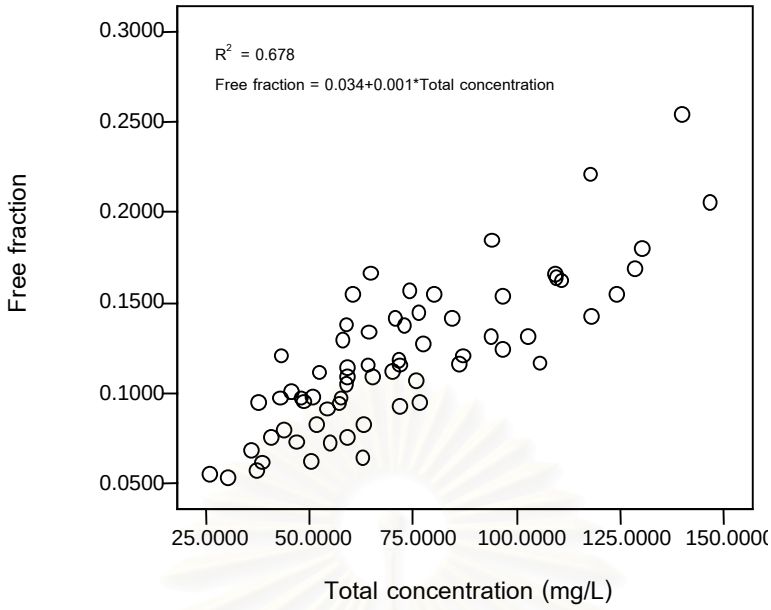


Figure 8 Correlation between total concentrations and free fraction obtained from Chrono dosage form (n= 33 x 2)

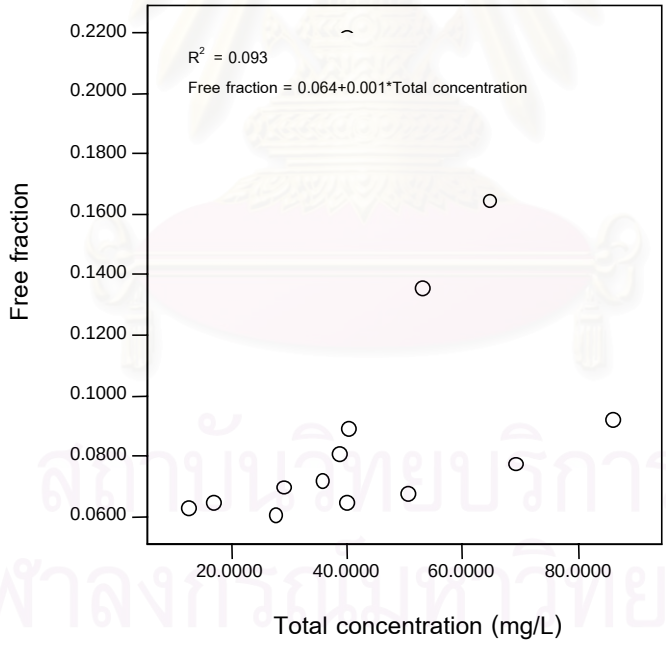


Figure 9 Correlation between total concentration and free fraction obtained from conventional dosage form (n = 7 x 2)

By dividing the data of this study into three groups of different concentration ranges which were <50 mg/L, 50-100 mg/L and > 100mg/L, the free fractions found in each group were 8.3%, 11.14% and 17.3%, respectively. There were significant differences in the free fractions among three groups as shown in Table 9.

Table 9 Comparison of the free fractions between different concentration ranges

Concentration range	Free fraction (%)	Comparison groups	p value
A < 50	8.3	A&B	0.000
B 50-100	11.4	B&C	0.000
C > 100	17.3	C&A	0.000

3. Pharmacokinetic Parameters

The PK parameters were calculated for both total and unbound VPA in plasma concentrations and were shown in Table 10 and 11 for seizure uncontrolled group and seizure controlled group, respectively. The PK parameters calculated were k, $t_{1/2}$, Cl, V_d.

Table 10 Pharmacokinetic parameters of total and unbound VPA in seizure-uncontrolled group (n = 15)

Patient No.	Total VPA conc.				Unbound VPA conc.			
	k (hr^{-1})	$t_{1/2}$ (hr)	V_d (L/kg)	Cl (L/kg/hr)	k (hr^{-1})	$t_{1/2}$ (hr)	V_d (L/kg)	Cl (L/kg/hr)
1	0.0266	26.044	0.2506	0.0067	0.0654	10.599	0.4571	0.0300
2	0.0345	20.070	0.1855	0.0064	0.0622	11.147	0.4469	0.0278
3	0.0255	27.228	0.4549	0.0116	0.0811	8.5399	1.0530	0.0854
4	0.0568	12.198	0.1162	0.0066	0.0560	12.368	0.7643	0.0428
5	0.0307	22.577	0.3308	0.0102	0.0278	24.917	3.2599	0.0906
6	0.0313	22.150	0.2460	0.0077	0.0873	7.9342	0.4147	0.0362
7	0.0371	18.671	0.2532	0.0094	0.0882	7.8545	0.6268	0.0553
8	0.0419	16.531	0.2291	0.0096	0.0793	8.7385	0.6772	0.0537
9	0.0457	15.159	0.1554	0.0071	0.0426	16.261	1.8122	0.0772
10	0.0277	25.024	0.3502	0.0097	0.0907	7.6432	0.4190	0.0380
11	0.0442	15.668	0.2014	0.0089	0.0509	13.615	2.5953	0.1321
12	0.0300	23.136	0.2493	0.0075	0.0828	8.3667	0.3834	0.0317
13	0.0604	11.471	0.2086	0.0126	0.0523	13.257	3.4704	0.1815
14	0.0598	11.591	0.3328	0.0199	0.1178	5.8810	1.9669	0.2317
15[#]	0.0432	16.043	0.2153	0.0093	0.0772	8.9759	1.9702	0.1521
Mean ±SD	0.0397 ±0.0119	18.9045 ±5.2989	0.2520 ±0.0854	0.0095 ±0.0034	0.0708 ±0.0228	11.0734 ±4.7377	1.3545 ±1.0813	0.0844 ±0.0627

Conventional dosage form

Table 11 Pharmacokinetic parameters of total and unbound VPA in seizure-controlled group (n = 25)

Patient No.	Total VPA conc.				Unbound VPA conc.			
	k (hr^{-1})	$t_{1/2}$ (hr)	V_d (L/kg)	Cl (L/kg/hr)	k (hr^{-1})	$t_{1/2}$ (hr)	V_d (L/kg)	Cl (L/kg/hr)
16	0.0220	31.4495	0.3909	0.0086	0.0619	11.2030	1.1260	0.0697
17	0.0153	46.2437	0.4837	0.0074	0.0578	11.9905	0.9221	0.0533
18	0.0249	27.8428	0.3454	0.0086	0.0399	17.3837	1.3709	0.0547
19	0.0258	26.8140	0.2868	0.0074	0.0736	9.4147	0.9837	0.0724
20	0.0261	26.5245	0.3487	0.0091	0.0056	123.839	10.4821	0.0587
21	0.0398	17.4045	0.3342	0.0133	0.0596	11.6205	2.0654	0.1231
22	0.0389	17.8014	0.2982	0.0116	0.0664	10.4325	1.7033	0.1131
23	0.0476	14.5463	0.2164	0.0103	0.0347	19.9724	3.0720	0.1066
24	0.0095	72.6026	0.5684	0.0054	0.0399	17.3612	0.6241	0.0249
25	0.0187	36.9769	0.4354	0.0081	0.0299	23.1524	1.6994	0.0508
26	0.0420	16.4913	0.2643	0.0111	0.0646	10.7302	1.3467	0.0870
27	0.0700	9.8984	0.1571	0.0110	0.1402	4.9412	0.4593	0.0644
28	0.0132	52.4886	0.4015	0.0053	0.0022	319.123	26.7727	0.0589
29	0.0332	20.8589	0.4699	0.0156	0.0988	7.0173	1.2500	0.1235
30	0.0275	25.1945	0.3455	0.0095	0.0758	9.1449	0.7533	0.0571
31	0.0380	18.2355	0.1935	0.0074	0.0573	12.1013	0.7978	0.0457
32	0.0133	52.2546	0.4812	0.0064	0.0683	10.1415	0.1772	0.0121
33	0.0462	14.9969	0.1234	0.0057	0.0534	12.9745	1.2228	0.0653
34	0.0306	22.6254	0.4706	0.0144	0.0251	27.5926	11.7291	0.2944
35#	0.0962	7.2053	0.0800	0.0077	0.0396	17.4946	0.1313	0.0052
36#	0.0396	17.4985	0.1313	0.0052	0.0996	6.9566	0.3815	0.0380
37#	0.0594	11.6620	0.1195	0.0071	0.0712	9.7341	1.5154	0.1079
38#	0.0405	17.1032	0.2222	0.0090	0.0614	11.2867	1.8078	0.1110
39#	0.0517	13.3980	0.1431	0.0074	0.0870	7.9661	0.1414	0.0123
40#	0.0435	15.9489	0.4460	0.0194	0.0473	14.6541	6.2600	0.2961
Mean ±SD	0.0365 ±0.0194	25.362 ±15.7173	0.2884 ±0.1246	0.0094 ±0.0034	0.0584 ±0.0298	29.529 ±64.4926	2.4778 ±4.6372	0.0843 ±0.0679

Conventional dosage form

4. Comparisons of Pharmacokinetic Parameters

As displayed in table 12 and 13, the PK parameters were compared between seizure-uncontrolled and seizure-controlled groups. Since the dosage formulation might produce some effects on the PK parameters, only the PK parameters obtained from chrono dosage form were used in the comparison between the seizure-uncontrolled and seizure-controlled groups. Two patients in seizure-controlled groups were excluded because they had very long $t_{½}$ of unbound VPA, therefore, the numbers of subjects included in the calculation become 14 and 17 for the seizure-uncontrolled and seizure-controlled group respectively.

For total concentrations, the mean value of $t_{½}$ of the seizure-uncontrolled group was shorter while the V_d was less extensive when compared to the seizure-controlled group with *p* value equaled to 0.142 and 0.032 respectively. For unbound concentrations, even through the mean value of $t_{½}$ in the seizure-uncontrolled group was less than the value in the seizure-controlled group while the V_d was smaller in the seizure-uncontrolled group as compared to the controlled group, these differences were not statistically significant at α = 0.05 since the PK parameters varied highly especially in the seizure-controlled group which two patients showed extreme values and were excluded from the calculation as mention above. If the two outliners were included, the p-value become 0.069 and 0.018 for comparison of the mean $t_{½}$ and V_d respectively.

