EFFECTS OF DOSAGE, EXCESS WEIGHT AND GENDER ON PHARMACOKINETICS OF SODIUM VALPROATE SUSTAINED RELEASE DOSAGE FORM IN PATIENTS WITH NEURO-PSYCHIATRIC DISORDERS

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A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Pharmaceutical Care
Department of Pharmacy Practice
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2009

ผลของขนาดยา น้ำหนักส่วนเกิน และเพศต่อเภสัชจลนศาสตร์ของยาโซเดียม วาลโปรเอท รูปแบบออกฤทธิ์เนิ่นในผู้ป่วยที่มีความผิดปกติของจิตประสาท

นางสาว ทัดตา ศรีบุญเรือง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรดุษฎีบัณฑิต
สาขาวิชาการบริบาลทางเภสัชกรรม ภาควิชาเภสัชกรรมปฏิบัติ
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2552

Thesis Title	EFFECTS OF DOSAGE, EXCESS WEIGHT AND GENDER ON PHARMACOKINETICS OF SODIUM VALPROATE SUSTAINED RELEASE DOSAGE FORM IN PATIENTS WITH NEURO-PSYCHIATRIC DISORDERS
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ทัดตา ศรีบุญเรือง : ผลของขนาดยา น้ำหนักส่วนเกิน และเพศต่อเภสัชจลนศาสตร์ของยาโซเดียม วาลโปรเอท รูปแบบออกฤทธิ์เนิ่นในผู้ป่วยที่มีความผิดปกติของจิตประสาท. (EFFECTS OF DOSAGE, EXCESS WEIGHT AND GENDER ON PHARMACOKINETICS OF SODIUM VALPROATE SUSTAINED RELEASE DOSAGE FORM IN PATIENTS WITH NEURO-PSYCHIATRIC DISORDERS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. ดร. ดวงจิตต์ พนมวัน ณ อยุธยา, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: นพ. สันติชัย ฉ่ำจิตรชื่น, 104 หน้า.

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของขนาดยา น้ำหนักส่วนเกิน และ เพศ ต่อเภสัชจลนศาสตร์ของโซเดียม วาลโปรเอท ในผู้ป่วยที่มีความผิดปกติทางจิตและประสาท อายุระหว่าง 18-60 ปี โดยมีรูปแบบการศึกษาแบบไปข้างหน้า การศึกษาแบ่งออกเป็น 3 ส่วน ได้แก่ **ส่วนที่1**) ศึกษาเปรียบเทียบ พารามิเตอร์ ทางเภสัชจลนศาสตร์ ของโซเดียม วาลโปรเอท ระหว่างสองขนาดยา (500 มกต่อวัน และ 1000 มก. ต่อวัน) จากผลการศึกษาพบว่าในกลุ่มที่ได้รับยาขนาด 1000 มก.ต่อวัน (จำนวน 38 ราย) ค่าเฉลี่ยของระดับยาน้อยกว่าที่คาดคะเน หรือไม่เป็นสัดส่วนกับขนาดยาที่เพิ่มขึ้น และพบมีอัตราการกำจัดยาเพิ่มขึ้นเมื่อเทียบกับกลุ่มที่ได้รับยา 500 มก.ต่อวัน (จำนวน 28 ราย)ในขณะที่การเพิ่มขึ้นของระดับยาอิสระเป็นสัดส่วนกับขนาดยาและไม่พบความแตกต่างของอัตราการกำจัดยาอิสระระหว่างสองขนาดยาข้างต้นซึ่งผลการศึกษานี้มีทั้งส่วนที่สอดคล้องและแตกต่างกับการศึกษาก่อนหน้า นอกจากนี้ได้นำเสนอช่วงของระดับยาอิสระเพื่อการติดตามระดับยาอิสระในเลือดเป็น 4-12 มก/ลิตร **ส่วนที่** 2) ศึกษาผลของเพศและน้ำหนักส่วนเกินต่อพารามิเตอร์ทางเภสัชจลนศาสตร์ ของโซเดียม วาลโปรเอท ในผู้ป่วยที่มีความผิดปกติทางจิตและประสาทจำนวน 99 ราย พบว่าปัจจัยทั้งสองส่งผลต่อระดับยาและอัตราการกำจัดยาโดยเฉพาะในกลุ่มผู้หญิงที่มีน้ำหนักเกินเกณฑ์ พบอัตราการกำจัดยาลดลงอย่างมีนัยสำคัญ **ส่วนที่** 3) สร้างสมการ เพื่อทำนายพารามิเตอร์ทางเภสัชจลนศาสตร์ ของโซเดียม วาลโปรเอท ได้แก่ ระดับยา และอัตราการกำจัดยาทั้งในรูปแบบยาอิสระและไม่อิสระ ในกลุ่มผู้ป่วยที่มีภาวะน้ำหนักเกินเกณฑ์ (ดัชนีมวลกาย มากกว่าเท่ากับ 25 กิโลกรัมต่อเมตร^2) โดยใช้ตัวแปรอิสระจากลักษณะทั่วไปของผู้ป่วย อันได้แก่ ขนาดยา เพศ อายุ น้ำหนักจริง ส่วนสูง ดัชนีมวลกาย พื้นผิว น้ำหนักกล้ามเนื้อ และระดับอัลบูมิน

ภาควิชา เภสัชกรรมปฏิบัติ ลายมือชื่อนิสิต .....

สาขาวิชา การบริบาลทางเภสัชกรรม ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ปีการศึกษา 2552 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

4576973833 : MAJOR PHARMACEUTICAL CARE

KEYWORDS : SODIUM VALPROATE / PHARMACOKINETICS / DOSAGE / EXCESS WEIGHT / GENDER

TATTA SRIBOONRUANG: EFFECTS OF DOSAGE, EXCESS WEIGHT AND GENDER ON PHARMACOKINETICS OF SODIUM VALPROATE SUSTAINED RELEASE DOSAGE FORM IN PATIENTS WITH NEURO-PSYCHIATRIC DISOREDERS. THESIS ADVISOR: ASSOC. PROF. DUANGCHIT PANOMVANA NA AYUDHYA, Ph.D. THESIS CO-ADVISOR: SANTICHAI CHAMCHITCHUN, M.D., 104 pp.

The purposes of this study were to determine the effects of dosage, excess weight and gender on pharmacokinetics of sodium valproate in patients with neuro-psychiatric disorders age ranged from 18 to 60 years old. The study design was open label, multiple dose, paralleled prospective study. The study could be divided into three parts as follow: First, the impact of different dosages (500 mg/day versus 1000 mg/day) of sustained release formulation on valproate clearance and plasma concentration were studied in psychiatric patients; analysis based on routine therapeutic drug monitoring data. The results have shown both consistent and contrast to previous studies reported. Mean of total concentration increased disproportionately with the increase in dosage, while free concentration increased proportionately. Moreover, higher clearance of total VPA while no difference in clearance of free VPA were found in patients receiving VPA 1000 mg/day as compared to patients receiving 500 mg/day of VPA. Free VPA concentration was proposed to be a more suitable parameter for future therapeutic level monitoring and the targeted level was proposed to be 4-12 mg/l. Second, the effects of gender and excess weight on VPA pharmacokinetics were demonstrated. Female with excess weight showed significantly lower in both clearances of total and free VPA, the effect of excess weight on VPA pharmacokinetics was less obvious in male. Third, predictive equations for total and free VPA concentrations and clearances from general demographic characteristics of patients, such as, dosage, gender, age, body weight, etc. have been generated. These predictive factors account for approximately 60% of the variances.

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ACKNOWLEDGEMENTS

I would like to express my deepest and sincere gratitude to my advisors, Associate Professor Dr. Duangchit Panomvana Na Ayudhya for her supervision, valuable instructions, excellent suggestion, kindness and assistance on the preparation of this dissertation. This accomplishment would not be possible without her help.

I would like to express my sincere gratitude to Mr. Santichai Chamchitchun, co-advisor and psychiatrist, Mrs Orawan Silpakit, neurologist and other physicians who provided supportive information on the clinical outcomes of the patients.

My appreciation is also extended to the staffs of Srithanya hospital, Department of Mental Health, Ministry of Public Health, Thailand including laboratory staffs for their help in specimen collection and laboratory analysis and OPD teams (all nurses, nurse aids, and register officer) for their help in recruitment subjects.

Unforgettably, this dissertation was supported by the grants from graduate school and from the Ph.D. Pharmaceutical care International Program both of Chulalongkorn University. In addition, permission to leave for working on this dissertation granted by Higher Education Committee is also appreciated.

I would also thank my colleagues for great co operation and wonderful relationship. Finally I would like to thanks my lovely family Mr. Rawat Tiasakul, Mr. Son Sriboonruang , Mrs Wanpen Sriboonruang, Mr Songyos Sriboonruang for their warm love, support, encouragement and understanding.

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LIST OF ABBREVIATIONS

% free	Percentage of free drug
ABW	Adjusted body weight
ADRs	Adverse drug reactions
AEDs	Antiepileptic drugs
Alb	Albumin level
ALT	Alanine aminotransferase
AST	Aspartate aminotransterase
BD	Bipolar disorder
BMI	Body mass index
BSA	Body surface area
BW	Body weight or (TBW) total body weight
C_{free}	Free or unbound concentration
CL_{free}	Clearance of free drug
CL_{int}	Intrinsic clearance
CL_{total}	Clearance of total drug
C_{max}	Maximum concentration
C_{peak}	Peak plasma total concentration
CR	Controlled release tablet
CSF	Cerebrospinal fluid
Css	Steady state concentration
C_{total}	Total concentration
C_{trough}	Trough plasma total concentration
D	Dosage
d	Day
dl	Deciliter
EC	Enteric coated formulation
F or BA	Bioavailability

FFA	Free fatty acid
FPIA	Fluorescence polarization immunoassay
Fu	Free fraction or unbound fraction
Ht	Height
hr	Hour
IBW	Ideal body weight
Ka	Absorption rate constant
K or Ke	Elimination rate constant
kg	Kilogram
l	Liter
m	Meter
mg	Milligram
ml	Milliliter
μl	Micro liter
n	Sample size
OB	Obesity
OW	Overweight
S	Salt factor
Scr	Serum creatinine
SR	Sustained release formulation
t_{max}	Time to maximum concentration
τ	Dosing interval
TBW	Total body weight
US FDA	United States Food and drug administration
VPA	Valproate or valproic acid
VPA-Na	Sodium valproate
Wt	Weight

CHAPTER I
INTRODUCTION

1.1 Rationale and significance of the problem

Valproate is the general term for either valproic acid (VPA), sodium valproate (VPA-Na) and semi-sodium valproate, Valproate is branch chain fatty acid with lipophilic properties.[1,2] The antiepileptic properties of valproate were found four decades ago. [2, 3] More recently, several clinical studies have shown the therapeutic effect of VPA in treatment of migraine, bipolar disorder, anxiety including panic disorder, social phobia and posttraumatic stress disorder, and psychotic disorder and alcohol withdrawal-dependence.[2-6] Now a day US FDA has been approved valproate for three indications including epilepsy, bipolar disorder manic phase and migraine prophylaxis. VPA has complete bioavailability (96-100%) from various formulations; syrup, enteric coated tablets, sustained release tablets, sprinkle, injection, etc.[7-9] The rate of absorption depends on formulations. Sustained release tablets will reach plateau of plasma concentration around 10-12 h after ingestion.[10-14] VPA is eliminated almost completely by hepatic metabolism, since it has a low hepatic extraction ratios. The hepatic clearance depends on the free fraction (Fu) and the intrinsic clearance (CL_{int}). The accepted therapeutic range of total VPA steady state concentration for general psychiatric conditions is 45-100 mg/l,[15] although some researchers suggest drug concentrations as high as 125 mg/l in mania.[16] VPA is 78-94% bound to plasma protein mainly with albumin and exhibits concentration dependent degree of binding which are due to the saturate of protein binding in the usual therapeutic range (45-100 mg/l).[16-19] If plasma total VPA concentration (C_{total}) is above 70-80 mg/l, the binding may decrease by approximately 67% which results in higher Fu or percentage of free drug (% free VPA).[16, 19, 20] C_{total} increase less than proportionately after a dosage increase; on the other hand, the unbound steady state drug serum concentration (C_{free}) increases in a proportional fashion.[19, 20] VPA is metabolized by liver through at least 3 main pathways; including glucuronidation 50%, mitochondrial beta oxidation 40% and cytochrome P450

10%.[1, 17, 21] Generally, total VPA clearance (CL_{total}) is 0.4- 0.6 l/h in healthy and 1.0-1.1 l/h in epileptic patients using other AEDs.[1, 17, 19] CL_{total} have been reported to decrease in old age, patients with liver disease and renal impairment, and increase in higher dosage.[17, 18, 22, 23] The increase in Fu and CL_{int} may result in increased CL_{total}. At the same time, this increased Fu is related to higher free drug relevant to clinical effects (both efficacy and toxicity).[1, 22, 24] Most drugs follow linear pharmacokinetics with small variability in Fu throughout the usual therapeutic dosage range; an increase in daily dose should result in increase proportion of C_{total} along with a proportional raising in the amount of free drug.[18] In such cases, therapeutic drug monitoring (TDM) of C_{total} only should be enough for clinical effects evaluation. The pharmacotherapy rule as mentioned may not apply to VPA due to the protein binding saturation over the range of usual dosage (500-3000 mg/d). The measurement of C_{total} only may underestimate the relevant free drug that exerts pharmacological effects when the daily dose is increased. Nevertheless, whether this will cause problems in clinical situations is still controversial. Few previous studies on this issue had been performed or were done among very small sample sizes ($n<10$). The subjects were healthy volunteers and the dosage forms consumed were capsule, enteric coated tablets, and conventional tablets.[19, 20, 22] Part of this study was therefore performed to examine the effect of dosage on clearance of both total and free VPA after consuming sustained release dosage form in psychiatric patients. Whether or not this effect was strong enough to cause impact on total or free VPA therapeutic concentrations and, in turn, on the clinical outcomes was investigated.

Moreover, previous reviews[23, 25, 26] suggested that glucuronidation in male showed higher than in female. Oxazepam and temazepam are examples of drugs which are mainly metabolized through glucuronidation and show higher clearance in male compare to female.[23] Few studies about the impact of gender on VPA pharmacokinetics had been reported. Kodama Y et.al.[27] illustrated that there was no significant difference in Fu between genders. Dutta S et.al.[28] found no differences in VPA pharmacokinetic parameters including AUC, C_{max}, C_{min} between gender. However, information about the effect of gender on VPA pharmacokinetics is still limited. Overweight (OW) and obesity

(OB) are found worldwide and are being a big problem in healthcare system.[29, 30] An expert panel and World health organization (WHO) identified overweight as a body mass index (BMI) of 25-29.9 kg/m^2 and obesity as BMI of 30 kg/m^2 or greater.[25, 31] OW refers to an excess of body weight compare to normal. The excess weight may come from muscle, bone, fat and/or body water. OB refers to having an abnormally high proportion of body fat. As a rule, female has more body fat than male.[26, 32] Most healthcares agree that male with more than 25% body fat and female with more than 30% body fat are consider obese[25, 30] At present, little is known about the influence of OW/OB on drug pharmacokinetics (PK) and pharmacodynamics (PD) since limited studies devoted to it and its PK/PD consequences.[33, 34] There is no systematic relationship between the degree of lipophilicity of lipophilic drug and their distribution or elimination in OW/OB individuals.[34] OW/OB causes numerous changes in pathophysiology that could affect both PK/PD.[29, 30] The adjustment in both loading and maintenance doses may be necessitated. Failure to modified doses in this population may result in either therapeutic failure or toxicity. Another goal of this study was therefore designed to determine the effects of both gender and excess body weight on VPA pharmacokinetics to gain information for more appropriate VPA dosage adjustment in the future.

1.2 Objectives

1.2.1 To determine the effect of dosages on valproate pharmacokinetics in psychiatric patients and to provide the free VPA concentration therapeutic range for monitoring.

1.2.2 To examine the effects of gender and excess weight on valproate pharmacokinetics in neuro-psychiatric patients.

1.2.3 To generate the predictive equations for sodium valproate pharmacokinetic parameters.

1.3 Scope of the study

1.3.1 The population in this study was patients who diagnosed with neuropsychiatric disorders treated with sodium valproate sustained release formulation.

1.3.2 The subjects in this study were patients who diagnosed with neuron-psychiatric disorders treated with sodium valproate sustained release formulation who attended the out patient clinic at Srithanya hospital during the study period.

1.3.3 Variables in this study consist of;

1.3.3.1 Dependent variables: Pharmacokinetic parameters included Concentrations, Clearances of both total and free VPA, Free Fraction, and percentage of free VPA

1.3.3.2 Independent variables:

1.3.3.2.1 Demographic data: age, gender, weight, height, body mass index (BMI), body surface area (BSA), diagnosis, and co-medications

1.3.3.2.2 Chemistry laboratory: Albumin level

1.3.3.2.3 Dosage regimens: Dosages of sodium valproate, frequency of administration

1.3.3.3 Control variables:

1.3.3.3.1 Underlying diseases: congestive heart failure (CHF), ascites, deep vein thrombosis (DVT), nephritic syndrome, untreated hypothyroid, liver dysfunction (AST or ALT>3 TUL), renal insufficiency (SCr >1.5 mg/dl)

1.3.3.3.2 Other drugs affecting sodium valproate pharmacokinetics: protein displacer: aspirin; enzyme inducers: rifampicin, phenytoin, carbamazepine; enzyme inhibitor: azole antifungals.

1.4 Operational definition

Psychiatric disorder:	Any psychiatric diseases or disorders diagnosed follow DSMV–IV or ICD-10 criteria.
Neuropsychiatric disorder:	Any diseases or symptoms that related to neurology or psychiatry such as epilepsy, bipolar disorder etc.
Idea body weight (IBW):	Ideal body weight can be calculated by; IBW (male) = 50 + 2.3 (height in inches over 5 feet), or IBW (female) = 45.5 + 2.3 (height in inches over 5 feet).
Overweight:	BMI$\geq$ 25 to 29.9 kg/m^2
Obese:	BMI$\geq$ 30 kg/m^2
Excess weight	Both of obese and over weight

1.5 Ethic consideration

This study was approved by the Ethical Committee of the Ministry of Public Health, Bangkok, Thailand. This study used the blood samples of patients treated with sodium valproate to analyze plasma sodium valproate concentration both total and free drugs. However, all patients or caregivers included in this study must provide written informed consent voluntarily. Patient's medical information was protected confidentially. The results of this study might be published in scientific journals or presented at medical meeting but the patients would not personally be identified.