Figure 10 and 11 displayed percentage of patients within different ranges of $t_{½}$ in the seizure-uncontrolled and seizure-controlled groups for total and unbound VPA respectively. From the graph, seizure-uncontrolled patients showed tendency to have shorter $t_{½}$ than seizure-controlled patients. Figure 12 and 13 displayed percentage of patients within different ranges of V_d in the seizure-uncontrolled and seizure-controlled groups for total and unbound VPA respectively. From the graph, seizure-uncontrolled patients had tendency to have smaller V_d of VPA than seizure-controlled patients.

Table 12 Comparisons of the mean pharmacokinetic parameters obtained from total concentrations between seizure-uncontrolled and seizure-controlled groups

Pharmacokinetic parameters	Total concentrations				*p* value[!]
	Seizure-uncontrolled group (n = 14)		Seizure-controlled group (n = 17)		
	Mean±SD	Median	Mean±SD	Median	
k (hr^{-1})	0.0394±0.0123	0.0358	0.0319±0.0151	0.0306	0.142
$t_{½}$ (hr)	19.1088±5.4373	19.3710	27.7787±16.2272	22.6254	0.142
V_d (L/kg)	0.2546±0.0880	0.2476	0.3450±0.1288	0.3454	0.032*
Cl (L/hr/kg)	0.0096±0.0035	0.00915	0.0095±0.0030	0.0086	0.905

[!] Mann-Whitney test

* significant different at α = 0.05

Table 13 Comparisons of the mean pharmacokinetic parameters obtained from unbound concentrations between seizure-uncontrolled and seizure-controlled groups

Pharmacokinetic parameters	Unbound concentrations				*p* value[!]
	Seizure-uncontrolled group (n = 14)		Seizure-controlled group (n = 17)		
	Mean±SD	Median	Mean±SD	Median	
k (hr^{-1})	0.0703±0.0235	0.0724	0.0616±0.0275	0.0596	0.234
$t_{½}$ (hr)	11.2232±4.8796	9.6690	13.3632±5.8947	11.6205	0.234
V_d (L/kg)	1.3105±1.1081	0.7208	1.8414±2.6351	1.2228	0.383
Cl (L/hr/kg)	0.0796±0.0621	0.0545)	0.0834±0.0630	0.0653	0.578

[!] Mann-Whitney test

* significant different at α = 0.05

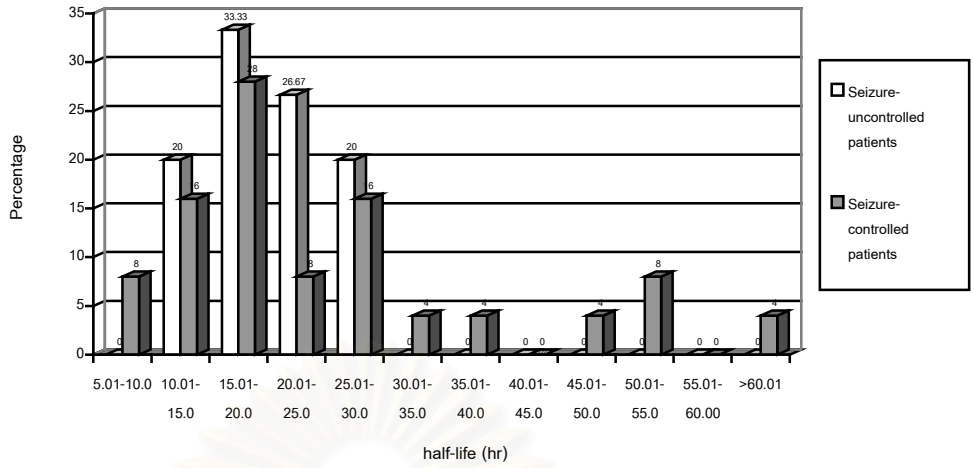


Figure 10 Percentage of seizure-uncontrolled and seizure-controlled patients categorized according to different half-lives obtained from total concentrations

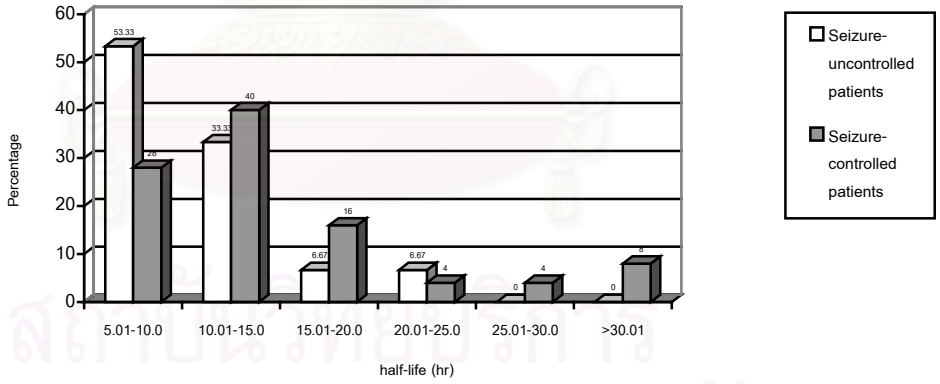


Figure 11 Percentage of seizure-uncontrolled and seizure-controlled patients categorized according to different half-lives obtained from unbound concentrations

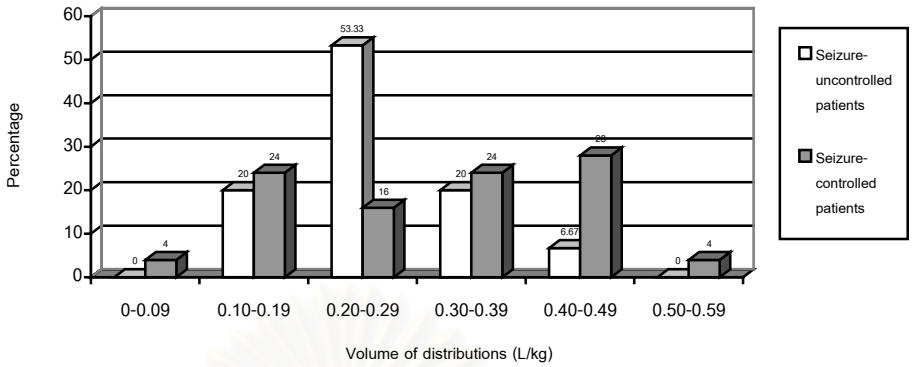


Figure 12 Percentage of seizure-uncontrolled and seizure-controlled patients categorized according to different volume of distributions obtained from total concentrations

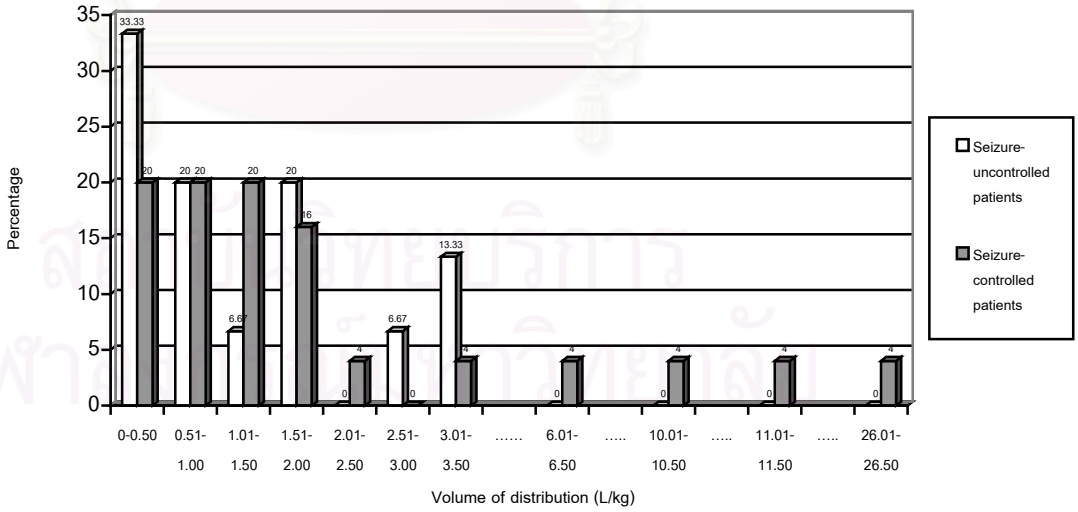


Figure 13 Percentage of seizure-uncontrolled and seizure-controlled patients categorized according to different volume of distributions obtained from unbound concentrations

In addition, as shown in Table 14 and 15, the PK parameters were also compared between dosage forms (Chrono tablet versus conventional tablet). The PK parameters of the seizure-controlled group only were analyzed. For total concentrations, k was lower, resulting in a longer $t_{½}$ in the chrono group as compared to the conventional group with *p* value equaled to 0.010 for both k and $t_{½}$. V_d of the chrono group was larger than that of the conventional group at p value equaled to 0.021. The same tendency was observed with unbound VPA PK parameter, however, the differences were not statistically significant at α = 0.05 since the standard deviations of the PK parameters were quite high in the seizure-controlled group.

Table 14 Comparisons of the mean pharmacokinetic parameters obtained from total concentrations between Chrono and conventional groups

Pharmacokinetic parameters[#]	Total concentrations				*p* value[!]
	Chrono group (n = 17)		Conventional group (n = 6)		
	Mean±SD	Median	Mean±SD	Median	
k (hr^{-1})	0.0320±0.0151	0.0306	0.0552±0.0215	0.0476	0.010*
$t_{½}$ (hr)	27.7787±16.2272	22.6254	13.8027±3.9352	14.6735	0.010*
V_d (L/kg)	0.3450±0.1288	0.3454	0.1904±0.1336	0.1372	0.021*
Cl (L/hr/kg)	0.0095±0.0030	0.0086	0.0093±0.0051	0.0076	0.419

[#] The pharmacokinetic parameters of the seizure-controlled group only were analyzed.

[!] Mann-Whitney test

* significant different at α = 0.05

Table 15 Comparisons of the mean pharmacokinetic parameters obtained from unbound concentrations between Chrono and conventional groups

Pharmacokinetic parameters[#]	Unbound concentrations				*p* value[!]
	Chrono group (n = 17)		Conventional group (n = 6)		
	Mean±SD	Median	Mean±SD	Median	
k (hr^{-1})	0.0616±0.0275	0.0596	0.0677±0.0230	0.0663	0.484
$t_{½}$ (hr)	13.3632±5.8947	11.6205	11.3487±4.0507	10.5104	0.484
V_d (L/kg)	1.8414±2.6351	1.2228	1.7062±2.3438	0.9485	0.575
Cl (L/hr/kg)	0.0834±0.0630	0.0653	0.0951±0.1086	0.0730	0.726

[#] The pharmacokinetic parameters of the seizure-controlled group only were analyzed.

[!] Mann-Whitney test

* significant different at α = 0.05

CHAPTER V

DISCUSSION

1. Demographic Data

The data were obtained from 40 adult patients using VPA. Twenty-five patients were in the seizure-controlled group; these patients were taking VPA as a monotherapy. On the other hand, 15 patients in seizure-uncontrolled group were either taking VPA monotherapy or added on some new generation antiepileptic drugs, such as topiramate, lamotrigine, gabapantin, oxcarbazepine and levetiracetam. The most common seizure type in the seizure-uncontrolled group was complex partial seizures (53.33 %). This result agreed with previous study, which indicated that about 60 % of patients with intractable epilepsy suffer from partial seizures. The most common seizure type in the seizure-controlled group was generalized tonic clonic seizures (68.00 %) which was consistent with a previous study which reported that VPA treatment in 808 adults and 585 children with epilepsy resulted in more than 75% reduction in seizure frequency in 78% of patients with generalized seizures *(4).* Most patients had their albumin values within normal ranges. There were no statistically significant differences in percentages of neurological abnormality detected from EEG and CT examinations between seizure-controlled and seizure-uncontrolled groups, which mean that the abnormality detected by these examinations could not be used as an indicator for seizure controlled or not controlled by VPA. For MRI, even though the difference in percentage of abnormality was not significant between the two groups at α = 0.05, due to small number of patients included in the observation, seizure-uncontrolled patients had higher tendency to show abnormality from MRI. This result was consistent with Fernando-dongas M.C. et al. who suggested that the VPA resistant group had a higher incidence of neurological and neuroimaging abnormalities *(13).*

2. Total, Unbound concentrations and Free Fraction

2.1 Therapeutic Concentrations

In seizure-uncontrolled group, of the 15 patients recruited, 14 patient received depakine chrono. Ten patients (71.43 %) whose blood samples were measured at 14th, 19th and 24th hours had total concentrations within the proposed therapeutic range (50-100 mg/L). These measured concentrations confirmed that depakine chrono could be used as once-daily regimen. Three patients (21.43%) whose blood samples were measured at 14th hour had total concentrations higher than 100 mg/L, no side effects were noted in these three patients. One patient (7.14%) whose blood sample was measured within 24 hours had total concentrations lower than 50 mg/L. However, high percentage of patients in seizure-uncontrolled group had total concentrations within or higher than the proposed therapeutic range, but the seizures still could not be controlled. It might imply that these patients did not response very well to VPA.

In seizure-controlled group, 8 patients (50.00 %) whose blood samples were measured at 14th, 19th and 24th hours had total concentrations within the proposed therapeutic range, while 4 patients (25.00 %) whose blood samples were measured at 24th hour had total concentrations less than 50 mg/L and 4 patients (25.00%) whose blood samples measured at 14th hour had total concentrations higher than 100 mg/L. Lower percentage of patients in seizure-controlled group had their total VPA concentrations within the proposed therapeutic range as compared with seizure-uncontrolled group some patients even though their total VPA concentrations were less than 50 mg/L, but the seizures were under controlled. One reason might due to the type of seizure. Majority of patients in the seizure-controlled group had generalized tonic clonic type of seizure. This finding consistent with previous study that complex partial seizures are usually refractory to antiepileptic drug therapy, carry a worse prognosis, and require higher AEDs blood level than that of generalized seizures *(19)*.

2.2 Relationship between Total and Unbound Concentrations

The unbound concentrations were found significantly positively correlated to the total concentrations (R^2 =0.853). In this study, there was a high degree of linear relationship between total and unbound VPA concentrations when the total concentrations ranged from 12.49 to 70.43 mg/L and the unbound concentrations ranged from 0.79 to 8.48 mg/L. The binding capacity of VPA had not reached saturation at the lower end of these concentrations. The graph showed obvious deviation from linearity when the concentrations were higher than 60-70 mg/L. The increment of free fraction tended to be markedly increased at higher concentrations. This finding agrees with previous research by Lagace D.C. et al. in 2004 who reported that the binding sites of VPA are saturable, and free fraction may increase as the total concentration increase *(31).* Guyot et al. in 1982 found that the correlation between free plasma VPA and total CSF concentration was higher than that between total plasma VPA concentration and total CSF concentration. The free level of VPA was therefore recommended to estimate instead of the total concentrations. The unbound concentrations of Chrono and conventional dosage forms were correlated to their corresponding total concentrations with correlation coefficient equals to 0.879 and correlation coefficient equals to 0.490, respectively. Correlation coefficient of conventional dosage form was low due to small number of subjects and narrow total concentration range within this group.

2.3 Free Fraction

The free fractions ranged from 0.054 to 0.254. The free fractions of chrono and conventional dosage forms were correlated to their corresponding total concentrations with correlation coefficient equals to 0.678 and correlation coefficient equals to 0.093, respectively. Correlation coefficient of conventional dosage form was low due to small number of subjects, narrow total concentration range and low total concentrations, since the binding capacity of VPA had not reached saturation at the low concentrations.

These findings were consistent with the results reported by Kodoma Y. et al. in 1995 and Cloyd J.C. et al. in 1993 who found that the free fractions were ranged from 0.057 to 0.16 and 0.08 to 0.17, respectively *(54, 55)*. The lower end was similar with the values reported previously while at upper end, the free fraction was higher which might due in part to the higher total concentration obtained in this study. Gal P. et al. in 1988 stated that six neonates with prolonged, intractable seizures were treated with VPA their VPA free fraction ranged from 11.3 to 31.6 % (mean, 19.2%) *(15)*. The free fractions seem to be higher in neonate who might have lower albumin concentration and/or have higher total VPA concentration.

By dividing the total concentrations into three groups of different concentration range, < 50 mg/L, 50-100 mg/L and >100 mg/L, their free fractions were 8.3%, 11.4% and 17.3%, respectively*)*. There were significant differences among the three groups. These results were consistent with Cramer J.A. et al. in 1986 who evaluated the albumin-binding characteristics of VPA in patients with epilepsy. According to their data, free fractions of VPA were approximately 7% at 50 mg/L, 15% at 100 mg/L, 22% at 125 mg/L and 30% at 150 mg/L *(56)*. Cloyd J.C. et al. in 2003 found that VPA unbound fraction decreased from 15% at maximum concentration to 9% at 45 mg/L *(37)*. Higher concentrations resulted in higher percentages of free fraction, which might imply that saturation had occurred.

3. Pharmacokinetic Parameters

3.1 Comparisons the Mean PK Parameters between Seizure- uncontrolled and Seizure-controlled Patients

a) Total VPA PK Parameters

This study found that there were significant differences of mean $t_{½}$ (p= 0.142) and V_d (p=0.032) between seizure-uncontrolled and seizure-controlled groups. The mean k of total VPA in seizure-uncontrolled group was higher than that of total VPA in the seizure-controlled group. The mean $t_{½}$ of VPA in seizure-uncontrolled group was shorter than that

obtained in seizure-controlled group. The mean V_d of seizure-uncontrolled group was lower than the value in seizure-controlled group. These might imply that less VPA could distribute into the brain of the seizure-uncontrolled by VPA group. The reason for low V_d of seizure-uncontrolled group is not fully understood. First reason, there might be some mechanism in blood brain barrier that decreased movement of VPA into CNS or the brain uptake process might become saturated more easily in some patients. Second reason, VPA might not bind to lipid or protein components of neural tissues *(49)*. Higher VPA concentration in plasma then resulted in higher k constant and shorter $t_{½}$ of VPA in seizure-uncontrolled group.

The mean ($\pm$SD) values of $t_{½}$ of total VPA concentration for seizure-uncontrolled patient and seizure-controlled patient were 19.1088$\pm$5.4373 hr and 27.7787$\pm$16.2272 hr, respectively. These $t_{½}$ seem to be longer than those reported in previous studies. Redenbaugh J.E. et al. in 1980 found the $t_{½}$ of 20 patients with intractable seizures of all types to be ranged from 5.4 to 13.5 hours (mean, 9.5 $\pm$ 2.1 hr) for adults and from 6.0 to 18.5 hr (9.2$\pm$3.5 hr) for children. *(14)*. Hergren L. et al. in 1991 reported the mean terminal $t_{½}$ of VPA in plasma and whole blood of infants to be 12.5 and 15.5 hr, respectively. *(10)*. Gidal B.E. and Graves N.M. stated that $t_{½}$ of neonates, infants, children and adults were 17.2 hr, 12.5$\pm$2.8 hr, 11$\pm$4 hr and 11.9$\pm$5.7 hr, respectively *(46)*. Gal P. et al. (1988) stated that the mean $t_{½}$ of six neonates with prolonged intractable seizures was 26.4 hr (ranged 8.6 to 48.5) *(15)*. The $t_{½}$ might be higher in neonates than in grown up. The longer $t_{½}$ found in this study might due in part to the dosage formulation taken. In this study, majority of the patients took chrono dosage form while in previous studies; mostly they were taken conventional dosage form. From previous report, total and unbound peak plasma concentrations were stable at plateau from 4-14 hours after administration of depakine chrono dosage form *(57)* which means that after 14th hour the absorption process should fade out and the elimination phase would dominate.

In order to prove that the absorption process was completed at 14th hour, four blood samples from a few patients had been collected at 14th, 19th, 24th, and 29th hours after administration of chrono dosage form, semi log plot of VPA concentration versus time showed linearity (as shown in appendix D) which means that these samples were all

collected during the elimination kinetic phase and that the absorption and distribution phase had been completed since the first blood sampling time which was at 14^{th} hour. However, in some patients, VPA concentrations obtained from the two blood samples collected 5 hours apart (14^{th} hour and 19^{th} hour, 24^{th} hour and 29^{th} hour, or 36^{th} hour and 41^{st} hour) showed only slightly decrement in VPA concentration of the second blood sample from the first blood sample, resulting in a very long $t_{½}$. This study found that there were no significant differences between means PK parameter obtained at 14^{th} – 19^{th} and 24^{th} – 29^{th} hours (as shown in appendix E).

Approximately 53 percent of patients in seizure-uncontrolled group and 44 percent of patients in seizure-controlled group had the $t_{½}$ of total VPA within the range of 10-20 hr. Seizure-uncontrolled patients had tendency to have shorter $t_{½}$ of total VPA than seizure-controlled patients.

The mean values of V_d of total VPA concentration for seizure-uncontrolled and seizure-controlled groups were 0.2546±0.0880 L/kg and 0.3450±0.1288 L/kg, respectively. A high percentage of VPA is bound to albumin relative to tissue proteins and ionization of VPA in blood lead to the relatively small V_d *(20).* These values were lower than those reported by Gal P. et al. in 1988 who stated that V_d tin neonates with intractable seizures was 0.4 L/kg (range, 0.36 to 0.47 L/kg) *(15).* The V_d might be higher in neonates than in grown up, neonate had lower albumin concentration.