1.6 Expected results

1.6.1. To obtain the results that show whether the interested factors i.e., dosage, gender, and excess weight could influence VPA pharmacokinetics in VPA treated patients.

1.6.2. Successful generating predictive equations for VPA concentration and clearance to implement in clinical setting.

CHAPTER II
REVIEW OF LITERATURE

2.1 Sodium valproate general information

2.1.1 History[16, 35]

Valproic acid (VPA) was first synthesized as an organic compound use as solvent for screening of new antiepileptic compounds in laboratory in 1882 by Burton. Pierre Eynard et al discovered its antiepileptic activity by accident and this finding was published was licensed in Europe in 1963. Successful therapy of generalized epilepsies led to approved of VPA in France in 1967 and by United States Food and drug administration (US FDA) in 1983. In 1995 and 1996, it has been approved by US FDA for bipolar mania and migraine, respectively.

2.1.2 Basic chemistry and basic physicochemical properties[35-37]

Valproic acid (VPA) or 2-propylvaleric acid or other rarely use descriptive names including di-n-propylacetic acid and 2-propylpentanoic acid is a simple eight branched chain carboxylic acid with properties of weak acid (pKa 4.95).[37, 38] VPA is a white, odorless deliquescent with solubility 1 in 5 in water and ethanol which is only slightly soluble in water, but highly soluble in organic solvents. It is liquid at room and body temperature with melting point 120-121°C. The partition coefficients of VPA between organic solvents and buffer at 7.4 have been reported as 0.013 for heptane, 0.064 for benzene and 0.21 for chloroform. According to VPA showed high degree of ionization at 7.4 so VPA is much less lipid soluble than any other standard AEDs. This explains why Vd of VPA is so low. However, this physicochemical property could not explain the rapid diffuse of VPA into brain which is thought to be mediated by active transport mechanisms.[36] Generally, there are three salt forms in different preparations that vary in different countries including valproic acid ($C_8H_{16}O_2$, MW=144.2), sodium valproate ($C_8H_{15}NaO_2$, MW=166.2) and semisodium valproate which is known divalproex (combined of 1:1 molar of VPA: VPA-Na) have been developed and formulated . All three chemical structures are illustrated in figure 1.

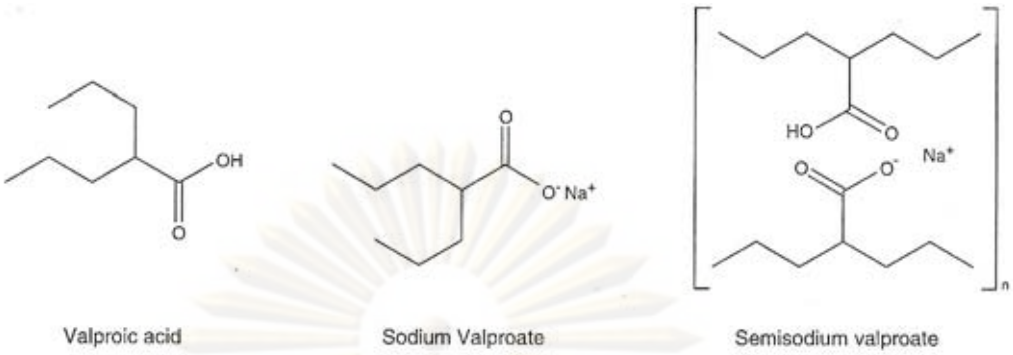

Figure 1 Chemical structure of valproic acid, sodium valproate and semisodium valproate.[6]

2.1.3 Mechanisms of Actions[2, 38-40]

After more than 40 years of clinical use, the mechanisms of action of valproate in epilepsy, bipolar disorder and migraine are still not fully understood. Its mode of action might involve several mechanisms.[41] It has been demonstrated that valproate potentiates gamma-aminobutyric acid inhibitory effects in the central nervous system. In addition, valproate might also act through attenuation of N-methyl-D-aspartate receptor-mediated excitation, although this does not explain the effect of valproate on absence seizures. It has also been proposed that valproate exerts its effect via blockade of voltage dependent sodium channels, although this has not been clearly confirmed. Acting to alter the balance of neuronal inhibition and excitation through more than one mechanism is clearly an advantage for an anticonvulsant and is likely to contribute to its broad spectrum of clinical effects.[2, 16, 36, 40, 42] Beyond the enhancement of gamma-aminobutyric acid-mediated neurotransmission, valproate has been found to affect signaling systems like the Wnt/b-catenin and ERK pathways and to interfere with inositol and arachidonate metabolism. Nevertheless, the clinical relevance of these effects is not always clear. Valproate treatment also produces marked alterations in the expression of multiple genes, many of which are involved in transcription regulation, cell survival, ion homeostasis, cytoskeletal modifications and signal transduction. These alterations may well be relevant to the therapeutic effects of valproate, and result from its enhancement of activator protein-1DNA binding and direct inhibition of histone deacetylases, and possibly additional, yet unknown, mechanism(s). Most likely, both

immediate biochemical and longer-term genomic influences underlie the effects of valproate in all three indications.[2]

2.1.4 Indications [4, 5, 36]

VPA have been approved by US FDA for three indications including epilepsy, bipolar disorder especially manic episodes and migraine. More recently, several clinical studies have shown the therapeutic effects of VPA in treatment of anxiety including panic disorder, social phobia and posttraumatic stress disorder, and psychotic disorder and alcohol withdrawal-dependence.[3, 6]

2.1.5 Compounds and preparations in Thailand[43]

Valproate is general term use to call any salt forms of VPA including sodium valproate, divalproex sodium, magnesium or calcium salt, or valpromide. These forms do not differ significantly. Now a day, there are two salt forms including valproic acid and sodium valproate with 3 different dosage forms including injection, syrup, tablets (enteric coated, controlled release tablets) available in Thailand. . It is available as VPA-Na in 200 mg, 300 mg enteric coated tablets (EC), 200 mg/mL syrup, and 400 mg/vial parenteral preparation for IV injection. Combinations of VPA-Na and VPA in different ratio are found in 200 and 500 mg controlled-release tablets.

Table 1 VPA products available in Thailand in year 2009.[43]

Company	Sanofi-Aventis	Pharmaland	Torrent
Brand name	Depakine Depakine Chrono	Encorate Encorate Chrono	Valparin
Syrup	VPA-Na 200 mg/ml	-	-
Enteric coated tablet (EC)	VPA-Na 200 mg/tab	VPA-Na 200, 300 mg/tab	VPA-Na 200
Controlled release tablet (CR)	VPA-Na 333 mg +VPA 145 mg Equivalent to VPA-Na 500 mg	VPA-Na 133.5 mg +VPA 58 mg Equivalent to VPA-Na 200 mg	-
IV injection	VPA-Na 400 mg/vial	-	-

2.1.6 Dosage and administration[19, 24]

In adult, the usual starting dose is 250 mg/day with a maintenance dose of 500-1500 mg/day (maximum dose 3000 mg/day). For treatment seizure and bipolar disorder[44], the initial dose of VPA is 15 mg/kg/day may increase up to 30 mg/kg/day to obtain target total VPA concentration approximately 50-100 mg/l, nevertheless, few cases may need VPA dose high as 60 mg/kg/day.[45] With a specific guide dose for migraine prevention is 500 mg daily for 7 days followed by 1000 mg daily after. For conventional tablet or enteric coated tablet or syrup, dosing 3 times per day is better tolerated, but twice-daily dosage is usual. For sustained release tablet only once or twice daily could sustain plasma drug level and provide sufficient outcomes. Rapid titration usually is well tolerated. In children, the usual dose is 20 mg/kg/d and the maintenance dose is 40 mg/kg/d. IV VPA should be administered as a 60-minute infusion with a rate not exceeding 20 mg/min.

2.1.7 Clinical Uses

2.1.7.1 Epilepsy[2, 24, 46, 47]

Valproate (VPA) is the drug of choice for primary generalized epilepsies, and is also approved for the treatment of partial seizures. VPA is a potent antiepileptic drug (AED), effective against a wide range of seizure types. It is the drug of choice in idiopathic generalized epilepsy. Open and comparative studies have shown excellent control rates in patients with newly diagnosed typical absence seizure. It is the drug of choice for juvenile myoclonic epilepsy and can be used in other types of myoclonus. Also, it is a first-line drug in photosensitive epilepsy and Lennox-Gastaut syndrome. It is a second choice in the treatment of infantile spasms. In focal epilepsy, VPA has been shown to be as effective as other first-line agents. VPA is one of the most commonly used AEDs around the world. A microdialysis study in humans demonstrated the pharmacokinetic rationale for acute treatment with VPA. It is based on the rapid distribution of VPA to the brain. Recently, Perucca[48]reviewed prospective, randomized, comparative studies of VPA monotherapy and concluded that VPA can be distinguished from other AEDs by its broad spectrum of efficacy against all seizure types.

2.1.7.2 Bipolar disorder[49-52]

Bipolar disorder (BD) is a major medical and social burden, whose cause, pathophysiology and treatment are not agreed on. It is characterized by recurrent periods of mania and depression (Bipolar I) or of hypomania and depression (Bipolar II). Its inheritance is polygenic, with evidence of a neurotransmission imbalance and disease progression. Patients often take multiple agents concurrently, with incomplete therapeutic success, particularly with regard to depression. Suicide is common. Five controlled trials have shown valproate to be efficacious as monotherapy for the short-term treatment of acute bipolar mania.[39, 40, 42, 53, 54] These studies include comparisons of valproate and a placebo in crossover trials without concomitant psychotropics[39, 54], valproate and a placebo in a parallel-group trial in lithium refractory or intolerant patients[42], valproate and lithium in a parallel-group trial[40], and valproate and a placebo and lithium in a parallel-group trial[53]. The last three studies, which enrolled the largest patient samples, allowed as-needed lorazepam at low dosages during the initial week of 3-week trials. Two of these trials[42, 53] led to valproate being the second drug approved by the FDA for the treatment of the manic episodes associated with bipolar disorder.

2.2 Sodium valproate pharmacokinetics

Valproate (VPA) has a complex pharmacokinetics due to the saturation of plasma protein binding occur within therapeutic range (C_{total} 50-100 mg/l).[16, 45] At concentrations more than 75 to 85 mg/L, the albumin binding sites become saturated, resulting in a nonlinear increase in free (unbound) VPA.[16] This can lead to greater pharmacological effect until new steady-state conditions are achieved. Because VPA serum concentrations of up to 150 mg/L are occasionally encountered in psychiatry, this saturation binding phenomenon is likely to to occur. The implication of this phenomenon is a more rapid clearance rate for VPA. This results in disproportionately larger dosage requirements for further increases in serum drug concentrations. VPA pharmacokinetics are reviewed as follow:

2.2.1 Pharmacokinetics of sodium valproate

2.2.1.1 Absorption[7, 16, 55]

The bioavailability (BA) of VPA is almost complete. It is 96 to 100% for all formulations of VPA in common use (conventional tablets, enteric coated tablets, sustained-release tablets, capsules, and oral and intravenous solutions). BA did not differ between healthy and patients with epilepsy receiving other antiepileptic drugs.[56] The rate of absorption, however, depends on the formulation. The time periods required to reach the peak serum concentrations of VPA vary with the formulations. With conventional tablets and solutions peak serum concentrations of VPA are reached at 1 to 2 h after administration, with enteric coated tablets at 3 to 6 h, and with sustained-release tablets at 10 to 12 h. Food could delayed the rate of absorption of VPA but not affected the extents.[55]Diurnal variation in VPA absorption has been observed in healthy receiving VPA enteric coated tablet 400 mg twice daily, higher C_{max} and shorter t_{max} were found after morning compared with evening.[57] Although food and evening administration slowed the absorption , these should be of little consequence in patients with steady state plasma drug concentrations.[58] Under steady state condition, BA of VPA sustained-release tablet and enteric coated tablet were not different; while, sustained release tablet provided more uniform VPA plasma concentration with less fluctuate of C_{peak} and C_{trough}.[59]

2.2.1.2 Distribution[16]

VPA is 85-95% bound to plasma proteins mainly with albumin. The plasma protein binding is concentration dependent and free fraction increased nonlinear with total plasma concentration (C_{total}). Several studies reported that proportion of free drug increased nonlinearly when C_{total} rose above 80-85 mg/l. In addition, free VPA concentration (C_{free}) was observed in epileptic adults receiving monotherapy to be 9.8-18.9 mg/l and in epileptic adults receiving other AEDs as co-medication to be 4.4-12.0 mg/l.[60, 61] Total serum VPA levels affect protein binding such that at serum concentrations less than 75 mg/l, the free fraction ranges from 7-9%. At serum concentrations of 100 mg/L, the free fraction increased to 15%. [17] Free fraction increases at higher levels, in renal and hepatic diseases, in elderly and during

pregnancy.[17] Some other drugs (eg, aspirin, phenylbutazone) displace VPA, but other AEDs do not. The volume of distribution (Vd) is 0.1-0.4 L/kg. VPA reaches the brain by an active transport process that is saturable. Free VPA concentration in plasma approximate those in cerebrospinal fluid (CSF) and tears.[62] In single dose studies, the apparent Vd of VPA ranged from 0.126 to 0.175 l/kg which reflected confinement principally to the circulation and extracellular fluid. Higher Vd 0.2-0.4 l/kg could observed in epileptic patients receiving other AEDs than healthy volunteers, and in children compared to adults.[17] Diurnal variation in protein binding of VPA has been presented and may be explained by fluctuation in levels of free fatty acids (FFA).[63, 64] FFA displace VPA from the binding sites, increasing C_{free}.[63, 65, 66]

2.2.1.3 Metabolism and excretion[21, 67]

VPA is almost completely metabolized in patients, with a very small amount of the parent compound being found in urine only 1-3% of administered dose of VPA.[68] At least three principle metabolic pathways for VPA have been described in humans including glucuronidation, mitochondrial-β-oxidation, and CYP P450 (2C9, 2C19) account for 50%, 40% and 10%, respectively. The metabolic fate of VPA is highly complex and results in the production of at least 50 different metabolites.[37]The excretion of VPA and its metabolites occurs primarily in the urine, with trace amounts in the bile, feces and expired air. Conjugation reactions account for around 50% of VPA metabolism and another 40% can be accounted for through metabolites formed by β-oxidation as shown in figure 2. Conjugation to Δ-glucuronic acid is one of the major routes of VPA biotransformation, but VPA also undergoes conjugation with carnitine, glycine, and coenzyme A as shown in figure 3. β -Oxidation of VPA occurs primarily in the mitochondria of liver cells[69]

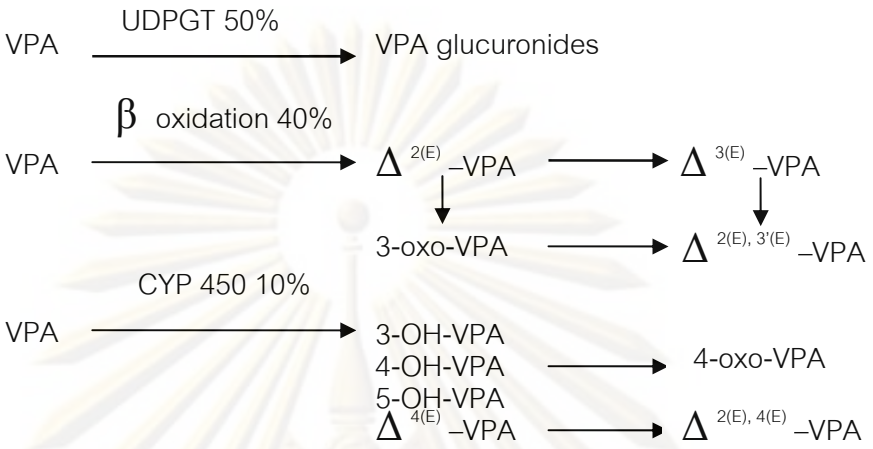

Figure 2 Major metabolic pathways of valproic acid[1]

The main metabolites formed from β -oxidation of VPA include $\Delta^{2(E)}$ –VPA, 3-hydroxyl-VPA, and 3-oxo-VPA. [21, 37] Several of the metabolites formed by β -oxidation exhibit anticonvulsant activity in animals models; however, the brain concentrations of these metabolites are likely too low to produce therapeutic effects. The $\Delta^{2(E)}$ –VPA metabolite is a more potent anticonvulsant and remains in the brain longer than VPA in rodent models; however even with these characteristics it is questionable whether the levels of this metabolite within the CNS are enough to produce therapeutic benefit. VPA also undergoes a variety of oxidation reactions within the endoplasmic reticulum (ER) to produce a variety of metabolites, including (1) hydroxylation products arising from cytochrome P450 metabolism (3-OH-VPA, 4-OH-VPA, 5-OH-VPA), (2) ketones arising from the oxidation of 3-OH-VPA or 4-OH-VPA (3-oxo- VPA, 4-oxo-VPA), and (3) dicarboxylic acids arising from oxidation of 4-OH-VPA and 5-OH-VPA (propylglutaric acid, propylsuccinic acid). A small proportion of VPA (0.3%) undergoes cytochrome P450 desaturation in liver microsomes to produce $\Delta^{4(E)}$ –VPA .

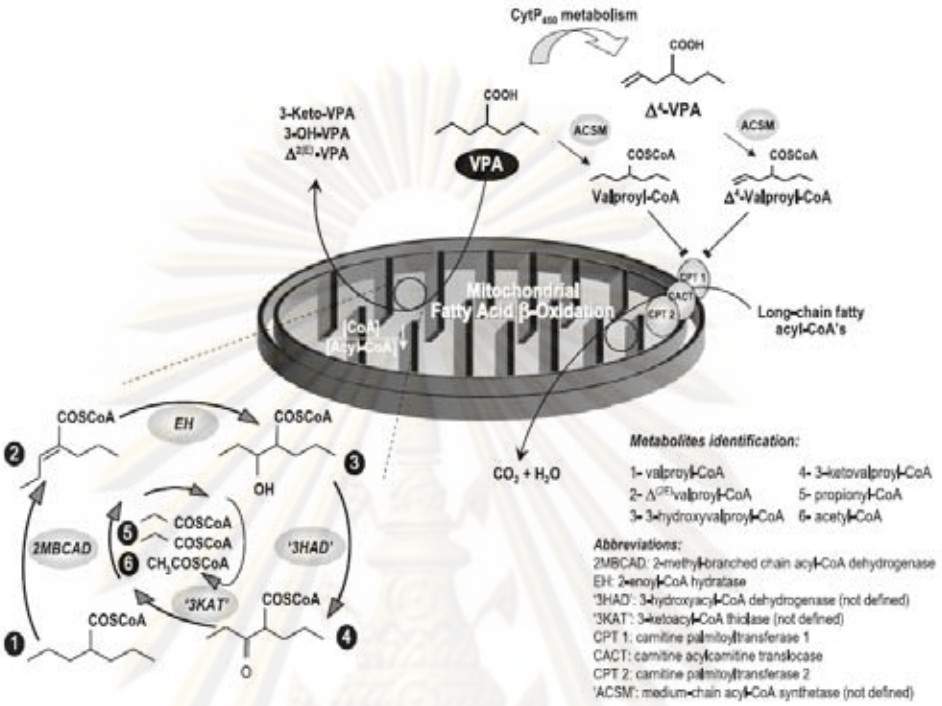

Figure 3. Schematic representation of mitochondrial β -oxidation of valproic acid (VPA) and potential interaction with the carnitine shuttle proteins[21]

Plasma clearance of total VPA (CL_{total}) in healthy volunteers ranged from 0.4 to 0.6 l/h[70] and was independent of hepatic blood flow which due to VPA has low extraction ratio.[7, 56] Higher CL_{total} 1.0 to 1.1 l/h were observed in epileptic patients receiving other concomitant AEDs compare with healthy volunteers.[19, 71] CL_{total} depends on both free fraction (Fu) and intrinsic clearance (CL_{int}) which represent the capacity of hepatic metabolism. For VPA CL_{int} is a clearance of free VPA (CL_{free}) which was approximately 72 to 90 ml/kg/h and decreased at higher dosage.[19] At steady, linear pharmacokinetics (dosage-dependent) has been reported[7, 18, 22], although increased VPA CL_{total} at higher dosages (nonlinear pharmacokinetics) has also been observed[45, 72]. During dosage changes accompanied by proportional changes in plasma concentrations; however, the linearity could be disrupted by the saturation of plasma protein binding at higher dosage, result in an increased in VPA CL_{total}. All VPA pharmacokinetic parameters are summarized in table 2.

Table 2 VPA pharmacokinetic parameters[45]

Pharmacokinetics Parameters	Value
Bioavailability (F)	80-90%: SR tablet 100%: all other forms
Salt factor (S) • VPA-Na • VPA	 0.86 1
Vd, l/kg	0.14 (0.1-0.5) l/kg
CL_{total}, l/hr CL_{total}, ml/kg/h • Children • Adults	0.4-0.6 l/h 13 ml/kg/h 8 ml/kg/h
Half – life, hr (range) • Children • Adults	 6-8 h 10-12 h
Plasma protein binding, %	85-95 % at low concentration decreasing to 70 % with higher dose
Time to reach plateau of SR, h	10-12 h
Time to reach steady-state, days	3-5 days
Therapeutic steady state plasma concentration, µg/ml	50-125
Urinary excretion unchanged, %	1-3%

2.2.2 Factors influence sodium valproate pharmacokinetics

2.2.2.1 Effect of age [73]

In neonates, half life ($t_{1/2}$), Vd and % free VPA are increased compared with in infants and children. Mean $t_{1/2}$ ranged from 30-60 h in untreated neonates born to epileptic mothers, and from 17-40 h in neonates treated with VPA. $t_{1/2}$ decreased in first few week of life. Factors such as age, C_{total}, albumin level, FFA could influence plasma protein binding. In epileptic infants study, %free VPA increased with C_{total}. Higher CL_{total} and lower $t_{1/2}$ were observed in epileptic children aged 2 to 10 years receiving VPA monotherapy or with other AEDs as co medications compared with adults.[74] CL_{total} decreased with increasing in children aged $\geq$ 10 years were similar to those reported in adults.[73] In study of six epileptic elderly patients, two fold prolongation of $t_{1/2}$ with an increase in Vd was observed; however, CL_{total} did not differ from 7 young healthy volunteers.[73] Baurer et.al. observed that mean CL_{free} was

significantly reduced in elderly (60-88 years) compared with healthy volunteers (22-25 years); CL_{free} in morning were 0.064 l/kg/h in elderly vs 0.106 l/kg/h in young healthy adults and were 0.075 l/kg/h in elderly vs 0.123 l/kg/h in young healthy volunteers.[71] One study of 146 elderly patients have reported that daily dose and C_{total} were not different in patients receiving inhibitory or inducing co-medications, between men and women, or by albumin level and CL_{total} was similar between men and women, among elderly age groups.[75]

2.2.2.2 Effect of gender[32, 76]

The Food and Drug Administration (FDA) reviewed 300 new drug applications between 1995 and 2000. Of the 163 that included a sex analysis, 11 drugs showed a 40% difference in pharmacokinetics between males and females, which was listed on the product label, yet no dosing recommendations were made based on sex. Female sex has been shown to be a risk factor for clinically relevant adverse drug reactions. Would simply dosing females based on their different pharmacokinetics decrease the incidence of adverse events? The answer is not known. Sex-dependent pharmacodynamic effects have been identified. The role of pharmacokinetics vs. pharmacodynamics is unclear, as is the impact of pharmacogenetics on both.[46] Some highlight review in gender based different in pharmacokinetics are focused as below:

On average, men are larger than women. Body size differences results in larger distribution volumes and faster total clearance of most medications in men compared to women. Greater body fat in women (until older ages) may increase distribution volumes for lipophilic drugs in women. Total drug absorption does not appear to be significantly affected by sex although absorption rates may be slightly slower in women. Bioavailability after oral drug dosing, for CYP3A substrates in particular, may be somewhat higher in women compared to men. For hepatic processes, drugs metabolized by Phase I metabolism (oxidation, reduction, and hydrolysis via cytochrome P450's 1A, 2D6, 2E1), Phase II conjugative metabolism (glucuronidation, conjugation, glucuronyltransferases, methyltransferases, dehydrogenases) and by combined oxidative and conjugation processes are usually

cleared faster in men compared to women (mg/kg basis). Metabolism by CYP2C9, CYP2C19, and N-acetyltransferase, appear to be similar in men and women (mg/kg). Clearance of p-glycoprotein substrates appear to be similar in men and women. In contrast, total clearance of a number of CYP3A substrates appear to be mildly or moderately faster (mg/kg) in women compared to men. The clinical significance of reported differences warrants consideration. Clearance reported on a per kg basis directly addresses organ or enzyme clearance. The difference in size between men and women means translating these results to clinical dosage rates should include an adjustment for body size. Unfortunately, this is not standard. Reports of sex differences that persist after considering weight may warrant further dosage adjustments. In addition, investigations are often performed in healthy fasting individuals yet medications are prescribed to patients with confounding influences of disease, co-medications, diet, and social habits.[32, 76]

For VPA, limited studies devoted to this aspect. One study showed the results that age, but not gender, had significant influences on the binding characteristics of VPA to serum proteins in epileptic adult patients.[27] Another one study by Dutta et.al have reported that gender did not have a significant influence on VPA pharmacokinetics following semisodium valproate and semisodium valproate ER administration after accounting for dose, differences in body weight.[28]

2.2.2.3 Effect of excess weight

Weight gain is a well-known adverse effect of valproic acid (VPA) treatment; it is reported in 57-71% of adults with epilepsy.[77, 78] None of study investigated the influences of excess weight on VPA pharmacokinetics and none of study shows the prevalence of excess weight in patients treated with VPA in Thailand.

Obese people have larger absolute lean body masses as well as fat masses than non-obese individuals of the same age, gender and height. However, the percentage of fat per kg of total bodyweight (TBW) is markedly increased, whereas that of lean tissue is reduced. Cardiac performance and adipose tissue blood flow may be altered in obesity. There is uncertainty about the binding of drugs to plasma proteins

in obese patients. Some data suggest that the activities of hepatic CYP P450 isoforms are altered, but no clear overview of drug hepatic metabolism in obesity is currently available. There is no systematic relationship between the degree of lipophilicity of markedly lipophilic drugs and their distribution in obese individuals. The distribution of a drug between fat and lean tissues may influence its pharmacokinetics in obese patients. Thus, the loading dose should be adjusted to the TBW or IBW, according to data from studies carried out in obese individuals. Adjustment of the maintenance dosage depends on the observed modifications in clearance.

Obesity is defined as an excess of fat tissue compared with normal values for age and gender, but the way data are expressed may differ from one study to another. Thus, standard definitions of excess bodyweight have been proposed. The most frequently used method was initially the ideal bodyweight (IBW), with a formula taking height and gender into account; an individual was said to be obese when the actual bodyweight exceeded the IBW by more than 20%. The international recommended classification of obesity recently published by the WHO[31] is based on the body mass index (BMI), calculated as bodyweight (in kg) divided by the square of the height in meters as shown in table 3. Overweight is defined as a BMI $\geq$25 to 29.9 kg/m2 and obesity as a BMI $\geq$30 kg/m2. Obesity is divided into 3 classes: moderate (BMI 30.0 to 34.9), severe (BMI 35.0 to 39.9) and morbid (BMI $\geq$ 40.0).

Table 3 Classification of BMI according to WHO and Asian guideline.[30, 79]

Categories	Risk of co-morbidities	BMI (WHO/NIH)	BMI (Asian)
Underweight	Low	<18.5	<18.5
Normal weight	Average	18.5-24.9	18.5-22.9
Overweight	Increase	25-29.9	23-24.9
Obesity class I	Moderate	30-34.9	25-29.9
Obesity class II	Severe	35-39.9	≥30
Obesity class III	Very severe	≥40	

Previous studies[79] have recently been carried out to collect information on trends in the prevalence of overweight and obesity in Thailand, as classified by the WHO and

Asian.[31]In 1991, the first report on National Health Examination Survey of Thailand[80] was conducted in 13,300 adults, aged > 20 years. The results revealed that 12% of men and 19.5% of women (total 16.7%) had BMI 25-30, whereas 1.7% of men and 5.6% of women (total 4.0%) had BMI >30. In 1997, the second report on National Health Examination Survey of Thailand[81] was conducted by the Ministry of Public Health as shown in table 4.

Table 4 Percentage of BMI in Thai adults in1997[81]

BMI	Female (%)	Male (%)	Total (%)
$\geq$ 25	33.9	19.2	28.3
$\geq$ 30	8.8	3.5	6.8

2.2.2.4 Effect of hypoalbuminemia[82, 83]

The protein binding of VPA is not linear. As documented in previously published studies, the risk of supratherapeutic and toxic levels of unbound VPA is higher in the presence of hypoalbuminemia[84, 85] Therefore, whole serum VPA levels may be unreliable when a low serum albumin level is present. Albumin is the most abundant plasma protein produced by hepatocytes. Rate of production is dependent on several factors, including supply of amino acids, plasma oncotic pressure, levels of inhibitory cytokines (particularly IL-6), and number of functioning hepatocytes. The half-life of plasma albumin is normally about 19-21 days. Plasma albumin concentrations are low in neonates, typically 28 to 44 g/L (2.8-4.4 g/dL). By the first week of life, adult values of 37 to 50 g/L (3.7-5.0 g/dL) are reached, rising to 45-54 g/L (4.5-5.4 g/dL) by age 6 and remaining at these concentrations through young adulthood before declining to typical adult values. There is no significant difference in reference limits between males and females. Increased albumin is typically due to hemoconcentration, caused either by dehydration, prolonged tourniquet use during collection, or specimen evaporation. The main causes for decreased albumin include protein loss (nephrotic syndrome, burns, protein losing enteropathy), increased albumin turnover (catabolic states, glucocorticoids), decreased protein intake (malnutrition, very low protein diets),

and liver disease. Plasma albumin is seldom decreased in acute hepatitis, due to its long half-life, but in chronic hepatitis albumin gradually falls with progression to cirrhosis. Albumin concentrations are a marker of decompensation and prognosis in cirrhosis.[83]

2.3 Adverse events of sodium valproate

Even though VPA has been used for many years, large controlled and blinded studies to determine the frequency of adverse effects have not been conducted. Based on clinical experience, dose-related adverse effects include nausea, vomiting (mainly during initiation of therapy and improved by administration of enteric-coated preparations), tremor, sedation, confusion or irritability, and weight gain.[77, 78] Metabolic effects from interference in mitochondrial metabolism include hypocarnitinemia, hyperglycinemia, and hyperammonemia.[86, 87] Severe sedation or even coma may result from hyperammonemia, typically with normal liver function tests. Patients with an underlying urea cycle enzyme defect may become encephalopathic from acute hyperammonemia, which may be fatal occasionally.[88-90] Hair loss or curling of hair may occur, which improved when the patients used baby shampoo and a multivitamin supplement.[91] VPA has adverse endocrine effects, including insulin resistance and change in sex hormone levels causing anovulatory cycles, amenorrhea, and polycystic ovary syndrome.[92] Bone marrow suppression with neutropenia and allergic rashes are rare.[93, 94] Acute pancreatitis is rare but potentially fatal and usually reverses after withdrawal of VPA.[95-97] The most serious idiosyncratic adverse effect is hepatotoxicity.[98, 99] This is observed mainly in patients younger than 2 years and with polytherapy.[100]

2.4 Drug-drug interactions of sodium valproate

Valproic acid (valproate, divalproex sodium) is a weak inhibitor of CYP2C9. Concomitant administration of valproate with drugs that are primarily metabolized by CYP2C9 (eg, phenobarbital, phenytoin, warfarin) should be monitored closely, particularly with narrow therapeutic ratio drugs, which may result in significant drug interactions. Concomitant administration of valproate and phenobarbital has been associated with higher serum levels of phenobarbital.[101-104] Subsequent mechanistic

studies have demonstrated that valproate inhibits the 2 major pathways of phenobarbital metabolism—CYP2C9 and N-glucosidation.[105] Serum levels of phenobarbital are typically reported to increase by 35% to 81%[106, 107] and phenobarbital clearance to decrease by 28% to 39%[108] with concomitant valproate administration. The prescribing information for valproate recommends that all patients receiving concomitant barbiturate therapy should be evaluated for neurological toxicity and monitored for serum barbiturate concentrations frequently. Reducing phenobarbital dosage 20% to 30% when introducing of valproate has also been recommended. The relationship between valproate and phenytoin levels is complex and time-dependent. Introducing valproate to patients stabilized on phenytoin has been associated with a transient decrease in total plasma phenytoin levels[102, 109] and elevations in free phenytoin levels.[110] Withdrawing valproate was associated with a decrease in the concentration of unbound phenytoin.[111] The fluctuations in bound and unbound phenytoin levels can be explained by a transient displacement of phenytoin from protein binding sites accompanied by a decrease in phenytoin metabolism due to inhibition of CYP2C9 by valproate. Breakthrough seizures occurring with the combination of phenytoin and valproate have been reported. Therefore, the dosage of phenytoin should be adjusted as required by the clinical situation. Patient management may best be guided by monitoring unbound phenytoin levels. Valproate has been reported to increase the international normalized ratio (INR) when administered concomitantly with warfarin.[112] Because warfarin is a narrow therapeutic ratio drug and its active enantiomer is metabolized by CYP2C9, INRs should be monitored closely when introducing and withdrawing valproate. Although CYP2C9 has many more substrates, such as non-steroidal anti-inflammatory drugs (NSAIDS), angiotensin II receptor blockers, sulfonylureas, and cyclooxygenase (COX)-2 inhibitors, the substrates would not be expected to have clinically significant interactions with valproate, because valproate is a weak inhibitor of CYP2C9. Valproic acid inhibited CYP3A4 in human liver microsomes, but only at concentrations higher than would be used during therapeutic treatment. with valproate. *In vivo*, valproate does not inhibit known substrates of CYP3A4, including oral contraceptives,[113]cyclosporine[114] and

tiagabine. Concomitant administration of valproic acid and nimodipine has been reported to increase nimodipine levels by 54% in epileptic patients as compared to drug-free controls.[115] It is known that nimodipine is metabolized by CYP3A4; however, *in vitro* and animal data suggest that nimodipine also undergoes glucuronidation. Therefore, it seems likely that the mechanism of nimodipine inhibition is via glucuronidation rather than CYP3A4. Clinically significant interactions between valproate and CYP3A4 substrates, such as cyclosporine, tacrolimus, sirolimus, sildenafil, atorvastatin, simvastatin, estradiol, alfentanil, eplerenone, or finasteride, would not be expected.