Approximately 53 percent of patients in seizure-uncontrolled and 28 percent of patients in seizure-controlled had V_d obtained from total concentrations within the range of 0.2-0.3 L/kg and 0.4-0.5 L/kg, respectively. Seizure-uncontrolled patients had tendency to have lower V_d than seizure-controlled patients.

In this study, the mean values of Cl of seizure-uncontrolled group and seizure-controlled group were 0.0096±0.0035 L/kg/hr and 0.0095±0.0030 L/kg/hr, respectively. These values were similar to previous study by Gidal B.E. and Graves N.M. who stated that Cl of adults were 0.009±0.005 L/kg/hr, respectively *(46).* But, Cl obtained from this study seem to be lower than those reported in Hergren L. et al. in 1991 who suggested that the mean Cl of total VPA in plasma and whole blood of infants was 0.0178 and 0.0289 L/kg/hr, respectively *(12).* Gal P. et al. in 1988 stated that the mean Cl of total VPA concentrations in six neonates with intractable seizures was 0.0144 L/kg/hr (0.0055

to 0.0182 L/kg/hr) *(15).* Sanchez-Alcaraz A. et al. (1998) reported that Cl in children who were receiving VPA monotherapy was 0.0245±0.0124 L/kg/hr (age <2 years), 0.0199±0.0061 L/kg/hr (age 2-4 years) and 0.0127±0.0030 L/kg/hr (age >4 years), respectively *(40).* This study was performed in adults while the last three studies were mostly performed in infants or children, the Cl might be higher in children than in adults. Since Cl is the product of k and V_d and these two values were both lower in this study than those previously reported, therefore, Cl was also lower.

b) Unbound VPA PK Parameters

All PK parameters of unbound VPA showed no statistically significant differences between seizure-uncontrolled group and seizure-controlled group at α = 0.05. This might due in part to the high inter-subject variations in unbound VPA PK parameters among the patients in seizure-controlled group, which lead to a requirement of higher number of subjects to be able to conclude for a significant difference. The p-values were 0.234 and 0.383 for $t_{½}$ and V_d, respectively.

The $t_{½}$ and V_d of seizure-uncontrolled group seem to be shorter and smaller than those of the seizure-controlled group, which might imply that the seizure- uncontrolled patients had tendency to have smaller amount of VPA distributed into the brain. These findings were in agreement with Shen D.D. in 1992 who studied the distribution of VPA between brain (gray matter) and serum in patients with intractable seizures who receiving chronic VPA therapy. There was a tendency for the brain-to-serum concentration ratio to be lower in tissues from the epileptic foci than in tissues from non-epileptic areas. The mean brain-to-serum ratio for non-epileptic brain specimens was 0.130±0.058 as compared with a mean of 0.100±0.044 for epileptic brain specimens *(50).* From this result, calculation of VPA PK parameter would be beneficial for future decision-making, whether VPA treatment dosage regimen should be altered (If the value falls in the seizure-controlled group) or the other AEDs should be added (If the value falls in the seizure-uncontrolled group).

The mean values of $t_{½}$ of unbound VPA concentration for seizure-uncontrolled patient and seizure-controlled patient were 11.2232±4.8796 hr and 13.3632±5.8947 hr, respectively. $T_{½}$ found in this study were quite different from those found by Hergren L.

et al. in 1991, they reported the mean terminal $t_{½}$ of free VPA in plasma and whole blood of infants to be 6.4 and 6.5 hr, respectively. Fifty-three percent of patients in seizure-uncontrolled group and 40 percent of patients in seizure-controlled group had $t_{½}$ obtained from unbound concentration within the range of 5-10 hr and 10-20 hr, respectively. Seizure-uncontrolled patients had tendency shorter $t_{½}$ than seizure-controlled patients. Patient with $t_{½}$ of unbound VPA equaled to or less than 10 hour, had higher tendency to be in the seizure-uncontrolled group while patient with $t_{½}$ of unbound VPA longer than 15 hour would mostly belong to the seizure-controlled group. $T_{½}$ within the range of 10.01-15.00 hour had nearly equal chance to be in either group.

The V_d of unbound VPA concentration for seizure-uncontrolled and seizure-controlled groups were 1.3105$\pm$1.1081 L/kg and 1.8414$\pm$2.6351 L/kg, respectively. Unbound VPA PK parameters found in this study were not much different from those reported by Gal P. et al. in 1988 who studied in six neonates with prolonged, intractable seizures and were treated with VPA. They reported the V_d to be 2.02 L/kg (range, 1.14 to 2.44 L/kg), serum Cl to be 0.1089 L/hr/kg (0.0420 to 0.2520 L/hr/kg) and $t_{½}$ to be 17.6 hr (6.7 to 34.2) *(26).* Fifty three percent of patients in seizure-uncontrolled group and 40 percent of patients in seizure-controlled group had V_d obtained from unbound concentrations within the range of 0.01-1 L/kg. Seizure-uncontrolled patients had tendency to have lower V_d than seizure-controlled patients. V_d of less than or equaled to 0.5 L/kg had a higher chance to be in the seizure-uncontrolled group while V_d of higher than 3.5 L/kg should be in the seizure-controlled group. V_d within the range of 0.51-3.50 L/kg had equal change to belong to either group.

3.2 Comparisons the Mean PK Parameters between Chrono and Conventional Dosage Forms

a) Total VPA PK parameters

There were significant differences of k (p=0.010), half-lives (p=0.010) and V_d (p=0.021) between Chrono and conventional groups. $T_{½}$ of Chrono and conventional groups were 27.7787$\pm$16.2272 hr and 13.8027$\pm$3.9352 hr, respectively. V_d of Chrono

and conventional groups were 0.3450±0.1288 L/kg and 0.1904±0.1336 L/kg, respectively. The mean values of V_d and the $t_{½}$ were higher in the chrono group. The patients who consumed chrono dosage form usually consumed in higher dosage as compared to conventional dosage form. The concentrations of VPA in the blood were therefore sustained in higher levels for prolong of time, and in consequence the free fractions were also higher for prolong of time, more free VPA could equilibrate with free VPA in the tissue compartment, resulting in a higher V_d. At the same time, redistribution back from the tissue compartment might cause in less decrement of VPA in plasma with time, resulting in lower k and longer $t_{½}$ of VPA after consumed sustained release dosage form as compared to conventional tablet.

The results obtained from this study did not in agreement with previous studies by Wangemann M. et al. In the year 2000, they reported the $t_{½}$ of VPA in healthy male volunteers after administration of 100, 150 and 300 mg sustained release formulations to be 15.9 hr (ranged 14.6-18.1 hr), 15.5 hr (ranged 13.2-18.1 hr) and 15.5 hr (ranged 13.9-17.2 hr), respectively *(10).* In the year 1999, they demonstrated that $t_{½}$ of VPA in healthy male volunteers after administration of 300 mg sodium valproate sustained release capsule (Orfiril long) and 5 ml of valproate solution (Orfiril salt) were similar and were equaled to 14.5±1.9 and 14.3±2.0 hr, respectively *(11).* Dulac O. et al. in 2005 concluded that $t_{½}$ of sodium valproate 1000 mg sustained release and modified release formulations in 24 healthy volunteers were 16.6±3.4 and 16.5±3.5 hr, respectively *(42).* The $t_{½}$ of all three studies were obtained from single dose VPA sustained release administration. $T_{½}$ obtained from single dose VPA was lower than multiple doses VPA.

This study was performed by collecting the blood samples at steady state after multiple doses. The VPA concentrations should be much higher than the single dose due to accumulation factors, besides the fact that the dosage administered in this study was higher than most of previous studies. Higher concentrations might result in higher free fractions and in turn higher V_d. Usually higher free drug means higher amount of drug is in the form that is available for distribution and for elimination. For VPA, higher fraction of the increased amount of free drug go for distribution while less amount available for elimination, resulting in wider Vd, slower k, then, longer $t_{½}$.

b) Unbound VPA PK parameters

All PK parameters of unbound VPA showed no statistically significant difference between Chrono and conventional groups at α = 0.05. The mean values of $t_{½}$ for chrono and conventional groups were 13.3632±5.8947 hr and 11.3487±4.0507 hr, respectively. The mean values of V_d of Chrono and conventional groups were 1.8414±2.6351 L/kg and 1.7062±2.3438 L/kg, respectively. The V_d and the $t_{½}$ were higher in the chrono group. Even though the mean values of some parameters were more than higher or lower than each other, they were not statistically significant at α = 0.05. This might due in part to the high inter-subject variation in VPA PK parameters among the patients, which lead to a requirement of higher number of subjects to be able to conclude for a significant difference.

CHAPTER VI

CONCLUSION

This study was performed in 40 patients receiving VPA. The treatment outcomes were classified into 2 groups that were seizure-controlled and seizure-uncontrolled patients. Blood samples were obtained just before the morning dose of VPA (trough) and at 5^{th} hour after administration for conventional dosage form. Patients who used Depakine chrono 500 mg, the blood samples were obtained at 14^{th} and 19^{th} hours after the study dosage administration if the dosage were given in the evening, however, if the once daily dosage were given in the morning, the blood samples would be collected at 24^{th} and 29^{th} hours after the study dosage was given. The PK parameters were calculated and compared between seizure-controlled and seizure-uncontrolled patients.

The conclusion of this study were

1. Majority of the total VPA concentrations measured within 24 hours were within the proposed therapeutic range (50-100 mg/L). These measured concentrations confirm that depakine chrono could be used as once-daily regimen. In seizure-uncontrolled group, 71.43 % of patients whose blood samples were measured at 14^{th}, 19^{th} and 24^{th} hours had their total VPA concentrations within the proposed therapeutic range, but the seizures still could not be controlled. It might imply that patients did not response to VPA. In seizure-controlled group, several patients had total VPA concentrations lower than 50 mg/L, but the seizures were under controlled. Approximately 20 % of blood samples collected at 14^{th} hour after drug administration of in either seizure-uncontrolled or seizure-controlled groups had VPA concentrations higher than 100 mg/L, no seizures side effect was observed.

2. There was a good linear relationship between total and unbound VPA concentrations especially when total VPA concentrations were lower than 60-70 mg/L. The reason could due to binding capacity of VPA have not quite reached saturation at

the lower end of these concentrations. The equations to predict unbound concentration and free fraction from total concentrations were:

Unbound concentration = - 5.61±0.213*Total concentration (R^2 = 0.853, p<0.001)

Free fraction = 0.041±0.001*Total concentration (R^2 = 0.569, p<0.001)

Higher concentrations resulted in higher percentage of free fraction. The correlation between total concentration and free fraction was better for concentrations obtained from chrono dosage form as compared to conventional. Correlation coefficient of conventional dosage from was low due to small number of subjects and narrow total concentration range.