CHAPTER III
METHODOLOGY

3.1 Conceptual framework

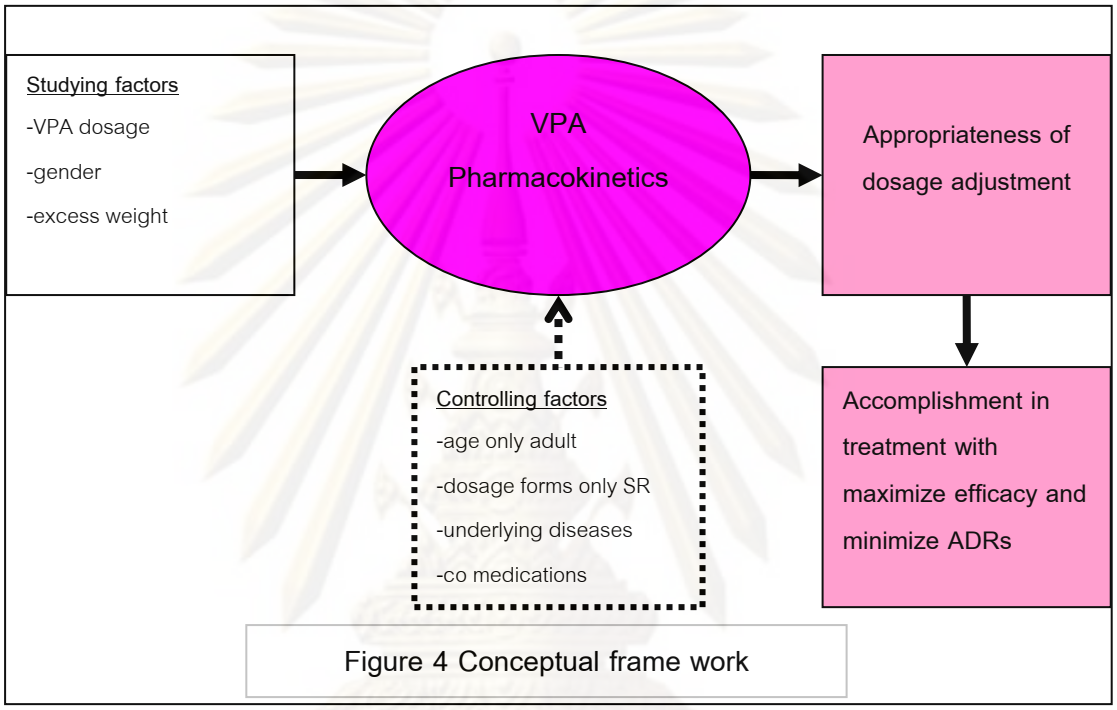

Figure 4 Conceptual frame work

From the frame work, this study focus on the influence of three main factors on VPA pharmacokinetics including VPA dosage, gender, and excess weight which are tested separately using appropriate statistics in data analysis. Besides the inclusion criteria, this study try to control others variables which may confound the results such as age (only adults age range 18 to 60 year-old), dosage forms (only SR) etc. Due to it is cross-sectional study, many factors could not be controlled; however, they will be tested for the balance or same base line characteristic before the other process begin. Starting with collecting all VPA treated patients who are willing to participate in this study. Ending with separate data analysis according to the objectives, so the power of test will be checked after instead of calculation of sample size at the beginning.

3.2 Populations: Neuro-psychiatric disorder receiving VPA-Na SR is the population that want to generalize the results of the study.

3.3 Place of study: Srithanya Hospital, Department of Mental Health, Ministry of Health, Nonthaburi Province, Thailand

3.4 Duration of study: 2006-2009

3.5 Study design: The study was an open-label, multiple-dose, parallel prospective study. The study protocol was approved by the ethical committee review board of the Thai Ministry of Public Health.

3.6 Subjects: All neuro-psychiatric patients, receiving VPA SR to control their seizure or moods, who came for follow up at Srithanya Hospital, Nonthaburi Province, Thailand, were recruited into the study. Eligible patients included both men and women with age ranging between 18 to 60 years who had been on VPA sustained release dosage form at a stable dose for at least 4 weeks. They were not receiving any concurrent medications known to affect VPA metabolism, had no clinically significant hepatic and renal impairment. Compliance was determined by interviewing the patient and/ or the caregiver. Informed written consent was obtained from each patient.

3.7 Sample collection: At steady state condition, after consuming the current stable dosage regimen of VPA for at least 4 weeks, a single 5 ml venous blood sample was obtained by venipuncture from the forearm into plain-tube with aseptic technique and immediately centrifuged for plasma separation. Analysis for the plasma VPA concentrations were performed within the same day. Blood samples were mostly obtained at trough or at longer than 10 hr after drug administration (the recommended time for general drug level monitoring). However, a few samples had been collected earlier than 10 hr after drug administration due to patient and/ or clinical convenience.

3.8 Bioanalytical methods: The blood sample was immediately centrifuged at 3000 rpm for 10 minutes for plasma separation. Concentrations of both total and free VPA in plasma (C_{total} and C_{free}) were determined by a fluorescence polarization immunoassay

(FPIA) using the TDx analyzer (Abbott laboratories, Abbott Park, IL, USA), according to the instruction of manufacturer. The lowest measurable concentration of total VPA that could be distinguished from zero with 95% confidence level was 0.7 mg/l. Precision was determined using total VPA control concentrations of 37.5, 75, and 125 mg/l; they all yielded coefficients of variation of < 5%, and the average recoveries were 100.7 ± 2.2 %. In addition, the lowest measurable concentration of free VPA that could be distinguished from zero with 95% confidence interval was 0.1 mg/l. Precision was determined using free VPA control concentrations of 4, 12, and 20 mg/l; they all yielded coefficients of variation of < 5%, and the average recoveries were 100.6 ± 1.9 %. Separation of Free VPA in plasma was done by ultra-filtration Centrifree® YM30 micro-partition devices with regenerated cellulose membrane 300,000 MWCO (Millipore Corporation, MA, USA) Lot no. L6DN3591B. Approximately 1 ml of plasma was transferred into the reservoir of the device and then was centrifuged with fixed angle at room temperature at 3000 rpm for 10 minutes. A plasma water sample (filtrate) 100-200 μl was collected from the cup and then analyzed.

3.9 **Pharmacokinetic analysis**: At steady state, after consuming VPA-Na sustained release formulation and VPA concentrations in plasma had been determined, both CL_{total} and CL_{free} could be calculated by applying the continuous infusion one-compartment with first-order elimination model as shown below:

$$CL = \frac{SFD}{\tau C_{SS}}$$

where:

CL = Valproate clearance, l/h

S = Salt factor of VPA-Na (0.86)

F = Bioavailability (1)

D = Valproate dosage, mg

Css = Steady state concentration of valproate (C_{total} or C_{free}), mg/l

τ = Dosing interval, hr

VPA dosage, concentration and clearance were also normalized for the patient's weight to the units of mg/kg/day, (mg/l of concentration)/(mg/kg of VPA dose) and ml/kg/h, respectively.

3.10 Adverse drug reactions (ADRs) assessment

To assess ADRs, patients and/or caregivers were questioned about any complaints or symptoms as in the ADR checklists which were interviewed directly by pharmacist at visit day. Type of ADRs, date, dose of drug at onset of the ADR, severity of ADR were recorded and summarized.

3.11 Data analysis 1: study of the impact of dosage of sustained release formulation on valproate clearance and plasma concentration in psychiatric patients: analysis based on routine therapeutic drug monitoring data.

Patients: Psychiatric patients receiving VPA SR to control their moods who came for follow up at Srithanya Hospital, Nonthaburi Province, Thailand, were recruited into the study. Eligible patients included both men and women age ranging between 18 to 60 years who had been on VPA sustained release dosage form at a stable dose for at least 4 weeks. They were prescribed with either 500 mg/day or 1000 mg/day once or twice daily as a suitable dose to control their psychiatric condition. They were not receiving

any concurrent medications known to affect VPA metabolism, had no clinically significant hepatic and renal impairment. Compliance was determined by interviewing the patient and/ or the caregiver. Informed written consent was obtained from each patient

Statistical analysis

Characteristics of patients and pharmacokinetic parameters between the two dosage groups were compared using Chi-square test and independent t-test where appropriate. The effects of dosing intervals (once daily versus twice daily for the 1000 mg/day group) and different sampling times (earlier than 10 hr versus equal/longer than 10 hr after drug administration) on the observed VPA concentrations, the consequently calculated VPA clearances, Fu and %free were tested using two-way analysis of variance (2-way ANOVA). If no differences were observed, then, the data could be pooled. Regression and correlation analysis between CL_{total}, CL_{free} and VPA dosage were generated using SPSS version 17 software (SPSS Inc., Chicago. IL, USA.)

3.12 Data analysis 2: study of the influences of both gender and overweight/obese on valproate pharmacokinetics.

Patients

All patients receiving VPA-Na sustained release (SR) oral dosage form to control their symptoms who came to follow up at Srithanya hospital, Nonthaburi province, Thailand, were recruited into the study. Eligible patients included both female and male, age ranged between 18 to 60 years, who had been on VPA sustained release dosage form at a stable dose for at least 4 weeks. They were not receiving any co-medications known to affect VPA metabolism, had no clinically significant hepatic and renal impairment. Compliance was determined by interviewing the patient and/ or the caregiver. Informed written consent was obtained from each patient or the caregiver.

Statistical analysis

Demograpic data and pharmacokinetic parameters between the two gender groups and two BMI groups were compared using Chi-square test and independent t-test where appropriate. The effects of gender and excess body weight (different BMI groups) on

VPA pharmacokinetics were analyzed both separately and together using independent t-test or Mann-Whitney U test and two way ANOVA which were performed using SPSS version 17 software (SPSS Inc., Chicago. IL, USA.).

3.13 Data analysis 3 prediction of valproate concentration and clearance in adults patients with excess weight.

Patients

Excess weight patients including overweight/obese receiving VPA-Na sustained release (SR) oral dosage form to control their symptoms who came to follow up at Srithanya hospital, Nonthaburi province, Thailand, were recruited into the study. Eligible patients included both female and male, age ranged between 18 to 60 years, who had been on VPA sustained release dosage form at a stable dose for at least 4 weeks. They were not receiving any co-medications known to either inhibit or induce VPA metabolism, had no clinically significant hepatic and renal impairment. Compliance was determined by interviewing the patient and/ or the caregiver. Informed written consent was obtained from each patient or the caregiver.

Statistical analysis

Demograpic data, clinical characteristic and pharmacokinetic parameters of patients were reported as mean ± SD. Correlation and multiple regression analysis were used to generate predictive equations for VPA concentration and clearance which were performed using SPSS version 17 software (SPSS Inc., Chicago. IL, USA.).

CHAPTER IV
RESULTS

4.1 Analysis1 : study of the impact of dosage of sustained release formulation on valproate clearance and plasma concentration in psychiatric patients: analysis based on routine therapeutic drug monitoring data

Abstract

Objective

To compare valproate (VPA) pharmacokinetic parameters between the two dosages of sustained-release formulation (SR) in psychiatric patients.

Methods

Psychiatric patients who have received 500 mg/day or 1000 mg/day of VPA SR at least 4 weeks were recruited into the study. Blood samples were collected and analyzed for total and free VPA concentrations. Pharmacokinetic parameters were then calculated and compared between the two dosage groups using independent t-test. Correlation and regression analysis of VPA clearance and dosage were generated.

Results

Sixty-six psychiatric patients (21 females and 45 males), with an age ranging between 18 to 60 years, were recruited. Twenty-eight patients received 500 mg/day and 38 patients received 1000 mg/day VPA SR. The increment in C_{total} per each mg/kg increment in dosage was decreased when the CL_{total} increased with the increasing dose; while, the increment in C_{free} and CL_{free} were not significant difference. Due to the saturation of protein binding, %free was significantly increased when the dose was increased.

Conclusions

An increase in VPA dose resulted in less than proportional increase in C_{total} while a proportional increase between dosage and C_{free} still existed. Therefore, our study proposed therapeutic range of C_{free} to be 4-12 mg/l based on the therapeutic range of C_{total} (45-100 mg/l)

Results

A total 66 psychiatric patients were recruited in the study. Twenty-eight patients received 500 mg/day and 38 patients received 1000 mg/day of VPA. Demographic data of all patients (21 females and 45 males) were collected. Age ranged from 18 to 60 years as summarized in Table 5. The mean age, weight, height, BMI and albumin levels were not significantly different between the two dosage groups. The 1000 mg/day group was further divided according to dosing interval into two subgroups of 1000 mg once daily (n= 13) versus 500 mg twice daily (n=25). In addition, the 1000 mg/day group was also categorized based on the sampling time into two subgroups (earlier than 10 hr (n=11) and equal/longer than 10 hr (n=27) after drug administration). Two way ANOVA showed that different dosing interval and different sampling time did not cause any significant difference in the concentrations, free fractions, or percentages of free and clearances of VPA obtained from different subgroups ($P>0.05$). The variances of each group were not statistically significantly different analyzed by using Levene's test ($p> 0.05$) as shown in Table 6. The data of the subgroups could thus be pooled into one group of 1000 mg/day. Table 7 illustrates pharmacokinetic parameters comparisons between two dosages. The increment in C_{total} per each mg/kg increase in dosage was significantly decreased in the higher dosage group. While for C_{free} , the increase in C_{free} was not significantly different between the two dosage groups. The mean CL_{total} was significantly elevated 46.26% from 6.15±2.62 to 8.99±3.11 ml/kg/h when the dosage was increased from7.65 to 15.50 mg/kg/day ($p=0.000$). A significant relationship was found between CL_{total} and VPA dose as shown in Figure 5. On the other hand, there was no significant difference in the mean CL_{free} between the two different dosage groups; the mean±SD were 80.39±41.53 ml/kg/h and 92.22± 47.57 ml/kg/h for 500 mg /day and 1000 mg/day, respectively. No relationship between CL_{free} and VPA dose was found as shown in Figure 6. The mean percentage of free VPA increased statistically significantly from 8.28±3.00 % to 10.71±2.88% when the dosage was increased from 500 mg/day to 1000 mg/day ($P<0.05$).

Table 8 presents C_{free} in each therapeutic category. The percentage of patients whose plasma VPA levels were within, higher and lower than the proposed therapeutic range (45-100 mg/l) were 74.24%, 4.55% and 21.21%, respectively. Based on mean, SD and range of the data obtained, a slight adjustment to the therapeutic range for C_{free} was proposed to be 4-12 mg/l as equivalent to the previously proposed therapeutic range for C_{total} as 45-100 mg/l.

The five most common adverse events (AEs) were gastrointestinal AEs (10.61%), weight gain (43.93%), hair loss (9.09%), tremor (34.85%) and sedation (50.00%). These adverse events tended to occur more often when the C_{total} or C_{free} increased, however, they were not statistically significantly different at $p < 0.05$ except for tremor which showed significantly higher occurrence in the higher C_{free} group ($p < 0.05$) as presented in table 9.

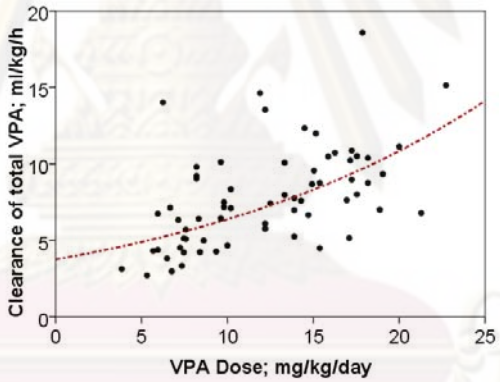

Figure 5 Non linear relationship between CL_{total} versus VPA dose

Model fit with exponential: $Y=3.758 \bullet e^{(0.053\ X)}$; $r = 0.585$; $r^2=0.342$ (p-value =0.000)

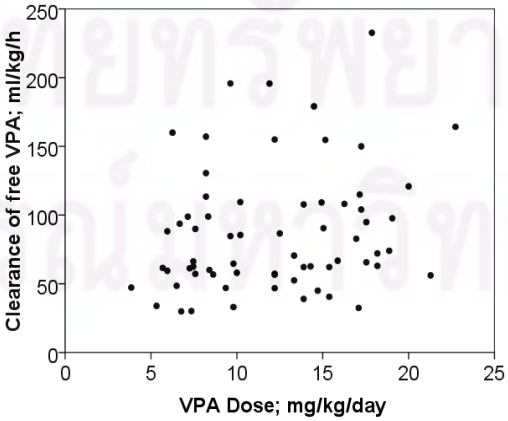

Figure 6 No relationship between CL_{free} versus VPA dose

Table 5 Demographic data of 66 psychiatric patients.

Demographic data	VPA 500 mg/day (n=28)	VPA 1000 mg/day (n=38)	p-value
	Mean ± SD (Range)	Mean ± SD (Range)	
Age; years	40.43 ± 10.94 (19-60)	39.05 ± 11.46 (18-56)	0.625[a]
Weight; kg	68.62 ± 17.22 (49-130)	67.06 ± 13.96 (44-109)	0.685[a]
Height; cm	163.21 ± 8.49 (150-179)	162.37 ± 6.82 (150-180)	0.655[a]
BMI; kg/m^2	25.63 ± 5.08 (15.47-40.57)	25.42 ± 5.01 (18.36-41.80)	0.867[a]
Albumin; gm/dl	4.49 ± 0.33 (3.70-5.10)	4.31 ± 0.39 (3.60-4.90)	0.053[a]
	Count (% of total)	Count (% of total)	p-value
Gender (n=66)			0.264[b]
Female (n=21)	11 (16.7)	10 (15.2)	
Male (n=45)	17 (25.8)	28 (42.4)	
Diagnosis (n=66)			0.146[b]
Bipolar disorder (n=31)	15 (22.7)	16 (24.2)	
Depression (n=3)	2 (3.0)	1 (1.5)	
Schizophrenia or Schizoaffective disorder (n=28)	9 (13.6)	19 (28.8)	
Psychosis (n=2)	2 (3.0)	0 (0.0)	
Others (n=2)	0 (0.0)	2 (3.0)	
Coffee consumer (n=25)	16 (24.2)	9 (13.6)	0.006[b**]
Smoking (n=25)	9 (13.6)	16 (24.2)	0.410[b]
Frequency of dosing			0.000[b**]
Once daily (n=41)	28 (42.4)	13 (19.7)	
Twice daily (n=25)	0 (0.0)	25 (37.9)	
Sampling time at			0.002[b**]
Before 10 hr after administration (n=11)	0 (0.0)	11 (16.7)	
After 10 hr after administration (n=55)	28 (42.4)	27 (40.9)	
Therapeutic status			0.014[b**]
Sub-therapeutics (total VPA <45 mg/l) (n=14)	10 (15.2)	4 (6.1)	
Therapeutics (total VPA 45-100 mg/l) (n=49)	18 (27.3)	31 (47.0)	
Over-therapeutics (total VPA >100 mg/l) (n=3)	0 (0.0)	3 (4.5)	
Co-medications			
Antipsychotics (n=37)	2 (31.8)	35 (53.0)	0.055[b]
Antidepressants (n=11)	5 (7.6)	6 (9.1)	1.000[b]
Anticholinergics (n=51)	20 (30.3)	31 (47.0)	0.331[b]
Mood stabilizers (n=15)	10 (15.2)	5 (7.6)	0.031[b**]
Propranolol (n=11)	1 (1.5)	10 (15.2)	0.014[b**]
Benzodiazepines (n=40)	15 (22.7)	25 (37.9)	0.315[b]

[a] Independent t-test [b] Chi square test

Table 6 The influence of dosing interval (OD vs BID) and sampling time (earlier than 10 hr vs equal/longer than 10 hr after drug administration) on concentration, clearance,% free of VPA in 1000 mg/ day (n=38).

Factors / Variables	Once daily	Twice daily		Two-way ANOVA p-value		Levene's test p-value
	Sampling time ≥ 10 hr after administration (n=13)	Sampling time < 10 hr after administration (n=11)	Sampling time ≥ 10 hr after administration (n=14)			
	Mean±SD (Range)	Mean±SD (Range)	Mean±SD (Range)	Factor1	Factor2	
VPA Concentration						
Total VPA concentration; C_{total}						
C_{total}; mg/l	69.52 ± 25.11 (29.18-122.77)	69.46 ± 12.49 (53.51-96.52)	63.32 ± 23.57 (32.28-118.90)	0.461	0.486	0.284
C_{total}; (mg/l)/(mg/kg)	4.57 ± 1.51 (2.45-7.98)	4.71 ± 1.52 (2.37-8.39)	4.14 ± 1.56 (1.93-6.96)	0.469	0.362	0.787
Free VPA concentration; C_{free}						
C_{free}; mg/l	7.99 ± 3.51 (2.18-13.60)	6.81 ± 2.17 (4.07-10.34)	7.34 ± 4.40 (2.75-18.92)	0.642	0.713	0.401
C_{free}; (mg/l)/(mg/kg)	0.53 ± 0.22 (0.18-0.88)	0.45 ± 0.16 (0.22-0.76)	0.48 ± 0.27 (0.15-1.11)	0.554	0.795	0.275
VPA protein binding						
Free fraction (Fu)	0.11 ± 0.04 (0.07-0.19)	0.10 ± 0.02 (0.07-0.14)	0.11 ± 0.03 (0.07-0.16)	0.632	0.293	0.273
Percentage of free VPA (%free)	11.41 ± 3.51 (7.25-19.22)	9.67 ± 2.00 (7.21-13.90)	10.89 ± 2.76 (6.89-15.91)	0.634	0.299	0.279
VPA clearance						
Total VPA clearance; CL_{total}						
CL_{total}; l/h	0.58 ± 0.23 (0.29-1.23)	0.53 ± 0.09 (0.37-0.67)	0.64 ± 0.23 (0.30-1.11)	0.475	0.194	0.160
CL_{total}; ml/kg/h	8.62 ± 2.72 (4.49-14.62)	8.35 ± 2.825 (4.27-15.14)	9.84 ± 3.65 (5.15-18.58)	0.318	0.245	0.563
Free VPA clearance; CL_{free}						
CL_{free}; l/h	5.73 ± 3.70 (2.63-16.44)	5.76 ± 1.78 (3.47-8.80)	6.64 ± 3.81 (1.89-13.03)	0.482	0.516	0.155
CL_{free}; ml/kg/h	84.81 ± 46.87 (40.54-195.68)	89.06 ± 33.48 (46.90-164.19)	101.59 ± 58.25 (32.38-101.59)	0.373	0.524	0.202

Factor1= dosing interval Factor2= sampling time

Table 7 Comparisons of clearance and concentration of VPA between the two standard doses (n=66).

Variables	VPA 500 mg/day (n=28)	VPA 1000 mg/day (n=38)	p-value
	Mean± SD (Range)	Mean± SD (Range)	
VPA Dose ; mg/kg/day	7.65 ± 1.61 (3.85-10.20)	15.50 ± 3.00 (9.35-22.73)	0.000 [a]**
VPA Concentration			
Total VPA concentration; C_{total}			
C_{total}; mg/l	50.09 ± 16.31 (15.98-81.25)	67.22 ± 21.23 (29.18-122.77)	0.001 [a]**
C_{total}; (mg/l)/(mg/kg)	6.84 ± 2.75 (2.56-13.22)	4.45 ± 1.51 (1.93-8.39)	0.000 [a]**
Free VPA concentration; C_{free}			
C_{free}; mg/l	4.22 ± 2.18 (1.40-10.63)	7.41 ± 3.51 (2.18-18.92)	0.000 [a]**
C_{free}; (mg/l)/(mg/kg)	0.56 ± 0.28 (0.18-1.20)	0.49 ± 0.22 (0.15-1.11)	0.214 [a]
VPA Protein Binding [a]			
Free fraction (Fu) [b]	0.08 ± 0.03 (0.05-0.22)	0.11 ± 0.03 (0.07-0.19)	0.002 [a]** 0.000 [b]**
Percentage of free VPA (%free)	8.28 ± 2.99 (5.17-21.72)	10.71 ± 2.88 (6.89-19.22)	0.001 [a]**
VPA Clearance			
Total VPA clearance; CL_{total}			
CL_{total}; l/h	0.41 ± 0.18 (0.22-1.12)	0.59 ± 0.20 (0.29-1.23)	0.000 [a]**
CL_{total}; ml/kg/h	6.15 ± 2.62 (2.71-14.01)	8.99 ± 3.11 (4.27-18.58)	0.000 [a]**
Free VPA clearance; CL_{free}			
CL_{free}; l/h	5.33 ± 2.61 (1.69-12.80)	6.08 ± 3.25 (1.89-16.44)	0.312 [a]
CL_{free}; ml/kg/h	80.39 ± 41.53 (29.85-195.77)	92.22 ± 47.57 (32.38-232.68)	0.296 [a]

[a] Independent t-test [b] Mann Whitney U test

Table 8 Free VPA concentration (Mean±SD (Range)) and therapeutic category.

	Therapeutic category		
	Sub therapeutics (C_{total} < 45 mg/l) (n=14)	Therapeutics (C_{total}= 45-100 mg/l) (n=49)	Over therapeutics (C_{total}>100 mg/l) (n=3)
Free VPA concentration; C_{free}	2.45 ±0.52 (1.40-3.34)	6.52 ±2.38 (3.02-12.78)	15.37 ±3.07 (13.60-18.92)

Table 9 Free VPA concentration and ADRs. Value in table are : Count (% within free VPA concentration group)

Adverse drug reaction (ADRs)	Free VPA concentration			Exact test p-value
	<4 mg/l (n=19)	4-12 mg/l (n= 43)	>12 mg/l (n=4)	
GI ADRS (n=7)	1 (5.3%)	5 (11.6%)	1 (25.0%)	0.527
Weight gain (n=29)	7 (36.8%)	19 (44.2%)	3 (75.0%)	0.418
Hair loss (n=6)	1 (5.3%)	4 (9.3%)	1 (25.0%)	0.474
Tremor (n=23)	4 (21.1%)	15 (34.9%)	4 (100.0%)	0.009**
Sedation (n=33)	8 (42.1%)	22 (51.2%)	3 (75.0%)	0.512

4.2 Analysis 2: study of the influences of both gender and overweight/obese on valproate pharmacokinetics.

Abstract

Valproate (VPA) has low hepatic extraction ratio, its hepatic clearance depends on free fraction (Fu) and intrinsic clearance (CL_{int}). Gender and overweight/obese (OW/OB) may affect either Fu or CL_{int}. This study is designed to study the impacts of gender and overweight/obese on VPA pharmacokinetics in patients consuming VPA sustained-release formulation (SR).

Objectives

To compare pharmacokinetic parameters of both total and free VPA between different gender (female and male) and different body mass index (BMI) groups (BMI <25 kg/m^2 vs BMI$\geq$ 25 kg/m^2).

Methods

Ninety nine patients (35 females and 64 males; age ranged 18 to 60 years) treated with stable dosage of VPA SR for at least 4 weeks were recruited into the study. Five ml of blood sample was colleted and immediately centrifuged for plasma separation. Separation of free VPA was performed using Centrifree® devices. Total and free VPA concentrations (C_{total} and C_{free}) were determined by TDx analyzer with fluorescence polarization immunoassay (FPIA) technique. The clearances of both total and free VPA (CL_{total} and CL_{free}) were calculated and compared between genders and BMI groups using independent t-test or Mann-Whitney U test where appropriate. Pharmacokinetic parameters were further categorized and compare between different BMI group within the same gender using appropriate statistics. Analyses were performed using SPSS version 17.

Results

Males showed statistically significantly higher CL_{total} (l/h) and CL_{free} (l/h) as compared to female; however, when these values were standardized to bodyweight (BW), the differences became none statistically significant. As a consequence, C_{total} (mg/l) was significantly lower in male. Statistically significantly lower mean CL_{total} (ml/kg/h) and CL_{free} (ml/kg/h) were found in female with BMI$\geq$ 25 kg/m^2 (n=12) as compared to females with

BMI<25 kg/m^2 (n=23) while mean Fu or %free was not significantly different. Each mg/kg increased in the dosage of VPA resulted in higher increase in both C_{total} and C_{free} in OW/OB than in normal weight females. Mean CL_{total} (ml/kg/h) and CL_{free} (ml/kg/h) were slightly lower in male with BMI$\geq$ 25 kg/m^2 (n=29) compared to male with BMI<25 kg/m^2 (n=35) but these differences did not reach the statistically significant levels while Fu or %free was also not significantly different. The results obtained imply that dosage adjustment in OW/OB female should be carefully considered.

Conclusion:

OW/OB female showed statistically significantly lower in both CL_{total} (ml/kg/h) and CL_{free} (ml/kg/h) of VPA than normal weight female, accordingly, each mg/kg increased in the dosage of VPA resulted in higher increase in both C_{total} (mg/l)/(mg/kg) and C_{free}(mg/l)/(mg/kg). The influences of OW/OB on VPA CL_{total} (ml/kg/h) and CL_{free} (ml/kg/h) were less obvious in male and did not reach statistically significant level.

Results

Gender effect

A total ninety-nine patients were recruited into the study, 35 patients were female and 64 patients were male. Demographic data of all patients were summarized in table 10. Mean age and BMI were not significantly different between the two gender groups; however, the means of weight, height and albumin level were significantly higher in male group. General characteristics were similar between the two groups except for cigarette smoking and some co-medications using. Number of smoker, antipsychotics and anticholinergic users were significant higher in male group compare to female group; nevertheless, they should not affect the interesting results in this study since they were metabolized through different pathway from VPA. As presented in table 12A, VPA plasma levels of all 99 patients were classified according to C_{total} to be approximately 66.7%, 26.3% and 7.1% in the therapeutic, subtherapeutic and supratherapeutic categories, respectively. While the dosage consumed were not significantly different (table 11A), male showed higher percentage of patients whose plasma level were in subtherapeutic while female showed higher percentage of patients whose plasma levels were in supratherapeutic as well as higher percentage in all common ADRs (table 12A). Both C_{total} (mg/l) and C_{free} (mg/l) showed somewhat higher mean concentrations in female than in male which the difference in C_{total} only was at the level of statistically significant. However, when C_{total} (mg/l) and C_{free} (mg/l) were standardized to each mg/kg of dosage given, no significant difference between gender of either C_{total} (mg/l)/(mg/kg) and C_{free} (mg/l)/(mg/kg) could be found (table 11A). Consequently, the mean CL_{total} (l/h) and CL_{free} (l/h) in female were significantly lower than male. Nevertheless, after corrected for body weight, CL_{total} (ml/kg/h) and CL_{free} (ml/kg/h) were not different. Fu and % free VPA were not significantly different between genders even though significant difference in albumin levels were found between female and male.

Overweight/obese effect

The prevalence of BMI$\geq$25-29.9 kg/m^2 and BMI$\geq$30 kg/$m^2$of patients using VPA SR in this study were approximately 27.3 % and 14.1%, respectively. Table 10 presents

demographics of patients categorized into two different BMI groups (BMI <25 kg/m^2 (G1) vs BMI≥25 kg/m^2 (G2)) and table 11B shows the impact of excess weight on VPA pharmacokinetics. No differences in the general demographic characteristics were found between the different BMI groups excepted for the albumin level which was higher in OW/OB group but still with in the normal range. VPA plasma protein binding focus on Fu and % free drug were not significantly different between the two BMI groups. VPA doses (mg/day) were not differ between the two BMI groups but VPA dose (mg/kg/day) was lower in higher BMI group, given C_{total} (mg/l) and C_{free} (mg/l) were not statistically significant different while C_{total} and C_{free} (mg/l)/(mg/kg) which were standardized for each mg/kg of VPA dose were statistically significantly different between BMI groups. Markedly increase in C_{total} and C_{free} for each mg/kg increase in VPA dose were found in OW/OB patients. CL_{total} and CL_{free} (l/h) were not significantly different between different BMI groups; however, when standardized for body weight CL_{total} and CL_{free} (ml/kg/h) were significantly lower in OW/OB patients.

Gender and overweight/obese effects

The effects of both gender and excess weight (OW/OB) were considered together by further categorized the patients into 4 subgroups. Demographics (table10) and pharmacokinetic parameters between different BMI groups for each gender were separately compared (table 11C). Most characteristics in each subgroups were comparable. Few baseline factors were found to be different, such as, higher percentage of antidepressant user in female with BMI ≥ 25 kg/m^2 and higher albumin level in male with BMI ≥ 25 kg/m^2. However, these differences should not cause impact on the interested results since antidepressants were metabolized through different pathways from VPA and even though albumin levels were significantly difference, the values were still with in normal range.

In female group (F1vsF2), the statistical significant differences in pharmacokinetic parameters including C_{total} (mg/l)/(mg/kg), C_{free} (mg/l)/(mg/kg), CL_{total} (ml/kg/h) and CL_{free} (ml/kg/h) were found between patients with different BMI. In male group (M1 vs M2),

VPA dose (mg/kg/day) was found to be statistically significantly different between different BMI subgroups. Even though higher C_{total} (mg/l)/(mg/kg), C_{free} (mg/l)/(mg/kg) and lower CL_{total} (ml/kg/h) and CL_{free} (ml/kg/h) were found in male with BMI $\geq$ 25 kg/m^2 but these did not reach statistically significant levels. In normal weight group (F1 vs M1), characteristics such as smoking and weight were significant higher in male (M1) compare to female (F1); however, none of pharmacokinetic parameters as shown in table 11C were significant differences. In OW/OB group (F2 vs M2), weight, height and albumin in OW/OB male (M2) were found significantly higher than in OW/OB female (F2). Significant lower C_{total} (mg/l), higher CL_{total} (l/h) and higher CL_{free} (l/h) were found in OW/OB male (M2) compared to OW/OB female (F2).

Clinical impacts

Based on the proposed therapeutic range of C_{total} (mg/l) of 50-100 mg/l, higher percentage of patients with supratherapeutic VPA levels were found in female while higher percentage of patients with subtherapeutic VPA levels were found in male. The significant association of therapeutic category and gender were found (table 12A). The association of therapeutic category and BMI group were not found (table 12B). Additionally, higher percentage of patients with supratherapeutic levels were found in OW/OB females (table 12C) but these findings were not reaching the statistically significant levels.

Five most common adverse events (AEs) found were gastrointestinal AEs (10.1%), weight gain (38.4%), hair loss (8.1%), tremor (34.3%) and sedation (49.5%). These adverse events were not statistically significantly different between gender and different BMI groups except for hair loss which showed significantly higher occurrence in female group ($P<0.05$) as presented in table 12A.

Table 10 Demographic data of 99 patients with neuropsychiatric disorders. (n=99)