There were significant differences in the free fractions between different concentration ranges (<50 mg/L, 50-100 mg/L and >100 mg/L). Higher concentrations resulted in higher percentages of free fraction, which might imply that saturation had occurred.

3. Comparisons the Mean PK Parameters

3.1 Comparisons the Mean PK Parameters between Seizure-Uncontrolled and Seizure Controlled Patients

There were significant differences of mean $t_{½}$ (p= 0.142) and V_d (p=0.032) of total VPA between seizure-uncontrolled and seizure-controlled groups. The mean values of $t_{½}$ of total VPA concentration for seizure-uncontrolled patient and seizure-controlled patient were 19.1088±5.4373 hr and 27.7787±16.2272 hr, respectively. The V_d of total VPA concentration for seizure-uncontrolled and seizure-controlled groups were 0.2546±0.0880 L/kg and 0.3450±0.1288 L/kg, respectively. The $t_{½}$ and V_d for seizure-uncontrolled group were less than $t_{½}$ and V_d for seizure-controlled group. The Cl of total VPA concentration for seizure-uncontrolled group and seizure-controlled group were 0.0096±0.0035 L/kg/hr and 0.0095±0.0030 L/kg/hr, respectively. The k of total VPA in seizure-uncontrolled group (0.0394±0.0123 hr^{-1}) was higher than that of VPA in the seizure-controlled group (0.0319±0.0151 hr^{-1}).

For unbound concentrations, the $t_{½}$ and V_d of seizure-uncontrolled group were also less than $t_{½}$ and V_d of seizure-controlled group, these differences were not

statistically significant at α= 0.05 since the standard deviations of the PK parameters were high in the seizure-controlled group. The p-values were 0.234 and 0.383 for $t_{½}$ and V_d, respectively. The $t_{½}$ of seizure-uncontrolled patient and seizure-controlled patient were 11.2232±4.8796 hr and 13.3632±5.8947 hr, respectively. The V_d of total VPA concentration for seizure-uncontrolled and seizure-controlled groups were 1.3105±1.1081 L/kg and 1.8414±2.6351 L/kg, respectively. The Cl of seizure-uncontrolled group and seizure-controlled group were 0.0796±0.0621 L/kg/hr and 0.0834±0.0630 L/kg/hr, respectively. The k of VPA in seizure-uncontrolled group (0.0703±0.0235 hr^{-1}) was higher than that of VPA in the seizure-controlled group (0.0616±0.0275 hr^{-1}).

3.2 Comparisons the Means PK Parameters between Chrono and Conventional Dosage Forms

There were significant differences in k (p=0.010), half-lives (p=0.010) and V_d (p=0.021) of total VPA after consumed Chrono and conventional dosage forms. $T_{½}$ of Chrono and conventional groups were 27.7787±16.2272 hr and 13.8027±3.9352 hr, respectively. V_d of Chrono and conventional groups were 0.3450±0.1288 L/kg and 0.1904±0.1336 L/kg, respectively. The V_d and the $t_{½}$ were higher in the chrono group.

All PK parameters of unbound VPA showed no statistically significant difference between Chrono and conventional groups at α = 0.05 the PK parameters varied highly especially in the Chrono group. The $t_{½}$ of unbound VPA concentrations for Chrono and conventional groups were 13.3632±5.8947 hr and 11.3487±4.0507 hr, respectively. The V_d of Chrono and conventional groups were 1.8414±2.6351 L/kg and 1.7062±2.3438 L/kg, respectively.

These results provided a more rational understanding of VPA PK in the clinical setting. Clinical characteristics combined with PK parameters data might help in predicting whether or not the epileptic patients will response favorably to VPA. Seizure-uncontrolled patients has a higher incidence of neurological and neuroimaging abnormalities by MRI, However, the MRI examination is quite expensive. V_d of total VPA was significantly different between the seizure-controlled and seizure-uncontrolled groups, therefore, narrow V_d could be an indicator that the seizure of that patient had

high tendency of could not be controlled by VPA. Besides, $t_{½}$ of VPA tended to be shorter in the seizure uncontrolled group as compared to the seizure controlled group. However, the definite range of values of PK parameters which could be used to identify between the seizure controlled and uncontrolled by VPA groups could not be specified by this study due to much too small number of patients included in the study while many confounding factors were involved. Further studies in higher number of subjects and more conservative designs are required before any definite conclusion can be made.

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APPENDICES

APPENDIX A

ลำดับที่ ……..

HN .....

แบบบันทึกข้อมูลผู้ป่วย

1. ข้อมูลทั่วไปของผู้ป่วย

ชื่อ.....อายุ.....ปี.....เดือน.....วันเกิด.....

เพศ ☐ ชาย ☐ หญิง น้ำหนัก.....กิโลกรัม ความสูง.....เซนติเมตร

ที่อยู่.....โทร.....

2. ประวัติโรค

โรคลมชัก

1. การวินิจฉัยโรคลมชักเป็นชนิด.....
2. ได้รับการวินิจฉัยโรคลมชักครั้งแรกอายุ.....ปี
3. การตอบสนองต่อวาลโปรอิกแอซิด

	ก่อนได้วาลโปรอิกแอซิด	หลังได้วาลโปรอิกแอซิด
อาการชัก		
1. ความถี่ในการชัก	.....	.....
2. ระยะเวลาในการชัก	.....	.....
การประเมินผู้ป่วย		
1. ลักษณะทาง	.....	.....
กายภาพ	.....	.....
2. EEG	.....	……..
3. CT scan	.....	……..
4. MRI	……..	……..

5. ผลตรวจทางห้องปฏิบัติการการ.....

.....

6. อาการไม่พึงประสงค์.....

7. ความร่วมมือในการใช้ยา ☐ ดี ☐ ไม่ดี

8. ประวัติการใช้ยา ☐ monotherapy ☐ polytherapy

วันที่	ยากันชัก	ขนาดและวิธีใช้ยา

9. การเก็บตัวอย่างเลือด

1) ยาเม็ดธรรมดาหรือยาน้ำ

เวลาที่เจาะเลือดก่อนให้ยามื้อเช้า.....ระดับยา.....mcg/ml

เวลาที่ให้ยามื้อเช้า.....

เวลาที่ชั่วโมงที่ 5 หลังให้ยามื้อเช้า.....ะดับยา..... mcg/ml

2) ยาเม็ด chrono

เวลา ……. ระดับยา.....mcg/ml

เวลา ..... ระดับยา.....mcg/ml

แบบบันทึกการรับประทานยา

ชื่อ/นามสกุล.....อายุ.....ปี.....HN…….

ขนาดยาที่รับประทาน.....

วันที่	เวลาที่กำหนดให้รับประทาน	เวลาที่รับประทานจริง
1.....	.....น.	.....น.
....	.....น.	.....น.
2.....	.....น.	.....น.
....	.....น.	.....น.
3.....	.....น.	.....น.
....	.....น.	.....น.
4.....	.....น.	.....น.
....	.....น.	.....น.
5.....	.....น.	.....น.
....	.....น.	.....น.
6.....	.....น.	.....น.
....	.....น.	.....น.
7.....	.....น.	.....น.
....	.....น.	.....น.

รายละเอียดที่ควรทราบ

1. ให้รับประทานยาในขนาดเดิม เวลาที่กำหนด และลงเวลาที่รับประทานจริง
2. งดยากันชักมื้อเช้าวันที่มาพบแพทย์
3. งดอาหารเช้าวันที่มาพบแพทย์จนกระทั่งมาเจาะเลือด
4. เตรียมยามาด้วย สำหรับรับประทานหลังเจาะเลือดแล้ว
5. หากมีข้อสงสัย กรุณาติดต่อ นพ. สมชาย โตวณะบุตร 0-2354-7091 ต่อ 1138

แบบฟอร์มหนังสือยินยอมเข้าร่วมโครงการวิจัย

วันที่.....เดือน.....พ.ศ.....(วันให้ความยินยอม)

ข้าพเจ้า นาย/นาง/นางสาว.....ได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ วิธีการวิจัย อันตรายหรืออาการที่อาจเกิดขึ้น รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยเรื่อง "การเปรียบเทียบค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของวาลโปรอิกแอซิดในผู้ป่วยโรคลมชักที่คุมอาการชักได้และคุมอาการชักไม่ได้" แล้วอย่างชัดเจน ไม่มีสิ่งใดปิดบังซ่อนเร้น และยินยอมเข้าร่วมการวิจัยครั้งนี้โดยสมัครใจ และข้าพเจ้ารู้ว่าถ้ามีปัญหาหรือข้อสงสัยเกิดขึ้น ข้าพเจ้าสามารถสอบถามผู้วิจัยได้ทุกเมื่อ

ข้าพเจ้าไม่สามารถเข้าร่วมโครงการวิจัยนี้เมื่อใดก็ได้ โดยไม่ต้องแจ้งเหตุผลและไม่มีผลกระทบต่อการรักษาโรคที่ข้าพเจ้าจะพึงได้รับต่อไป

ข้าพเจ้ายินยอมให้ผู้กำกับดูแลการวิจัย ผู้ตรวจสอบและกรรมการพิจารณาจริยธรรมสามารถเข้าไปตรวจสอบบันทึกข้อมูลทางการแพทย์ของข้าพเจ้าเพื่อเป็นการยืนยันถึงขั้นตอนในการวิจัยทางคลินิก โดยไม่ล่วงละเมิดเอกสิทธิ์ในการปิดบังข้อมูลตามกรอบที่กฎหมายและกฎระเบียบได้อนุญาตไว้

ผู้วิจัยรับรองว่าหากมีข้อมูลเพิ่มเติมทั้งด้านประโยชน์และโทษที่เกี่ยวข้องกับการวิจัยนี้ จะแจ้งให้ข้าพเจ้าทราบอย่างรวดเร็วโดยไม่ปิดบังซ่อนเร้น และข้าพเจ้าได้รับทราบจากผู้วิจัยว่าจะเก็บข้อมูลเฉพาะที่เกี่ยวกับตัวผู้ถูกวิจัยเป็นความลับและจะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย

หากข้าพเจ้าได้รับความผิดปกติเนื่องจากการทดลอง ข้าพเจ้าจะได้รับความคุ้มครองตามกฎหมายและหากข้าพเจ้ามิได้แจ้งให้แพทย์ทราบในทันทีถึงความผิดปกติของร่างกายที่เกิดขึ้น จะถือว่าข้าพเจ้าทำให้ความคุ้มครองความปลอดภัยเป็นโมฆะ (ตามที่กฎหมายกำหนด)

ลงชื่อ.....ผู้ยินยอม

( )

ลงชื่อ.....พยาน

( )

ลงชื่อ.....อาจารย์ที่ปรึกษา

(นพ.สมชาย โตวณะบุตร)

ลงชื่อ.....ผู้วิจัย

(น.ส.ลักขณา บุญมาก)

ข้าพเจ้าไม่สามารถอ่านหนังสือได้แต่ผู้วิจัยได้อ่านข้อความในใบยินยอมนี้ให้แก่ข้าพเจ้าฟังจนเข้าใจดีแล้ว ข้าพเจ้าจึงลงนาม หรือประทับลายนิ้วหัวแม่มือขวาของข้าพเจ้าในใบยินยอมนี้ด้วยความเต็มใจ

ลงชื่อ.....ผู้ยินยอม

( )

ลงชื่อ.....พยาน

( )

ลงชื่อ.....อาจารย์ที่ปรึกษา

(นพ.สมชาย โตวณะบุตร)

ลงชื่อ.....ผู้วิจัย

(น.ส.ลักขณา บุญมาก)

แบบประเมินอาการไม่พึงประสงค์จากการใช้ยา (Naranjo's Algorithm) เลขที่ ☐☐☐

ชื่อ-สกุล.....HN..... อายุ.....ปี

ชื่อยาที่สงสัย.....ประวัติการแพ้ยา ☐ NKDA

วันที่เริ่มใช้ยา..... ☐ แพ้ยา

วันที่หยุดใช้ยา..... วันที่ประเมิน.....

รายการประเมิน	ใช่	ไม่ใช่	ไม่ทราบ
1. เคยมีสรุปหรือรายงาน ADR เกี่ยวกับยาที่สงสัยมาแล้ว	+1	0	0
2. อาการไม่พึงประสงค์เกิดขึ้นหลังได้รับยาที่สงสัย	+2	-1	0
3. อาการไม่พึงประสงค์ดีขึ้นเมื่อหยุดยาที่สงสัยหรือเมื่อให้ยาต้านที่เฉพาะเจาะจง	+1	0	0
4. อาการไม่พึงประสงค์ดังกล่าวเกิดขึ้นอีกเมื่อได้รับยาที่สงสัยเข้าไปใหม่	+2	-1	0
5. อาการไม่พึงประสงค์สามารถเกิดจากสาเหตุอื่นนอกเหนือจากยาที่สงสัย	-1	+2	0
6. อาการไม่พึงประสงค์เกิดขึ้นได้ใหม่เมื่อได้รับยาหลอก	-1	+1	0
7. สามารถตรวจวัดระดับยาในเลือดหรือของเหลวในร่างกายว่ามีความเข้มข้นที่ทำให้เกิดพิษ	+1	0	0
8. อาการไม่พึงประสงค์รุนแรงขึ้นเมื่อเพิ่มขนาดยาหรือลดลงเมื่อลดขนาดยา	+1	0	0
9. ผู้ป่วยเคยเกิดอาการไม่พึงประสงค์เช่นนี้มาแล้วเมื่อได้รับยาในครั้งก่อน	+1	0	0
10. อาการไม่พึงประสงค์นั้นมีหลักฐานที่ได้รับการยืนยันโดยวิธีอันเหมาะสม	+1	0	0
รวมคะแนน			

ผลการประเมิน ☐ ใช่แน่นอน (Definite) > 9 คะแนน ☐ น่าจะใช่ (Probable) 5-8 คะแนน

☐ เป็นไปได้ (Possible) 1-4 คะแนน ☐ ไม่น่าจะใช่ (Doubtful) < 0 คะแนน

APPENDIX B

Patients' data

Table 1 Characteristics of the patients in seizure-uncontrolled group

Patient	Sex	Age	Weight	Dosage form	Albumin	Seizure type
1	F	22.09	43.50	Chrono +Tablet	4.1	CPS c 2nd GTC
2	F	49.02	54.20	Chrono	3.3	CPS
3	F	28.02	46.00	Chrono	4.6	CPS c 2nd GTC
4	F	23.10	74.50	Chrono	4.1	GTC
5	F	49.03	51.50	Chrono	3.3	CPS
6	M	19.03	60.00	Chrono		CPS
7	M	26.02	62.00	Chrono + Tablet		CPS
8	M	27.11	70.00	Chrono	3.9	CPS
9	M	45.02	63.60	Chrono	4.2	GTC
10	F	30.00	86.00	Chrono	3.2	CPS
11	M	16.02	88.00	Chrono	4.1	CPS c 2nd GTC
12	F	34.10	62.00	Chrono	3.1	SPS
13	M	24.09	63.50	Chrono	4.1	GTC
14	M	31.03	64.00	Chrono	4.1	CPS
15	F	17.07	56.00	Tablet (Depakine)	4.0	CPS
Meann SD	-	29.38 ±10.75	62.99 ±12.75	-	**3.85 ±0.44**	-

CPS = complex partial seizures

CPS c 2nd GTC = complex partial seizures

GTC = Generalized tonic clonic seizures

SPS = Simple partial seizures

Table 2 Characteristics of the patients in seizure-controlled group

Patient No.	Sex	Age (year)	Body weight (kg)	Dosage form	Albumin (g/dl)	Seizure type
16	F	15.03	57.00		4.7	CPS c 2nd GTC
17	M	42.11	55.00		4.0	GTC
18	F	42.01	50.00		3.7	GTC
19	M	23.09	73.00		4.2	GTC
20	F	23.09	61.00		3.9	CPS
21	M	39.04	52.00		4.1	GTC
22	F	48.01	46.00		3.7	GTC
23	F	41.10	46.00		3.8	GTC
24	F	30.03	57.00		3.8	SPS
25	F	45.00	55.00		3.5	CPS
26	F	28.09	63.00		3.9	CPS
27	F	33.04	49.00		3.7	GTC
28	M	56.05	75.60		3.8	GTC
29	F	22.01	51.00		4.0	GTC
30	F	18.06	88.00		3.7	GTC
31	F	42.02	75.00		4.1	GTC
32	F	27.11	57.70		3.6	GTC
33	M	18.00	55.00		4.1	GTC
34	F	48.00	56.80		3.9	GTC
35	F	65.02	44.40		3.7	CPS c 2nd GTC
36	F	39.06	62.00		3.3	GTC
37	M	20.08	47.00		4.0	GTC
38	F	15.06	50.00		4.2	ATONIC
39	F	29.04	66.00		4.7	GTC
40	M	19.08	53.00		4.6	ABSENCE
Mean ±SD	-	33.13 ±13.52	57.82 ±10.76	-	3.95 ±0.35	-

Table 3 Demographic data of seizure-uncontrolled groups

Pt No.	EEG	MRI	CT	First seizure age(m)	Frequency (/month)	Seizure duration (min)	ADR	Add on medication	Folic
1	Abnormal	Abnormal	No data	15	6	3	Wt gain	Topiramate	Yes
2	No data	No data	No data	1	1	1	Tremor	Lamotrigine, Clonazepam	No
3	Abnormal	Abnormal	No data	6	8	1	-	Topiramate,Oxcarbazepine	No
4	Normal	Abnormal	Normal	21	1	7	Wt gain	Topiramate	Yes
5	Abnormal	No data	Normal	29	10	2	-	Oxcarbazepine	Yes
6	Abnormal	No data	No data	0.01	1	5	-	-	No
7	No data	No data	No data	3	7	1	-	Oxcarbazepine	Yes
8	Normal	Normal	No data	14	10	1	Wt gain, hair lose	Lamotrigine	Yes
9	No data	Abnormal	No data	42	2	1	-	Gabapantin	No
10	Abnormal	No data	Normal	8	15	1	Wt gain	Oxcarbazepine, Topiramate	No
11	Abnormal	Abnormal	No data	15	2	1	Wt gain	Topiramate	No
12	No data	No data	No data	2	1	2	-	-	Yes
13	No data	No data	No data	6	4	2	-	Gabapantin, Levetiracetam Topiramate	No
14	Abnormal	Abnormal	Abnormal	5	7	5	-	Topiramate, Oxcarbazepine	Yes
15	No data	No data	No data	14	3	3	-	Oxcarbazepine	No

Table 4 Demographic data of seizure-controlled groups

Patient No.	EEG	MRI	CT	First seizure	Frequency (/month)	Seizure duration	ADR	Add on medication	folic
16	Abnormal	Normal	Abnormal	15	5	5	Wt gain, drowsiness	-	No
17	No data	No data	Abnormal	3	1	10	Wt gain	-	No
18	No data	No data	Abnormal	39	2	4	Wt gain tremor	-	No
19	Abnormal	No data	No data	20	3	10	-	-	Yes
20	Abnormal	No data	No data	22	1	5	Wt gain	-	No
21	Normal	No data	Normal	28	2	1	-	-	No
22	Abnormal	Normal	No data	36	1	1	Wt gain	-	No
23	Abnormal	Abnormal	Normal	39	3	10	-	-	Yes
24	No data	No data	No data	25	1	1	-	-	No
25	Abnormal	Normal	Normal	26	1	15	tremor	clonazepam	No
26	Abnormal	No data	No data	27	1	3	-	-	Yes
27	No data	No data	No data	30	1	1	Tremor	-	No
28	No data	No data	No data	60	1	5	-	-	No
29	Abnormal	No data	No data	18	1	1	-	-	No
30	No data	No data	No data	8	1	1	Wt gain	-	No
31	No data	No data	No data	40	3	2	-	-	No
32	No data	No data	Normal	22	1	1	Wt gain	-	No
33	No data	No data	No data	1	1	5	-	-	No
34	Abnormal	No data	No data	13	3	1	Wt gain	-	Yes
35	Abnormal	No data	No data	61	2	1	-	-	Yes
36	Normal	No data	Normal	40	1	2	Wt gain	-	Yes
37	No data	No data	No data	5	3	1	-	-	No
38	No data	No data	No data	10	1	7	-	-	No
39	Normal	Abnormal	Abnormal	26	1	30	-	-	No
40	Abnormal	No data	No data	5	1-2	3	-	-	No

Table 5 Dosage regimen of seizure-uncontrolled group

Patient No.	Dosage regimen		Dose/kg/day
	Chrono 500 mg	Conventional 200 mg	
1	1xhs	1x3	25.29
2	2xhs	-	18.45
3	21/2xhs	-	27.17
4	11/2xhs	-	10.07
5	2-0-11/2	-	33.98
6	2xhs	-	16.67
7	1x2	1x2	22.58
8	3xhs	-	21.43
9	2xhs	-	15.72
10	3xhs	-	17.44
11	3xhs	-	17.05
12	1x2	-	16.13
13	3xhs	-	23.62
14	3xhs	-	23.44
15	-	1x3	10.71
Mean ±SD	-	-	**19.98 ±6.30**