Demographic data	Gender						BMI groups		p-value of comparison between					
	Female (n=35)			Male (n=64)										
	F1: BMI<25 kg/m^2 (n=23)	F2: BMI≥ 25 kg/m^2 (n=12)	F3: Total female (n=35)	M1: BMI<25 kg/m^2 (n=35)	M2: BMI≥ 25 kg/m^2 (n=29)	M3: Total male(n=64)	G1: BMI<25 kg/m^2(n=58)	G2: BMI≥ 25 kg/m^2(n=41)	F1 vs F2	M1 vs M2	F3 vs M3	G1 Vs G2	F1 vs M1	F2 vs M2
Age; years	40.70±13.31 (22-60)	43.50±7.31 (34-59)	41.66 ± 11.56 (22-60)	36.09±9.83 (19-54)	40.17±10.69 (18-56)	37.94 ± 10.35 (18-56)	37.91 ± 11.45 (19-60)	41.15 ± 9.85 (18-59)	NS[a]	NS[a]	NS[a]	NS[b]	NS[a]	NS[a]
Height; cm	155.83±4.96 (150-165)	155.75±4.54 (150-162)	155.80 ± 4.75 (150-165)	165.63±5.80 (155-180)	165.34±6.80 (151-180)	165.50 ± 6.22 (151-180)	161.74 ± 7.28 (150-180)	162.54 ± 7.59 (151-180)	NS[a]	NS[b]	S[a]**	NS[b]	S[b]**	S[b]**
Weight; kg	52.88±6.77 (43.00-65.00)	68.43±7.44 (59.20-87.00)	58.21 ± 10.18 (43.00-87.00)	60.40±7.63 (45.00-75.00)	82.86±15.00 (64.00-130.00)	70.58 ± 16.08 (45.00-130.00)	57.42 ± 8.13 (43.00-75.00)	78.64 ± 14.73 (59.00-130.00)	S[b]**	S[b]**	S[a]**	S[b]**	S[a]**	S[b]**
BMI; kg/m^2	21.74±2.19 (17.90-24.14)	28.23±3.01 (25.15-36.21)	23.96 ± 3.98 (17.90-36.21)	22.01±2.45 (15.47-24.96)	30.20±4.32 (25.00-41.80)	25.72 ± 5.33 (15.47-41.80)	21.90 ± 2.34 (15.47-24.96)	29.62 ± 4.05 (25.00-41.80)	S[a]**	S[b]**	NS[a]	S[b]**	NS[a]	NS[b]
Albumin; gm/dl	4.29±0.29 (3.70-4.80)	4.26±0.32 (3.70-4.80)	4.28 ± 0.30 (3.70-4.80)	4.36±0.41 (3.60-5.30)	4.63±0.30 (3.90-5.30)	4.48 ± 0.38 (3.60-5.30)	4.33 ± 0.37 (3.60-5.30)	4.52 ± 0.35 (3.70-5.30)	NS[a]	S[a]**	S[a]**	S[b]**	NS[a]	S[a]**
Diagnosis														
Bipolar disorder	13 (56.5%)	6 (50.0%)	19 (54.3%)	12 (34.3%)	15 (51.7%)	27 (42.2%)	25 (43.1%)	21 (51.2%)						
Depression	0 (0.0%)	2 (16.7%)	2 (5.7%)	2 (5.7%)	0 (0.0%)	2 (3.1%)	2 (3.4%)	2 (4.9%)						
Schizophrenia or Schizoaffective disorder	5 (21.7%)	3 (25.0%)	8 (22.9%)	16 (45.7%)	13 (44.8%)	29 (45.3%)	21 (36.2%)	16 (39.0%)	NS[d]	NS[d]	NS[d]	NS[d]	NS[d]	NS[d]
Psychosis	1 (4.3%)	1 (8.3%)	2 (5.7%)	1 (2.9%)	0 (0.0%)	1 (1.6%)	2 (3.4%)	1 (2.4%)						
Epilepsy	1 (4.3%)	0 (0.0%)	1 (2.9%)	3 (8.6%)	0 (0.0%)	3 (4.7%)	4 (6.9%)	0 (0.0%)						
Others	3 (13.0%)	0 (0.0%)	3 (8.6%)	1 (2.9%)	1 (3.4%)	2 (3.1%)	4 (6.9%)	1 (2.4%)						
Coffee consumer	9 (39.%)	4 (33.3%)	13 (37.1%)	9 (25.7%)	9 (31.0%)	18 (28.1%)	18 (31.0%)	13 (31.7%)	NS[d]	NS[c]	NS[c]	NS[c]	NS[c]	NS[d]
Smoking	1 (4.3%)	0 (0.0%)	1 (2.9%)	20 (57.1%)	11 (37.9%)	31 (48.4%)	21 (36.2%)	11 (26.8%)	NS[d]	NS[c]	S[c]**	NS[c]	S[c]**	S[d]*
Co-medications														
Antipsychotics	14 (60.9%)	7 (58.3%)	21 (60.0%)	27 (77.1%)	25 (86.2%)	52 (81.2%)	41 (70.7%)	32 (78.0%)	NS[d]	NS[c]	S[c]*	NS[c]	NS[c]	NS[d]
Antidepressants	2 (8.7%)	5 (41.7%)	7 (20.0%)	7 (20.0%)	5 (17.2%)	12 (18.8%)	9 (15.5%)	10 (24.4%)	S[d]*	NS[c]	NS[c]	NS[c]	NS[d]	NS[d]
Anticholinergics	15 (65.2%)	6 (50.0%)	21 (60.0%)	28 (80.0%)	24 (82.8%)	52 (81.2%)	43 (74.1%)	30 (73.2%)	NS[d]	NS[c]	S[c]*	NS[c]	NS[c]	NS[d]
Mood stabilizers	6 (26.1%)	5 (41.7%)	11 (31.4%)	3 (8.6%)	8 (27.6%)	11 (17.2%)	9 (15.5%)	13 (31.7%)	NS[d]	NS[d]	NS[c]	NS[c]	NS[d]	NS[d]
Propranolol	3 (13.0%)	2 (16.7%)	5 (14.3%)	3 (8.6%)	7 (24.1%)	10 (15.6%)	6 (10.3%)	9 (22.0%)	NS[d]	NS[d]	NS[c]	NS[c]	NS[d]	NS[d]
Benzodiazepines	17 (73.9%)	7 (58.3%)	24 (68.6%)	20 (57.1%)	21 (72.4%)	41 (64.1%)	37 (63.8%)	28 (68.3%)	NS[d]	NS[c]	NS[c]	NS[c]	NS[c]	NS[d]
VPA Dose														
< 1000 mg/day (n=51)	13 (56.5%)	7 (58.3%)	20 (57.1%)	17 (48.6%)	14 (48.3%)	31 (48.4%)	30 (51.7%)	21 (51.2%)	NS[c]	NS[c]	NS[c]	NS[c]	NS[c]	NS[c]
≥ 1000 mg/day (n=48)	10 (43.5%)	5 (41.7%)	15 (42.9%)	18 (51.4%)	15 (51.7%)	33 (51.6%)	28 (48.3%)	20 (48.8%)						
Frequency of dosing														
Once daily	12 (52.2%)	9 (75.0%)	21 (60.0%)	19 (54.3%)	14 (48.3%)	33 (51.6%)	31 (53.4%)	23 (56.1%)						
Twice daily	8 (34.8%)	2 (16.7%)	10 (28.6%)	14 (40.0%)	14 (48.3%)	28 (43.8%)	22 (37.9%)	16 (39.0%)	NS[d]	NS[d]	NS[d]	NS[d]	NS[d]	NS[d]
Thrice daily	3 (13.0%)	1 (8.3%)	4 (11.4%)	2 (5.7%)	0 (0.0%)	2 (3.1%)	5 (8.6%)	1 (2.4%)						
Four times daily	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (3.4%)	1 (1.6%)	0 (0.0%)	1 (2.4%)						
Sampling time after drug administration at														
<10 hr	7 (30.4%)	1 (8.3%)	8 (22.9%)	5 (14.3%)	7 (24.1%)	12 (18.8%)	12 (20.7%)	8 (19.5%)	NS[d]	NS[c]	NS[c]	NS[c]	NS[d]	NS[d]
≥ 10 hr	16 (69.6%)	11 (91.7%)	27 (77.1%)	30 (85.7%)	22 (75.9%)	52 (81.2%)	46 (79.3%)	33 (80.5%)						

Values in table are mean±SD (min-max) or count (percentage within column)., [a] Independent t-test, [b] Mann Whitney U test, [c] Chi square test, [d] Exact test; * Statistical significant difference with p-value< 0.05, ** Statistical significant difference with p-value< 0.01, S-statistically significant difference; NS-not statistically significant difference.

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Table 11A Comparisons of pharmacokinetic parameters between Genders.

Pharmacokinetic Parameters	Female (n=35)	Male (n=64)	p-value
VPA dose; mg/day	790.00 ± 383.44 (200-2,000)	795.31 ± 317.20 (200-1,500)	0.774 [b]
VPA dose; mg/kg/day	13.74 ± 6.19 (4.60-27.78)	11.92 ± 6.18 (3.17-33.33)	0.163 [a]
VPA concentration			
C_{total}; (mg/l/)	67.39 ± 27.96 (29.96-143.59)	55.74 ± 21.49 (29.18-122.77)	0.023 [a]*
C_{total}; (mg/l/)/ (mg/kg)	5.33 ± 1.84 (2.37-10.76)	5.37 ± 2.45 (1.66-13.22)	0.924 [a]
C_{free}; mg/l	7.65 ± 6.24 (1.87-32.62)	5.51 ± 3.24 (1.34-13.60)	0.122 [b]
C_{free}; (mg/l/)/ (mg/kg)	0.55 ± 0.28 (0.22-1.41)	0.48 ± 0.22 (0.14-1.20)	0.227 [a]
VPA protein binding			
Free fraction	0.10± 0.04 (0.05-0.23)	0.09 ± 0.03 (0.05-0.19)	0.272 [b]
% free	10.36±4.17 (4.65-22.72)	9.35 ± 2.97 (5.17-19.22)	0.309 [b]
VPA clearance			
CL_{total}; l/h	0.43 ± 0.13 (0.21-0.67)	0.56 ± 0.25 (0.22-1.40)	0.014 [b]*
CL_{total}; ml/kg/h	7.45 ± 2.37 (3.33-15.14)	8.17 ± 3.87 (2.71-21.61)	0.255 [a]
CL_{free}; l/h	4.64 ± 2.08 (1.65-10.51)	6.41 ± 3.51 (2.21-16.80)	0.011 [b]*
CL_{free}; ml/kg/h	81.03 ± 36.14 (25.35-164.19)	93.18 ± 50.58 (29.85-262.45)	0.212 [a]

Values in table are mean±SD (min-max)., [a] Independent t-test, [b] Mann Whitney U test, * Statistical significant difference with p-value< 0.05, ** Statistical significant difference with p-value< 0.01.

Table 11B Comparisons of pharmacokinetic parameters between BMI groups.

Pharmacokinetic Parameters	BMI<25 kg/m^2 (n=58)	BMI≥ 25 kg/m^2 (n=41)	p-value
VPA dose; mg/day	765.52 ± 327.57 (200-1,500)	832.93 ± 357.53 (200-2,000)	0.569 [b]
VPA dose; mg/kg/day	13.63 ± 6.43 (3.17-33.33)	11.04 ± 5.62 (3.85-27.78)	0.011 [b]*
VPA concentration			
C_{total}; mg/l	60.28 ± 23.81 (18.74-122.77)	59.27 ± 25.69 (15.98-143.59)	0.876 [b]
C_{total}; (mg/l)/ (mg/kg)	4.87 ± 1.67 (1.93-8.47)	6.05 ± 2.74 (1.66-13.22)	0.029 [b]*
C_{free}; mg/l	6.14 ± 3.88 (1.34-18.92)	6.44 ± 5.55 (1.40-32.62)	0.867 [b]
C_{free}; (mg/l)/ (mg/kg)	0.46 ± 0.21 (0.15-1.11)	0.58 ± 0.28 (0.14-1.41)	0.015 [a]*
VPA protein binding			
Free fraction	0.10± 0.03 (0.05-0.22)	0.10 ± 0.04 (0.05-0.23)	0.631 [b]
% free	9.59±3.40 (4.65-21.72)	9.87 ± 3.58 (5.35-22.72)	0.654 [b]
VPA clearance			
CL_{total}; l/h	0.47 ± 0.17 (0.21-1.04)	0.56 ± 0.28 (0.22-1.40)	0.230 [b]
CL_{total}; ml/kg/h	8.29 ± 3.01 (4.23-18.58)	7.38 ± 3.90 (2.71-21.61)	0.028 [b]*
CL_{free}; l/h	5.40 ± 2.56 (1.69-13.03)	6.32 ± 3.88 (1.65-16.80)	0.322 [b]
CL_{free}; ml/kg/h	93.98 ± 41.63 (32.38-232.68)	81.68 ± 51.60 (25.35-262.45)	0.009 [b]**

Values in table are mean±SD (min-max)., [a] Independent t-test, [b] Mann Whitney U test, * Statistical significant difference with p-value< 0.05, ** Statistical significant difference with p-value< 0.01.

Table 11C Comparisons of pharmacokinetic parameters between subgroups categorized by Genders and BMI groups.

Pharmacokinetic Parameters	Female (n=35)		Male (n=64)		p-value			
	F1: BMI<25 kg/m^2 (n=23)	F2: BMI≥25 kg/m^2 (n=12)	M1: BMI<25 kg/m^2 (n=35)	M2: BMI≥ 25 kg/m^2 (n=29)	F1 vs F2	M1 vs M2	F1 vs M1	F2 vs M2
VPA dose; mg/day	739.13 ± 286.42 (200-1,250)	887.50 ± 524.02 (400-2,000)	782.86 ± 354.99 (200-1,500)	810.34 ± 270.05 (400-1,500)	0.858 [b]	0.694 [b]	0.881 [b]	0.846 [b]
VPA dose; mg/kg/day	13.98±5.16 (4.63-22.73)	13.28±8.04 (4.60-27.78)	13.40±7.20 (3.17-33.33)	10.12±4.09 (3.85-23.08)	0.754 [a]	0.026 [a]*	0.741 [a]	0.218 [a]
VPA concentration								
C_{total}; mg/l	63.70±24.87 (29.96-118.90)	74.46±33.12 (31.87-143.59)	58.02±23.18 (18.74-122.77)	52.99±19.29 (15.98-81.25)	0.287 [a]	0.355 [a]	0.379 [a]	0.013 [a]*
C_{total}; (mg/l/)/ (mg/kg)	4.78±1.30 (2.37-7.32)	6.39±2.29 (3.89-10.76)	4.93±1.89 (1.93-8.47)	5.91±2.94 (1.66-13.22)	0.040 [a]*	0.254 [b]	0.905 [b]	0.616 [a]
C_{free}; mg/l	6.47±4.16 (1.87-18.92)	9.90±8.80 (2.25-32.62)	5.92±3.73 (1.34-13.60)	5.01±2.52 (1.40-9.35)	0.385 [b]	0.529 [b]	0.530 [b]	0.083 [a]
C_{free}; (mg/l/)/ (mg/kg)	0.47±0.24 (0.22-1.11)	0.71±0.31 (0.27-1.41)	0.45±0.19 (0.15-0.92)	0.53±0.25 (0.14-1.20)	0.005 [b]**	0.184 [a]	0.709 [b]	0.095 [a]
VPA protein binding								
Free fraction	0.10±0.04 (0.05-0.22)	0.12±0.05 (0.06-0.23)	0.09±0.03 (0.05-0.17)	0.09±0.03 (0.05-0.19)	0.310 [b]	0.922 [b]	0.761 [b]	0.161 [b]
% free	9.73±3.72 (4.65-21.72)	11.58±4.85 (6.37-22.72)	9.51±3.22 (5.17-16.75)	9.16±2.69 (5.35-19.22)	0.218 [a]	0.976 [b]	0.830 [b]	0.144 [b]
VPA clearance								
CL_{total}; l/h	0.43±0.13 (0.21-0.67)	0.42±0.12 (0.23-0.60)	0.50±0.18 (0.23-1.04)	0.62±0.31 (0.22-1.40)	0.865 [a]	0.167 [b]	0.116 [a]	0.042 [b]*
CL_{total}; ml/kg/h	8.06±2.31 (4.89-15.14)	6.27±2.10 (3.33-9.22)	8.44±3.42 (4.23-18.58)	7.84±4.39 (2.71-21.61)	0.032 [a]*	0.254 [b]	0.642 [a]	0.492 [b]
CL_{free}; l/h	4.94±2.27 (1.69-10.51)	4.04±1.61 (1.65-7.96)	5.70±2.72 (2.63-13.03)	7.26±4.16 (2.21-16.80)	0.231 [a]	0.070 [b]	0.395 [b]	0.002 [b]**
CL_{free}; ml/kg/h	92.02±35.89 (32.38-164.19)	59.96±26.88 (25.35-130.54)	95.26±45.46 (38.94-232.68)	90.68±56.87 (29.85-262.45)	0.010 [a]**	0.721 [a]	0.709 [b]	0.109 [b]

Values in table are mean±SD (min-max)., [a] Independent t-test, [b] Mann Whitney U test,* Statistical significant difference with p-value< 0.05, ** Statistical significant difference with p-value< 0.01

Table 12A Comparisons of clinical effects between Gender.

Clinical effects		Female (n=35)	Male (n=64)	p-value
Therapeutic category				
Sub-therapeutics (total VPA <50 mg/l)	n=26	6 (17.1%)	20 (31.2%)	
Therapeutics (total VPA 50-100 mg/l)	n=66	23 (65.7%)	43 (67.2%)	0.007 [b]**
Supra-therapeutics (total VPA >100 mg/l)	n=7	6 (17.1%)	1 (1.6%)	
ADRs				
Gastrointestinal ADRs	n=10	5 (14.3%)	5 (7.8%)	0.487 [b]
Weight gain	n=38	17 (48.6%)	21 (32.8%)	0.123 [a]
Hair loss	n=8	7 (20.0%)	1 (1.6%)	0.003 [b]**
Tremor	n=34	15 (42.9%)	19 (29.7%)	0.187 [b]
Sedation	n=49	21 (60.0%)	28 (43.8%)	0.122 [a]

Values in table are count (percentage within column, a-Chi square test, b Exact test,* Statistical significant difference with p-value< 0.05, ** Statistical significant difference with p-value< 0.01

Table 12B Comparisons of clinical effects between BMI groups.

Clinical effects		BMI<25 kg/m^2 (n=58)	BMI≥ 25 kg/m^2 (n=41)	p-value
Therapeutic category				
Sub-therapeutics (total VPA <50 mg/l)	n=26	14 (24.1%)	12 (29.3%)	
Therapeutics (total VPA 50-100 mg/l)	n=66	40 (69.0%)	26 (63.4%)	0.891 [b]
Supra-therapeutics (total VPA >100 mg/l)	n=7	4 (6.9%)	3 (7.3%)	
ADRs				
Gastrointestinal ADRs	n=10	6 (10.3%)	4 (9.8%)	1.000 [b]
Weight gain	n=38	21 (36.2%)	17 (41.5%)	0.596 [a]
Hair loss	n=8	5 (8.6%)	3 (7.3%)	1.000 [b]
Tremor	n=34	22 (37.9%)	12 (29.3%)	0.371 [a]
Sedation	n=49	30 (51.7%)	19 (46.3%)	0.598 [a]

Values in table are count (percentage within column, a-Chi square test, b Exact test,

* Statistical significant difference with p-value< 0.05, ** Statistical significant difference with p-value< 0.01

Table 12C Comparisons of clinical effects between subgroups categorized by both Gender and BMI groups.

Clinical effects		Female (n=35)		p-value	Male (n=64)		p-value
		BMI<25 kg/m^2 (n=23)	BMI≥ 25 kg/m^2 (n=12)		BMI<25 kg/m^2 (n=35)	BMI≥ 25 kg/m^2 (n=29)	
Therapeutic category							
Sub-therapeutics (total VPA <50 mg/l)	n=26	4 (17.4%)	2 (16.7%)		10 (28.6%)	10 (34.5%)	
Therapeutics (total VPA 50-100 mg/l)	n=66	16 (69.6%)	7 (58.3%)	0.868[b]	24 (68.6%)	19 (65.5%)	0.884[b]
Supra-therapeutics (total VPA >100 mg/l)	n=7	3 (13.0%)	3 (25.0%)		1 (2.9%)	0 (0.0%)	
ADRs							
Gastrointestinal ADRs	n=10	2 (8.7%)	3 (25.0%)	0.313[b]	4 (11.4%)	1 (3.4%)	0.366[b]
Weight gain	n=38	10 (43.5%)	7 (58.3%)	0.404[a]	11 (31.4%)	10 (34.5%)	0.796[a]
Hair loss	n=8	4 (17.4%)	3 (25.0%)	0.670[b]	1 (2.9%)	0 (0.0%)	1.000[b]
Tremor	n=34	9 (39.1%)	6 (50.0%)	0.537[a]	13 (37.1%)	6 (20.7%)	0.152[a]
Sedation	n=49	12 (52.2%)	9 (75.0%)	0.282[b]	18 (51.4%)	10 (34.5%)	0.174[a]

Values in table are count (percentage within column, a-Chi square test, b Exact test,

* Statistical significant difference with p-value< 0.05,

** Statistical significant difference with p-value< 0.01

4.3 Analysis 3 :prediction of valproate concentration and clearance in adult patients with excess weight.

Abstract

Objective:

Few studies have shown valproate (VPA) pharmacokinetics in adult patients with excess weight. Weight change causes pathophysiological alterations as well as pharmacokinetics alterations including distribution and elimination processes. These changes result in change in efficacy and tolerability of most drugs especially lipophillic drug such as VPA. We tried to clarify and predict the VPA concentration and clearance in this special population for clinical monitoring application.

Patients and methods

Patients who have received VPA SR at least 4 weeks at Srithanya Hospital, Nonthaburi Province, Thailand, were recruited into the study. Five ml of blood sample was collected into EDTA tube and immediately centrifuged for plasma. Separation free VPA was further done using Centrifree® micro-partition devices. Both total and free VPA concentrations (C_{total} and C_{free}) were analyzed by TDx analyzer with fluorescence polarization immunoassay (FPIA) technique. Pharmacokinetic parameters were then calculated. The associations of Pharmacokinetic parameters (C_{total}, C_{free}, CL_{total} and CL_{free}) and patients characteristics (age, albumin level, gender, weight, height, BMI, IBW, BSA and VPA dose) were examined by using correlation and stepwise multiple regression analysis by using SPSS version 17. (SPSS Inc., Chicago. IL, USA.)

Results

Total of forty one adult patients with BMI $\geq$ 25 kg/m were participated in the study. Stepwise multiple regression analysis showed that VPA dose (mg/kg/day), age (year), BSA (m^2) and gender were significant independent variables associated with C_{total} (mg/l) (r^2_a=0.619, p=0.000). Also VPA dose (mg/ day), age (year), and gender were associated with C_{free}(r^2_a=0.645, p=0.000). Moreover, CL_{total} (l/h) could be predicted with VPA dose (mg/kg/day), age (year), gender and IBW (kg) (r^2_a=0.432, p=0.000).

Conclusion

This study provides several equations with higher regression determinant coeffecients to explain C_{total} , C_{free} , CL_{total} and CL_{free} of VPA in patients with excess weight. It might be useful in clinical setting application for monitoring patients . However, some other factors beside in the equations should always consider for some left sources of variations.

Results

Characteristics of study population

A total forty one VPA treated patients with BMI$\geq$ 25 kg/m^2 (12 females and 29 males), age ranged 18-60 years old, were participated in this study. Patients characteristics are summarized in table 13. Twenty- seven (65.85%) and fourteen (34.15%) patients exhibited overweight (BMI =25-29.9 kg/m^2) and obese (BMI $\geq$ 30 kg/m^2), respectively as illustrated in figure 7. All patients received VPA with other co-medications as presented in table 13; however, they did not interact with VPA. All eleven smokers were male. In figure 8, approximately 36 % of patients were categorized out of therapeutic range (50-100 mg/l of C_{total}). Five common ADRs were reported by patients as demonstrated in figure 9.

Correlation analysis

Without controlling other factors, the correlation analysis was examined as in table 14. Age correlated positively with C_{total} (mg/l)and C_{free} (mg/l) but correlated negatively with CL_{total} (l/h) and CL_{free}(l/h). Albumin had a negative correlation with C_{total} (mg/l)and C_{free} (mg/l). Weight and BSA correlated negatively with CL_{total} (ml/kg/h) while IBW correlated positively with CL_{free} (l/h). Finally, both VPA doses (mg/kg/day) and (mg/day) correlated positively with C_{total} (mg/l), C_{free} (mg/l), CL_{total} (l/h) and CL_{total} (ml/kg/h).

Multiple regression analysis (MRA)

To assess the relative contribution of different variables to the inter-individual variability in VPA concentrations and clearances of both total and free drugs in patients with excess weight. The influences of age, gender, body weight-size (weight, height, BMI, IBW, BSA), albumin level, smoking, coffee consuming, and type of co-medication were tested by MRA. All predictive equations analyzed by MRA were summarized in table 15.

The analysis indicated that C_{total} was significantly correlated highest with dose (mg/kg/day), age (year), BSA (m^2) and gender according to Eq.No. 1 (adjusted r^2=0.619, p=0.000). Other possible regression models that could explain variances of C_{total} were shown in Eq.No.2-6, table 15. Of the variables tested, BMI, height, albumin level, smoking, coffee consuming, and type of co-medication did not appear to contribute significantly to the prediction of C_{total}.

For prediction of C_{free} as in Eq.No 20, dose (mg/kg/day), age (year) and gender explained approximately 64.5% variance of C_{free}. Moreover, Eq.No. 7-12 explained variance of CL_{total} (l/h) with various percentages from 34.8-43.2% according to the predictors in models. Age (year), dose (mg/kg/day), gender and IBW (kg) were selected into the model No.7 and provided highest explanation of CL_{total} (l/h) variance (adjusted r^2=0.432, p=0.000). Additionally, CL_{total} (ml/kg/h) could be predicted with Eq.No 13-19. The best predictive model for CL_{total} (ml/kg/h) recruited age (year), BSA (m^2), gender and dose (mg/day) into model with adjusted r^2=0.513, p-value=0.000. For Eq.No. 22-23 and 24-26 explained approximately 30% of the variance of CL_{free} (l/h) and CL_{free} (ml/kg/h) respectively.

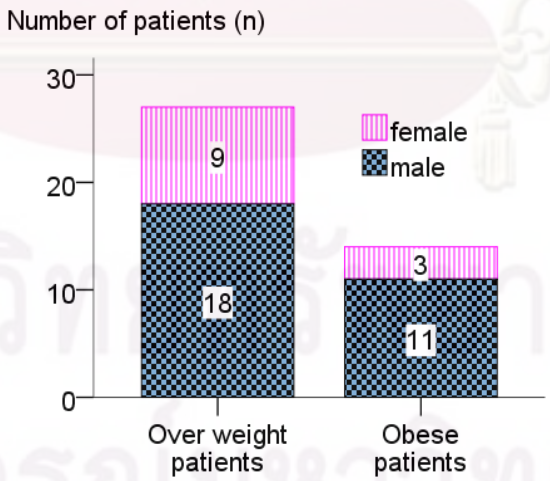

Figure 7 Bar chart presents number of patients in each diagnosis and BMI categories.

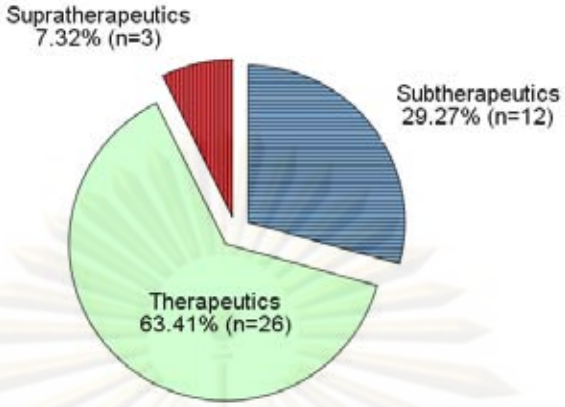

Figure 8 Pie graph represents number and percentage of patients in each therapeutic categories.

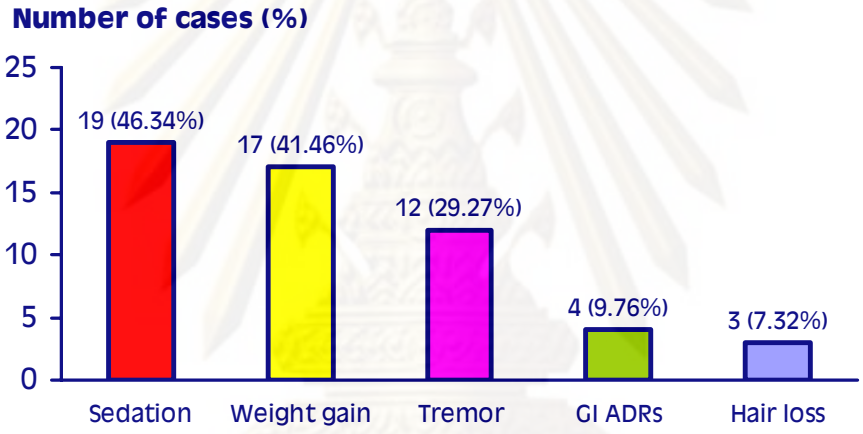

Figure 9 Bar chart presents number and percentage of patients in each ADRs.

Table 13 Characteristics of patients with excess weight (n=41).

Characteristics	Count	(%total)
Female:Male	*12:29*	
Cigarette smoking	11	26.83%
Coffee consuming	13	31.71%
Diagnosis		
Bipolar disorder	21	51.22%
Schizophrenia or schizoaffective disorder	16	39.02%
Depression	2	4.88%
Psychosis	1	2.44%
Others	1	2.44%
Co-medication		
Antipsychotics	32	78.05%
Antidepressants	10	24.39%
Mood stabilizes	13	31.71%
Benzodiazepines	28	68.29%
Anticholinergics	30	73.17%
Characteristics	***Mean±SD***	***(Min-Max)***
Age; year	41.15±9.85	(18.00-59.00)
Albumin; gm/dl	4.52±0.35	(3.70-5.30)
Height; cm	162.54±7.59	(150.00-180.00)
Weight; kg	78.64±14.73	(59.20-130.00)
BMI; kg/m^2	29.62±4.05	(25.00-41.80)
IBW; kg	58.34±7.54	(47.80-74.99)
BSA; m^2	1.88±0.20	(1.57-2.54)
Dose; mg/day	832.93±357.53	(400.00-2,000.00)
Dose; mg/kg/day	11.04±5.62	(3.85-27.78)
C_{total}; mg/l	59.27±25.69	(15.98-143.59)
CL_{total}; l/h	0.56±0.28	(0.22-1.40)
CL_{total}; ml/kg/h	7.38±3.90	(2.71-21.61)
C_{free}; mg/l	6.44±5.55	(1.40-32.62)
CL_{free}; l/h	6.32±3.88	(1.65-16.80)
CL_{free}; ml/kg/h	81.68±51.60	(25.35-262.45)

Table14 Correlations of pharmacokinetic parameters with general demographic characteristics of patients.

Predictors (X)	Statistics	Dependent variables (Y)					
		C_{total}; mg/l	CL_{total}; l/h	CL_{total}; ml/kg/h	C_{free}; mg/l	CL_{free}; l/h	CL_{free}; ml/kg/h
Age;year	r	[a]*0.388	*-0.313	-0.202	*0.353	*-0.466	-0.294
	p-value	0.012	0.046	0.204	0.024	0.002	0.062
Albumin;gm/dl	r	[a]*-0.387	0.232	0.146	*-0.329	0.335	0.294
	p-value	0.012	0.145	0.361	0.036	0.032	0.062
Height; cm	r	-0.193	0.062	-0.196	-0.219	0.263	-0.011
	p-value	0.226	0.698	0.220	0.169	0.097	0.944
Weight; kg	r	-0.127	-0.043	*-0.374	-0.233	0.237	-0.199
	p-value	0.428	0.789	0.016	0.142	0.136	0.212
BMI; kg/m^2	r	-0.033	-0.022	-0.294	-0.129	0.130	-0.251
	p-value	0.836	0.892	0.062	0.422	0.417	0.114
IBW; kg	r	-0.230	0.139	-0.133	-0.251	*0.343	0.044
	P-value	0.149	0.385	0.407	0.114	0.028	0.784
BSA; m^2	r	[a]-0.199	0.011	*-0.328	-0.281	0.302	-0.120
	p-value	0.213	0.944	0.036	0.075	0.055	0.454
Dose; mg/day	r	*0.464	*0.465	*0.442	*0.603	-0.107	0.009
	p-value	0.002	0.002	0.004	0.000	0.507	0.957
Dose; mg/kg/day	r	[a]*0.602	*0.436	*0.535	*0.638	-0.196	0.066
	p-value	0.000	0.004	0.000	0.000	0.220	0.683

a. Pearson correlation coefficient

b. Spearmans' rho correlation coefficient

* Significant correlate at p value<0.005

Table 15 Multiple Regression Equations predicting pharmacokinetic parameters based on general demographic data for overweight/ obese patients (n=41)

Eq No.	Dependent variable	Predictors	R^2_a	R^2
1	C_{total} = (mg/l)	-119.153+3.242 Dose (mg/kg/day)+1.224 Age (year)+56.665 BSA (m^2)-20.025 Male	**0.619**	**0.657**
2		-70.391+3.204 Dose (mg/kg/day)+1.212 Age (year)+0.725 Weight (kg)-17.785 Male	**0.610**	**0.649**
3		-73.821+0.046 Dose (mg/day)+1.183 Age (year)-21.445 Male+32.765 BSA (m^2)	**0.610**	**0.649**
4		-70.367+0.045 Dose (mg/day)+1.159 Age (year)-27.179 Male+1.090 IBW	**0.610**	**0.649**
5		0.043 Dose (mg/day)+1.012 Age (year)-14.808 Male	0.573	0.605
6		2.745 Dose (mg/kg/day)+1.006 Age (year)	0.485	0.511
7	CL_{total} = (l/h)	1.597-0.014 Age (year)+0.015 Dose (mg/kg/day)+0.381 Male-0.015 IBW (kg)	**0.432**	**0.489**
8		1.692-0.014 Age (year)+0.000 Dose (mg/day)+0.375 Male-0.017 IBW (kg)	**0.430**	**0.487**
9		1.663 -0.014 Age (year)+0.000 Dose (mg/day)+0.277 Male-0.472 BSA (m^2)	0.416	0.474
10		2.830-0.014 Age (year)+0.000 Dose (mg/day)+0.296 Male-0.013 Height (cm)	0.413	0.471
11		0.704 -0.012 Age (year)+0.018 Dose (mg/kg/day) +0.219 Male	0.376	0.422
12		0.707-0.012 Age (year)+0.000 Dose (mg/day)+0.182 Male	0.348	0.397

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Table 15 Multiple Regression Equations predicting pharmacokinetic parameters based on general demographic data for overweight/ obese patients (n=41) (Cont)

Eq. No.	Dependent variable	Predictors	R^2_a	R^2
13	CL_{total} = (ml/kg/h)	33.106-0.200 Age (year)-12.031 BSA (m^2)+3.848 Male+0.003 Dose (mg/day)	**0.513**	**0.561**
14		30.399-0.198 Dose (mg/kg/day)-0.197 Age (year)-10.584 BSA (m^2)+3.935 Male	**0.512**	**0.561**
15		21.120+0.207 Dose (mg/kg/day)-0.194 Age (year)-0.134 Weight (kg)+3.498 Male	0.494	0.544
16		22.797-0.197 Age (year)-0.154 weight (kg)+3.355 Male+0.003 Dose (mg/day)	0.492	0.543
17		21.429-0.176 Age (year)-0.320 BMI (kg/m^2)+0.003 Dose (mg/day)	0.328	0.378
18		10.708+0.287 Dose (mg/kg/day)-0.158 Age (year)	0.294	0.329
19		10.348-0.148 Age (year)+0.004 Dose (mg/day)	0.237	0.275
20	C_{free} = (l/h)	-7.607+0.010 Dose (mg/day)+0.194 Age (year)-3.457 Male	**0.645**	**0.672**
21		-9.009+0.686 Dose (mg/kg/day)+0.192 Age (year)	**0.578**	**0.599**
22	CL_{free} = (l/h)	2.369-0.024 Age (year)+0.443 Male	**0.354**	**0.387**
23		-0.152 Age (year)+3.716 Albumin (gm/dl)	**0.272**	**0.309**
24	CL_{free} = (ml/kg/h)	58.810 Albumin (gm/dl)-2.159 Age (year)-89.065 BSA (m^2)	**0.304**	**0.356**
25		69.881 Albumin (gm/dl)-2.023 Age (year)-2.447 Height (cm)	**0.297**	**0.350**
26		49.297 Albumin (gm/dl)-1.649 Age (year)	0.209	0.248

CHAPTER V
DISCUSSIONS

The power of each data analysis were checked using Primer of Biostatistics: statistical software program version 5.[116] The results shown that in analysis1 and analysis 2 which independent t-test were used to compared the difference of pharmacokinetic parameters between two groups. At significant level =0.05 the analysis could provide power of the test more than 0.8.

5.1 Analysis1: study of the impact of dosage of sustained release formulation on valproate clearance and plasma concentration in psychiatric patients: analysis based on routine therapeutic drug monitoring data

In Table 5, few variables showed significant difference between the two dosage groups, such as, coffee drinking and using of co-medications. Since they are not affect VPA pharmacokinetics, these variables should not cause any effect to the results of our study.[1, 101, 104, 117] For sustained-release formulation of VPA at the steady-state condition, blood sampling obtained either earlier or equal/longer than 10 h after administration did not result in any significant difference in C_{total} or C_{free} as long as the same dosage per day was consumed. In addition, whether the sustained-release tablet was taken once daily or twice daily, the same C_{total} and C_{free} could be obtained if the dosage per day was kept the same. These findings indicated that the plateau condition had been reached and could be maintained with low fluctuation between peak and trough levels throughout the dosing interval. VPA is highly protein bound to albumin, and this binding is saturated within the recommended plasma therapeutic concentration range (45-100 mg/l). Valproic acid or 2-propylpentanoate has a structure of a branched-chain fatty acid[21, 67], and therefore, its variations in plasma free fatty acids can displace VPA from albumin and affect unbound VPA concentration and clearance.[67] Free fatty acids are known to be increased at the fasting state; plasma sampling strategies may contribute

to variability in free drug concentrations.[20]However, in this study, plasma sampling at different times did not show any significant impact on the free drug concentration and clearance when the 1000 mg/day dosage was consumed in psychiatric patients. Our data suggest that the mean CL_{total} is significantly different between the two dosage groups. Higher CL_{total} was found in the higher dosage group which is consistent with other reports. [19, 71, 75] VPA is a low-extraction ratio drug; the CL_{total} therefore depends on its Fu and CL_{int}.[45] As VPA dosage is increased and the protein binding to albumin is saturated, Fu increased, CL_{total} increased and in turn resulted in a decrease in C_{total}.[118] The mean CL_{free} in our findings were not statistically significantly different between the two dosage groups. CL_{free} represents the CL_{int} which depends on the liver metabolism ability. Since VPA is an extensively metabolized drug and no change in hepatic function was recorded, no change in CL_{int} is presumed. No significant difference in CL_{free} is expected, a dose-concentration linear relationship should be obtained. In contrast to the study of Bowdle et. al.[19] and Felix et.al.[22] who reported significant decrease in CL_{free} with increased dose, and explained this with the theory of auto-inhibition of CL_{int} by higher dose of VPA. However, the sample sizes of both studies were small (n=6) compare to our study (n=66). Within the dosage of 500 mg/day to 1000 mg/day consumed by the patients in our study, the auto-inhibition effect was not observed. The usual CL_{total} is approximately 6-10 ml/kg/hr with average of 8 ml/kg/h in healthy adults and CL_{total} may be higher as 10-13 ml/kg/h in epileptic patients using other AED inducers.[45] In psychiatric patients from our study, CL_{total} is approximately 0.41 l/h or 6.15 ml/kg/h in the 500 mg/day group and 0.59 l/h or 8.99 ml/kg/h in the 1000 mg/day group which is similar to the healthy adult in the general population. Since CL_{total} increased as the dosage increased, this resulted in less than proportional increases in C_{total} when the dosage was increased. This same situation did not occur with C_{free}. Therefore, the monitoring of C_{free} and by adjusting the dosage accordingly, the target therapeutic concentration should be more accurately reached. We proposed the therapeutic range of C_{free} to be 4-12 mg/l as equal to the total VPA therapeutic range of 45-100 mg/l.