Table 6 Dosage regimen of seizure-controlled group

Patient No.	Dosage regimen		Dose/kg/day
	Chrono 500 mg	Conventional 200 mg	
16	21/2xhs	-	21.93
17	2xhs	-	18.18
18	2xhs	-	20.00
19	2xhs	-	13.70
20	2xhs	-	16.39
21	2xhs	-	19.23
22	11/2xhs	-	16.30
23	1xhs	-	10.87
24	2xhs	-	17.54
25	1x2	-	18.18
26	21/2xhs	-	19.84
27	2xhs	-	20.4
28	1xhs	-	16.61
29	2xhs	-	19.61
30	2xhs	-	11.36
31	2-0-1	-	20.00
32	2xhs	-	17.33
33	2xhs	-	18.18
34	2xhs	-	17.61
35	-	1x3	13.51
36	-	1x2	6.45
37	-	1x2	8.51
38	-	1x2	8.00
39	-	1x2	6.67
40	-	1x2	7.55
Mean ±SD	-	-	**15.36 ±4.85**

APPENDIX C

Calculation Volume of Distribution

1. Patients number 1 who taken depakine 200 mg at 7.00 a.m. , 12.00 a.m., 5 p.m. and depakine chrono 500 mg at 8 p.m.

1) Calculation volume of distribution of total concentration

$$Vd = \frac{FSD_1 (e^{-kt1} + e^{-kt2} + e^{-kt3})}{C\ (1-e^{-k\tau})} + \frac{FSD_2\ (1-e^{-ktinf})\ e^{-k(t-tinf)}}{k\ C\ t_{inf}} \left[\frac{1}{(1-e^{-k\tau})} - \frac{FSD_1 e^{-kt4}}{C}\right]$$

D_1 = 200 mg, D_2 = 500 mg, C = 146.5, k = 0.0266, τ = 24, t_{inf} = 14,
t_1 = 3, t_2 = 22, t_3 = 17, t_4 = 3, F = 1, S = 1

$$Vd = \frac{200 (e^{-0.0266x3} + e^{-0.0266x22} + e^{-0.0266x17})}{146.5\ (1-e^{-0.0266x24})} + \frac{500\ (1-e^{-0.0266x14})\ e^{-0.0266(14-14)}}{0.0266x146.5x\ 14} \left[\frac{1}{(1-e^{-0.0266x24})} - \frac{200\ e^{-0.0266x3}}{146.5}\right]$$

= 10.8996 /43.5 kg

= 0.2506 L/kg

2) Calculation volume of distribution of unbound concentration

$$Vd = \frac{FSD_1 (e^{-kt1} + e^{-kt2} + e^{-kt3})}{C\ (1-e^{-k\tau})} + \frac{FSD_2\ (1-e^{-ktinf})\ e^{-k(t-tinf)}}{k\ C\ t_{inf}} \left[\frac{1}{(1-e^{-k\tau})} - \frac{FSD_1 e^{-kt4}}{C}\right]$$

D_1 = 200 mg, D_2 = 500 mg, C = 30.16, k = 0.0654, τ = 24, t_{inf} = 14,
t_1 = 3, t_2 = 22, t_3 = 17 t_4 = 3 F = 1, S = 1

$$Vd = \frac{200 (e^{-0.0654x3} + e^{-0.0654x22} + e^{-0.0654x17})}{30.16\ (1-e^{-0.0654x24})} + \frac{500\ (1-e^{-0.0654x14})\ e^{-0.0645(14-14)}}{0.0654x30.16x\ 14} \left[\frac{1}{(1-e^{-0.0654x24})} - \frac{200\ e^{-0.0654x3}}{30.16}\right]$$

= 19.8849 /43.5 kg

= 0.4571 L/kg

2.Patients number 5 who taken depakine chrono 1000 mg at8.00 a.m. and 750 at 20.00 p.m.

1) Calculation volume of distribution of total concentration

$$Vd = \frac{FSD_1}{C\,k\,t_{inf}}(1-e^{-ktinf})\,e^{-k(t-tinf)} + \frac{FSD_2}{C\,k\,t_{inf}}(1-e^{-kt'inf}) - \frac{FSD_2}{C\,k\,t_{inf}}(1-e^{-kt'inf})(1-e^{-k\tau}) \Big/ (1-e^{-k\tau})$$

D_1 = 1000 mg, D_2 = 750 mg, C = 85.79, k = 0.0307, τ = 24, t_{inf} = 14, t'_{inf} = 12 t = 24

$$Vd = \frac{1000\,(1-e^{-0.0307x14})\,e^{-0.0307(24-14)}}{85.79x0.0307x14} + \frac{750(1-e^{-0.0307x12})}{85.79x0.0307x14} - \frac{1000\,(1-e^{-0.0307x12})(1-e^{-0.0307x24})}{85.79x0.0307x14} \Big/ (1-e^{-0.0307x24})$$

= 17.0353 /51.5 kg

= 0.3308 L/kg

2) Calculation volume of distribution of unbound concentration

$$Vd = \frac{FSD_1}{C\,k\,t_{inf}}(1-e^{-ktinf})\,e^{-k(t-tinf)} + \frac{FSD_2}{C\,k\,t_{inf}}(1-e^{-kt'inf}) - \frac{FSD_2}{C\,k\,t_{inf}}(1-e^{-kt'inf})(1-e^{-k\tau}) \Big/ (1-e^{-k\tau})$$

D_1 = 1000 mg, D_2 = 750 mg, C = 10.02, k = 0.0278, τ = 24, t_{inf} = 14, t'_{inf} = 12 t = 24

$$Vd = \frac{1000\,(1-e^{-0.0278x14})\,e^{-0.0278(24-14)}}{10.02x0.0278x14} + \frac{750(1-e^{-0.0278x12})}{10.02x0.0278x14} - \frac{1000\,(1-e^{-0.0278x12})(1-e^{-0.0278x24})}{10.02x0.0278x14} \Big/ (1-e^{-0.0278x24})$$

= 167.8899 /51.5 kg

= 3.2599 L/kg

3. Patients number 8 who taken depakine 200 mg at 8.00 a.m. , 20.00 p.m. and depakine chrono 500 mg at 8.00 a.m. , 20.00 p.m.

1) Calculation volume of distribution of total concentration

$$Vd = \frac{D_1}{C}\frac{(e^{-kt1}+e^{-kt2})}{(1-e^{-k\tau})} + \frac{D_2}{k\,C\,t_{inf}}(1-e^{-ktinf})\,e^{-k(t-tinf)}\,\frac{1}{(1-e^{-k\tau})} - \frac{D_1\,e^{-kt3}}{C} - \frac{D_2\,(1-e^{-kt3})}{k\,C\,t_{inf}}$$

D_1 = 200 mg, D_2 = 1000 mg, C = 75.57, k = 0.0371, τ = 24, t_{inf} = 14,

t_1 = 1, t_2 = 13, t_3 = 1

$$Vd = \frac{200\,(e^{-0.0371x1}+e^{-0.0371x13})}{75.57\,(1-e^{-0.0371x24})} + \frac{1000(1-e^{-0.0371x14})e^{-0.0371\,(24-14)}}{0.0371x75.57x\,14\,(1-e^{-0.0371x24})}\,1 - \frac{200\,e^{-0.0371x1}}{75.57} - \frac{1000\,(1-e^{-0.0371x1})}{0.0371x75.57x14}$$

= 15.7006 /62 kg

= 0.2532 L/kg

2) Calculation volume of distribution of unbound concentration

$$Vd = \frac{D_1\,(e^{-kt1}+e^{-kt2})}{C\,(1-e^{-k\tau})} + \frac{D_2\,(1-e^{-ktinf})\,e^{-k(t-tinf)}}{k\,C\,t_{inf}}\,1 - \frac{D_1\,e^{-kt3}}{(1-e^{-k\tau})\,C}$$

D_1 = 200 mg, D_2 = 500 mg, C = 8.13, k = 0.0882, τ = 24, t_{inf} = 14,

t_1 = 1, t_2 = 13, t_3 = 1

$$Vd = \frac{200\,(e^{-0.0882x1}+e^{-0.0882x13})}{8.13\,(1-e^{-0.0882x24})} + \frac{1000\,(1-e^{-0.0882x14})\,e^{-0.0882(24-14)}}{0.0882x8.13x\,14\,(1-e^{-0.0882x24})}\,1 - \frac{200\,e^{-0.0882x1}}{8.13} - \frac{1000\,(1-e^{-0.0882x1})}{0.0882x8.13x14}$$

= 38.8622 /62 kg

= 0.6268 L/kg

4.Patients number13 who taken depakine chrono500 mg at8.00 a.m. and 500 at 20.00 p.m.

1) Calculation volume of distribution of total concentration

$$Vd = \left[\frac{FSD_1\,(1-e^{-ktinf})\,e^{-k(t-tinf)}}{C\,k\,t_{inf}} + \frac{FSD_2\,(1-e^{-kt'inf})}{C\,k\,t_{inf}} - \frac{FSD_2\,(1-e^{-kt'inf})\,(1-e^{-k\tau})}{C\,k\,t_{inf}}\right] \Big/ (1-e^{-k\tau})$$

D_1 = 500 mg, D_2 = 500 mg, C = 58.63, k = 0.0300, τ = 24, t_{inf} = 14,

t'_{inf} = 12 t = 24

$$Vd = \left[\frac{500\,(1-e^{-0.0300x14})\,e^{-0.0300(24-14)}}{58.63x0.0300x14} + \frac{500(1-e^{-0.0300x12})}{58.63x0.0300x14} - \frac{500\,(1-e^{-0.0300x12})\,(1-e^{-0.0300x24})}{58.63x0.0300x14}\right] \Big/ (1-e^{-0.0300x24})$$

= 15.4556 /62 kg

= 0.2493 L/kg

2) Calculation volume of distribution of unbound concentration

$$Vd = \frac{FSD_1 (1-e^{-ktinf})\ e^{-k(t-tinf)}}{C\ k\ t_{inf}} + \frac{FSD_2\ (1-e^{-kt'inf})}{C\ k\ t_{inf}} - \frac{FSD_2\ (1-e^{-kt'inf})\ (1-e^{-k\tau})}{C\ k\ t_{inf}} \Big/ (1-e^{-k\tau})$$

D_1 = 500 mg, D_2 = 500 mg, C = 8.12, k = 0.0828, τ = 24, t_{inf} = 14, t'_{inf} = 12 t = 24

$$Vd = \frac{500\ (1-e^{-0.0828x14})\ e^{-0.0828(24-14)}}{8.12x0.0828x14} + \frac{500(1-e^{-0.0828x12})}{8.12x0.0828x14} - \frac{500\ (1-e^{-0.0828x12})\ (1-e^{-0.0828x24})}{8.12x0.0828x14} \Big/ (1-e^{-0.0828x24})$$

= 23.7738 /62 kg

= 0.3834 L/kg

5. Patients number 23 who taken depakine chrono1000 mg at8.00 a.m. and 500 at 20.00 p.m.

1) Calculation volume of distribution of total concentration

$$Vd = \frac{FSD_1 (1-e^{-ktinf})\ e^{-k(t-tinf)}}{C\ k\ t_{inf}} + \frac{FSD_2\ (1-e^{-kt'inf})}{C\ k\ t_{inf}} - \frac{FSD_2\ (1-e^{-kt'inf})\ (1-e^{-k\tau})}{C\ k\ t_{inf}} \Big/ (1-e^{-k\tau})$$

D_1 = 1000 mg, D_2 = 500 mg, C = 77.26, k = 0.0380, τ = 24, t_{inf} = 14, t'_{inf} = 12 t = 24

$$Vd = \frac{1000\ (1-e^{-0.0380x14})\ e^{-0.0380(24-14)}}{77.26x0.0380x14} + \frac{500(1-e^{-0.0380x12})}{77.26x0.0380x14} - \frac{500\ (1-e^{-0.0380x12})\ (1-e^{-0.0380x24})}{77.26x0.0380x14} \Big/ (1-e^{-0.0380x24})$$

= 14.5140 /75 kg

= 0.1935 L/kg

2) Calculation volume of distribution of unbound concentration

$$Vd = \frac{FSD_1 (1-e^{-ktinf})\ e^{-k(t-tinf)}}{C\ k\ t_{inf}} + \frac{FSD_2\ (1-e^{-kt'inf})}{C\ k\ t_{inf}} - \frac{FSD_2\ (1-e^{-kt'inf})\ (1-e^{-k\tau})}{C\ k\ t_{inf}} \Big/ (1-e^{-k\tau})$$

D_1 = 1000 mg, D_2 = 500 mg, C = 9.88, k = 0.0573, τ = 24, t_{inf} = 14, t'_{inf} = 12 t = 24

$$Vd = \frac{1000\,(1-e^{-0.0573\times14})\,e^{-0.0573(24-14)}}{9.88\times0.0573\times14} + \frac{500(1-e^{-0.0573\times12})}{9.88\times0.0573\times14} - \frac{500\,(1-e^{-0.0573\times12})\,(1-e^{-0.0573\times24})}{9.88\times0.0573\times14} \Big/ (1-e^{-0.0573\times24})$$

= 59.8320 /75 kg

= 0.7978 L/kg

6. Patients number 25 who taken depakine chrono 500 mg at 8.00 a.m. and 500 at 20.00 p.m.

1) Calculation volume of distribution of total concentration

$$Vd = \frac{FSD_1\,(1-e^{-ktinf})\,e^{-k(t-tinf)}}{C\,k\,t_{inf}} + \frac{FSD_2\,(1-e^{-kt'inf})}{C\,k\,t_{inf}} - \frac{FSD_2\,(1-e^{-kt'inf})\,(1-e^{-k\tau})}{C\,k\,t_{inf}} \Big/ (1-e^{-k\tau})$$

D_1 = 500 mg, D_2 = 500 mg, C = 70.43, k = 0.0187, τ = 24, t_{inf} = 14,

t'_{inf} = 12 t = 24

$$Vd = \frac{500\,(1-e^{-0.0187\times14})\,e^{-0.0187(24-14)}}{70.43\times0.0187\times14} + \frac{500(1-e^{-0.0187\times12})}{70.43\times0.0187\times14} - \frac{500\,(1-e^{-0.0187\times12})\,(1-e^{-0.0187\times24})}{70.43\times0.0187\times14} \Big/ (1-e^{-0.0187\times24})$$

= 23.9455 /55 kg

= 0.4354 L/kg

2) Calculation volume of distribution of unbound concentration

$$Vd = \frac{FSD_1\,(1-e^{-ktinf})\,e^{-k(t-tinf)}}{C\,k\,t_{inf}} + \frac{FSD_2\,(1-e^{-kt'inf})}{C\,k\,t_{inf}} - \frac{FSD_2\,(1-e^{-kt'inf})\,(1-e^{-k\tau})}{C\,k\,t_{inf}} \Big/ (1-e^{-k\tau})$$

D_1 = 500 mg, D_2 = 500 mg, C = 10.00, k = 0.0299, τ = 24, t_{inf} = 14,
t'_{inf} = 12 t = 24

$$Vd = \frac{500\,(1-e^{-0.0299\times14})\,e^{-0.0299(24-14)}}{10.00\times0.0299\times14} + \frac{500(1-e^{-0.0299\times12})}{10.00\times0.0299\times14} - \frac{500\,(1-e^{-0.0299\times12})\,(1-e^{-0.0299\times24})}{10.00\times0.0299\times14} \Big/ (1-e^{-0.0299\times24})$$

= 93.4689 /55 kg

= 1.6994 L/kg

APPENDIX D

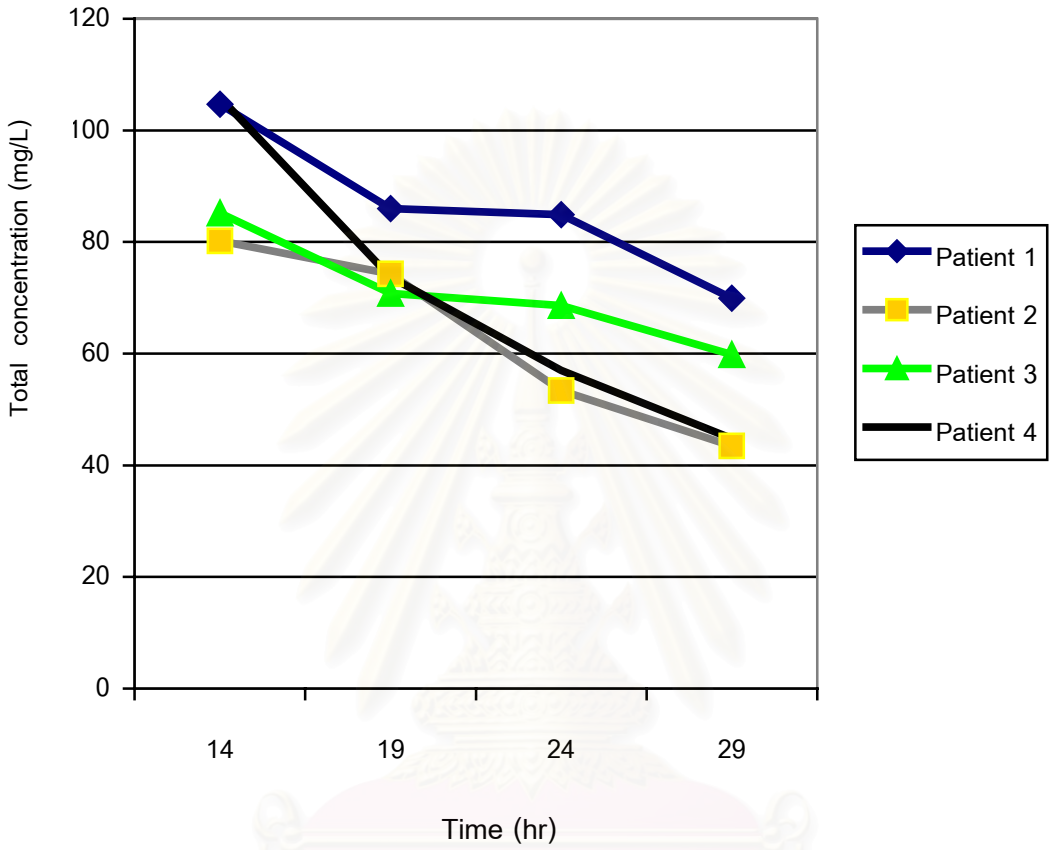

Figure 1 Total concentrations of Depakine chrono obtained from 14th, 19th, 24th and 29th hours

APPENDIX E

Table 1 Compared the mean pharmacokinetic parameters of total VPA concentrations obtained from 14th - 19th hours to 24th - 29th hours in seizure-uncontrolled group

Pharmacokinetic parameters	Total concentrations Mean±SD		*p* value*
	14th hour group (n = 4)	24th hours group (n = 9)	
k (hr^{-1})	0.0359±0.0145	0.0388±0.0105	0.414
$t_{½}$ (hr)	21.3855±6.8793	18.9323±4.5392	0.414
Vd (L/kg)	0.2518±0.1461	0.2471±0.0612	0.825
Cl (L/hr/kg)	0.0078±0.0025	0.0092±0.0017	0.148

* Mann-Whitney U test

Table 2 Compared the mean pharmacokinetic parameters of unbound VPA concentrations obtained from 14th - 19th hours to 24th - 29th hours in seizure-uncontrolled group

Pharmacokinetic parameters	Unbound concentrations Mean±SD		*p* value*
	14th hour group (n = 4)	24th hours group (n = 9)	
k (hr^{-1})	0.0662±0.0107	0.0669±0.0235	0.940
$t_{½}$ (hr)	10.6638±1.5973	12.0654±5.7631	0.940
Vd (L/kg)	0.6803±0.2888	1.5177±1.2897	0.825
Cl (L/hr/kg)	0.0465±0.0268	0.0774±0.0505	0.199

* Mann-Whitney U test

Table 3 Compared the mean pharmacokinetic parameters of total VPA concentrations obtained from 14^{th} - 19^{th} hours to 24^{th} - 29^{th} hours in seizure-controlled group

Pharmacokinetic parameters	Total concentrations Mean±SD		*p* value*
	14^{th} hour group (n = 8)	24^{th} hours group (n = 7)	
k (hr^{-1})	0.0301±0.0108	0.0306±0.0208	0.867
$t_{½}$ (hr)	26.0783±10.1142	33.5016±22.2740	0.867
Vd (L/kg)	0.3380±0.0786	0.3774±0.1362	0.463
Cl (L/hr/kg)	0.0095±0.0021	0.0094±0.0036	0.955

* Mann-Whitney U test

Table 4 Compared the mean pharmacokinetic parameters of unbound VPA concentrations obtained from 14^{th} - 19^{th} hours to 24^{th} - 29^{th} hours in seizure-controlled group

Pharmacokinetic parameters	Unbound concentrations Mean±SD		*p* value*
	14^{th} hour group (n = 8)	24^{th} hours group (n = 7)	
k (hr^{-1})	0.0499±0.0221	0.0645±0.0460	0.536
$t_{½}$ (hr)	26.9821+39.3061	55.9244±116.2303	0.536
Vd (L/kg)	2.7157±3.2170	4.7008±9.7428	0.281
Cl (L/hr/kg)	0.0815±0.0283	0.0667±0.0311	0.463

* Mann-Whitney U test

VITAE

Miss Lakkana Boonmark was born on the 12th of June in 1977 at Chachoengsao. She graduated with Bachelor degree in Pharmacy in 2000 from Faculty of Pharmacy, Chulalongkorn University. Her current position is a pharmacist at Sananchaiket Hospital.