5.2 **Analysis 2:** study of the influences of both gender and overweight/obese on valproate pharmacokinetics.

Gender effect

CL_{total} (l/h) and CL_{free} (l/h) in males were statistically significant higher than females which agree with other studies[119]and could be explained with the larger volume of distribution (Vd) from larger body weight in male compare to female; therefore, after standardized for body weight, CL_{total} (ml/kg/h) and CL_{free} (ml/kg/h) in males and females were not statistically significantly different. Nevertheless, males still showed slightly higher CL_{total} (ml/kg/h) and CL_{free} (ml/kg/h) of approximately 10% and 15%, respectively which cause some impact on clinical effects; such as, higher percentage of patients in subtherapeutic category and lower percentage of ADRs were found in male group. Higher CL (ml/kg/h) in male might associated with the higher number of cigarette smokers in male group and smoking is known enzyme inducer. Previous studies had also reported higher glucuronidation (one pathway of VPA metabolism account for 50%) in male.[23, 120] As a consequence, C_{total} (mg/l) was significantly lower in males than in females and the percentage of patients who had their C_{total} in the subtherapeutic level was higher in male than in female when the therapeutic range of C_{total} was proposed to be 50-100 mg/l. Even not reaching statistically significantly different level, greater percentage of ADRs were shown in females as a consequence of higher C_{total} (mg/l). These results are consistent with other reports which indicated that ADRs were found more often in female compare to male.[120] Hair loss showed statistically significantly higher in females which may due in part to the more concern about beauty of female.

Overweight/ obese effect

CL_{total} (l/h) and CL_{free} (l/h) of patients with BMI$\geq$ 25 kg/m^2 (or OW/OB group) did not differ from patients with BMI<25 kg/m^2 . However, when standardized for body weight, CL_{total} (ml/kg/h) and CL_{free} (ml/kg/h) were significantly lower in OW/OB group which lead to significantly higher C_{total} (mg/l)/(mg/kg) and C_{free} (mg/l)/(mg/kg). However, C_{total} (mg/l) and C_{free} (mg/l) which should be higher in the OW/OB group as a consequence of lower clearance showed no significant differences in the OW/OB group as compare to normal

weight group. These might due to the lower dose (mg/kg/day) of VPA consumed in the OW/OB group. The lower mean dosage (mg/kg/day) consumed in the OW/OB group might because the maximum recommended dose/day had been reach in the OW/OB group. Another reason for not finding significantly higher C_{total} (mg/l) and C_{free} (mg/l) in OW/OB group despite of lower clearance might due to the confounding of gender effect. Higher percentage of male was found in the OW/OB group (29/41= 70.73%) as compared to the normal weight group (35/58 =60.34%) and male showed some significantly impact on pharmacokinetics of VPA as previously described. Clinical effects including percentage of patients whose VPA levels were within, supra or subtherapeutic levels and percentage ADRs found were not different between different BMI groups; these might due to the similar C_{total} (mg/l) and C_{free} (mg/l) found between different BMI groups. Higher albumin level was found in OW/OB; however, this higher level was still within normal range and did not affect VPA plasma protein binding parameters including Fu and %free drug. The results was consistent with previous studies with other drug preliminary bound to albumin (phenytoin) and showed no significant change in protein binding in obese patient.[33]

Gender and overweight/ obese effects

In female (F1 vs F2), statistically significant lower CL_{total} (ml/kg/h) and CL_{free} (ml/kg/h) were found in higher BMI group. Although the differences in C_{total} (mg/l) and C_{free} (mg/l) did not reach statistical significance, higher C_{total} (mg/l) and C_{free} (mg/l) could be observed in OW/OB female, accordingly, higher percentage of patients in supratherapeutic level and higher percentage of ADRs were found in OW/OB female. The reason for not reaching statistically significant levels should due to the small sample size while high variations among patients were observed.

In male (M1 vs M2), the VPA pharmacokinetics differences were affected by OW/OB in the same direction as in female. CL_{total} (ml/kg/h) and CL_{free} (ml/kg/h) were lower in OW/OB but did not reach statistical significance. Higher BMI males received lower VPA dose (mg/kg/day) which may due to the maximum VPA dose/day had been reached; therefore,

no differences in C_{total} (mg/l) and C_{free} (mg/l) and its consequence, the clinical effects, were observed. Higher albumin levels were found in OW/OB patients but Fu and % free VPA did not showed significantly different between different BMI subgroups.

For patients with BMI < 25 kg/m^2 (F1 vs M1), the effect of gender was less obvious and was not statistically significant; nevertheless, the results were in the same direction as previously mentioned; i.e., female showed lower of both total and free VPA clearances and in turn, higher of both total and free VPA concentrations (mg/l) were observed.

For patients with BMI$\geq$ 25 kg/m^2 (F2 vs M2), female showed significantly lower CL_{total} (l/h) and CL_{free} (l/h) and in turn statistically significantly higher C_{total} and higher C_{free} without reaching statistical difference. Higher ADRs were observed in female compared to male. These might cause by variations in volume of distribution (Vd) of VPA in fat versus lean muscle mass; VPA distributed to fat mass in a lesser proportion than to lean muscle mass. Since clearance is the product of elimination rate constant (Ke) and Vd, a lesser Vd results in a lower clearance. Higher proportion of fat mass per total body weight in female as compared to male especially in OW/OB patients might be the main explanation for more obvious OW/OB effects on VPA clearance found in female and might be part of the reason for a lower clearance found in female as compared to male even in patients with BMI < 25 kg/m^2. The results obtained from our study were consistent with previous study [121] which reported that OW children were significantly more likely to experience supratherapeutic drug levels when receiving approximately 15 mg/kg/day of VPA loading dose based on actual weight. They suggested that loading dose should be given based on adjusted ideal body weight (IBW) rather than actual body weight (ABW); the adjusted weight was: IBW + 40% (observed weight-IBW). This might imply that VPA distributed to the fat mass approximately 40% only of the amount distributed to the lean muscle mass.

5.3 Analysis 3: prediction of valproate concentration and clearance in adult with excess weight.

An attempt was made to correlate numerous demographic factors with C_{total}, C_{free}, CL_{total}, CL_{free}. The results showed that non pharmacokinetic approaches could be used to predict C_{total}, C_{free}, CL_{total}, CL_{free} with some accuracy in VPA treated patients with excess weight. A stepwise multiple regression was conducted in a manner similar to other studies. Prior to present investigation, limited information on the pharmacokinetics of VPA in adults with excess weight had been revealed.

Since pharmacokinetic parameters including C_{total}, C_{free}, CL_{total}, CL_{free} were important for routine therapeutic monitoring for efficacy, tolerability and dosage adjustment. These regression models obtained could help significantly as a guide for monitoring in this special population. To our knowledge, this is the first study that shows the relationships of patient factors including age, albumin, gender, body size or weight and dosage on VPA pharmacokinetic parameters in patients with excess weight.

Practically, C_{total} of VPA after treatment with sustained release formulation could be obtained by direct measurement of VPA level in blood or predict from the pharmacokinetic formula which require the known value of CL_{total}. CL_{total} of normal adults has been reported to be approximately 0.1-0.4 l/h and 8 ml/kg/h in general population.[45] Our preliminary data found that there were significant decline in CL_{total} (ml/h/kg) and CL_{free} (ml/h/kg) of approximately 22% and 34% in female with excess weight while the decrement were 7% and 4% in male with excess weight compare to normal weight. These findings lead to the developing of the predictive equations for C_{total}, $C_{free,}$ CL_{total} and CL_{free} in these special population.

From equations, the increased C_{total} (mg/l) is influenced by the increase in age, weight, IBW, BSA, gender and dosage. Lower C_{total} was found in male compared to female. In agreement with previous studies[46, 122], age was found to be a factor that affected VPA pharmacokinetics. Older aged patients may have some impair hepatic metabolism and these may be the reason of age related increase in C_{total} and decrease in CL_{total}. However, those studies which reported changes in C_{total} and CL_{total} from different age

groups compared among neonate, child, adult and elderly. In our study, we specified the influence of age within the range of adults (18-60 years). Our results had shown the increase in C_{total} (mg/l) along with the increase in weight, IBW, BSA which converse to normal theory[29, 30, 34] which might due to the change in metabolism process which affects Ke more than Vd. Basically, CL_{total} depends on Ke and Vd, any factor such a greater weight could cause the increase in Vd and lead to the increased CL_{total} resulting in the decrease in C_{total}. The obvious increased C_{total} is a consequence of the decreased CL_{total} in heavy weight. The gender differences in C_{total} and CL_{total} are related to the differences of body weight and body size, hormonal system and metabolisms. [23, 123] Factors such as dose and age had been recruited into equations for prediction of CL_{total} which were similar to previous reported equations developed for normal adult and elderly.[118, 122] These results could be support with the changing in CL_{total} (ml/h/kg) in our preliminary study and gender differences based review. The CL_{total} (l/h) and CL_{total} (ml/kg/h) were influenced by age, dose, gender, IBW, and BSA. Dose, age, gender were influences C_{free}. The increase in C_{free} with the increase in age could be explained with the less function of hepatic metabolism in excess weight patients when the age increase. Male had lower C_{free} compared to female which may be the result of the lower CL_{free} in female with excess weight and higher glucuronidation in male.[123]Age, gender, albumin and BSA affected CL_{free} (l/h) .

Theoritically, C_{total} is increased by the reduction of CL_{total} and/or the enhance of VPA dosage. Moreover, for low extraction ratio drug such as VPA, CL_{total} depends on free fraction (Fu) and intrinsic clearance (CL_{int}), Fu is the ratio of C_{free} over C_{total} . Any factor that could increase Fu will increase CL_{total} accordingly. CL_{int} is equaled to CL_{free} and represent the hepatic metabolic capacity.

A significant positive correlation between age and C_{total} and C_{free} (mg/l) and negative correlation between age and CL_{total}, CL_{free} had also been previously shown in normal adults and elderly group.[46, 47, 73] A significant positive correlation between albumin and CL_{free} (l/h), (ml/kg/h) were observed in our study which consistent with other previous study. Higher albumin level will cause lower Fu follow by increase in CL_{int} or CL_{free}.

These support the association between liver function and albumin level. Since C_{total} and CL_{total} had been reported to be increased with increasing in dosage[124, 125] ; therefore, VPA dosage (mg/day and mg/kg/day) were recruited as a predictor in most of the regression models except for CL_{free} (ml/kg/h and l/h). However, the regression coefficients (b) between CL_{total} (l/h and ml/kg/h) and dosage predictor were small which might due to the relationship between VPA dose and CL_{total} were also appear to be curvilinear. Various body size or weight parameters including weight, height, BMI, IBW, BSA were examined in the analysis. Interestingly, the negative correlations of these parameters were significantly found in predictive model for CL_{total} (l/h and ml/kg/h) and CL_{free} (l/h). Most weight based reviewers revealed the alterations of pharmacokinetic processes in general; moreover, some had reported higher clearances of several drugs in obese.[29] Gender was a influence predictor for almost dependent variables studied except for CL_{free} (ml/kg/h). These findings were inconsistent with previous reports which no differences in pharmacokinetic parameters between genders. [28, 126] Nevertheless, few papers mentioned that there were differences in pharmacokinetic metabolism between genders, for example, higher glucuronidation; one of major hepatic pathway for VPA metabolism, is higher in male compare to female.[23, 123]

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CHAPTER VI
CONCLUSIONS

Analysis 1: study of the impact of dosage of sustained release formulation on valproate clearance and plasma concentration in psychiatric patients: analysis based on routine therapeutic drug monitoring data

The results obtained from this study were based on the data obtained from clinically routine therapeutic drug monitoring of VPA in psychiatric patients. These results indicated that monitoring of C_{total} only may lead to some error in the design/ adjustment of dosage regimen. The CL_{total} was significantly increased when the dosage was increased from 500 mg/day to 1000 mg/day, resulting in less than proportionally increased C_{total} of VPA when the dosage was increased. In contrast, CL_{free} was not significantly different between the 500 mg/day and 1000 mg/day groups. Therefore, C_{free} of VPA seems to be proportionally increased with increasing dosage, i.e., a linear relationship remained within the dosage range studied. Since it is the free drug that is pharmacologically active, it might be beneficial to set up a therapeutic range for C_{free} in place of C_{total}. The therapeutic drug level monitoring of VPA is aimed for this free-level therapeutic range to avoid all the complications caused by the saturation in protein binding of VPA within the concentrations of the therapeutic range.

Analysis 2: study of the influences of both gender and overweight/obese on valproate pharmacokinetics.

Gender and OW/OB both cause some impacts on VPA pharmacokinetics. Significant decrease in CL_{total} (ml/kg/h) and CL_{free}(ml/kg/h) were found in OW/OB especially female. From these finding VPA dosage adjustment might not be appropriated to calculated based on total body weight especially in OW/OB patients. Further prospective investigation in larger sample size should be performed to confirm the result. We recommend not to adjust the dosage of VPA according to TBW in these OW/OB female. Maintenance dose should be adjusted follow the observed C_{total} and calculated CL_{total}.

Analysis 3:

Our finding demonstrate the predictive multiple regression equations for C_{total}, C_{free}, CL_{total}, CL_{free}. These findings must be intepreted and applied in the context of the evidence in the study which are patients present excess weight, on maintenance VPA dose and reach steady state, age range 18-60 years old. Larger sample size and liver function tests may need to confirm the results in future study.

CHAPTER VII
THE LIMITATIONS OF STUDY

Limitations of the study in analysis 1: study of the impact of dosage of sustained release formulation on valproate clearance and plasma concentration in psychiatric patients: analysis based on routine therapeutic drug monitoring data

The analysis was based on the data of patients consuming VPA within the dosage range of 500 mg/day to 1000 mg/day. Extrapolation of the findings to higher dosage should be carefully interpreted. The proposed C_{free} range for monitoring was done according to C_{total} and Adverse drug reactions. The further studies should plan to correlate C_{free} and efficacy.

Limitations of the study in analysis 2: study of the influences of both gender and overweight/obese on valproate pharmacokinetics.

The analysis was based on the data of patients treated with VPA SR. Due to it is observational study, the classification of excess weight patients was done with BMI according to WHO criteria. None of other methods were used to test metabolic changing in excess weight patients associated which may confirm and give more detailed related to our assumption that patients with excess weight lost some of fatty oxidation which is one major metabolic pathway of VPA. The implement of specific methods to measure hepatic metabolic capacity should be plan in future study.

Limitations of the study in analysis 3: prediction of valproate concentration and clearance in adult with excess weight.

Predictive equations were generated based on the remained data, some left of unexplained variations of dependent variables should be examine in further study. Factors such as liver function, genetics are interesting factors that might combined into equation and may provide more accurately predictive equation.

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APPENDICES

APPENDIX A: Raw data of all ninety nine subjects

No of patients	Date of sampling	Gender	Age (years)	Weight (kg)	Height (cm)	BP (mmHg)	HR time	BMI ($kg/m^{2)}$
1	22/6/2549	F	44	51	150	124/84	86	22.67
2	22/6/2549	F	44	51	150	124/85	87	22.67
3	23/6/2549	M	29	56	160	120/78	92	21.88
4	21/7/2549	M	38	75	165	132/85	80	27.55
5	21/7/2549	M	38	75	165	131/65	72	27.55
6	18/8/2549	F	28	57	160	109/59	71	22.27
7	18/8/2549	F	28	57	160	109/59	71	22.27
8	25/8/2549	M	47	45	165	121/70	81	16.53
9	25/8/2549	M	47	45	165	121/71	82	16.53
10	9/1/2551	M	33	107	160	111/69	69	41.80
11	8/9/2549	F	60	61	160	121/59	100	23.83
12	8/9/2549	F	60	61	160	121/59	100	23.83
13	18/9/2549	F	22	60	158	134/79	90	24.03
14	15/9/2549	M	24	55	160	136/89	90	21.48
15	15/9/2549	M	24	55	160	136/90	91	21.48
16	26/9/2549	M	56	82	170	117/91	109	28.37
17	26/9/2549	M	56	82	170	117/91	109	28.37
18	26/9/2549	F	50	68	150	147/83	73	30.22
19	26/9/2549	M	52	66	160	128/93	87	25.78
20	26/12/254	M	49	57	165	127/78	77	20.94
21	28/11/255	F	30	46	158	118/76	102	18.43
22	20/10/254	M	39	63	165	111/70	77	23.14
23	28/11/255	M	52	72	160	114/70	65	28.13
24	6/10/2549	M	52	70	160	110/68	68	27.34
25	9/2/2550	M	28	62	170	114/77	109	21.45
26	5/1/2550	M	54	72	170	153/98	104	24.91
27	9/2/2550	M	38	67	170	105/59	83	23.18
28	9/1/2550	M	49	61.5	165	128/89	80	22.59
29	9/2/2550	M	42	67	165	100/67	70	24.61
30	15/9/2549	M	20	65	160	131/70	82	25.39
31	28/11/255	M	36	53	156	110/79	65	21.78
32	28/11/255	F	49	84	160	110/73	97	32.81
33	29/11/255	M	45	58	170	145/88	68	20.07
34	29/11/255	F	32	65	165	149/10	97	23.88
35	29/11/255	M	36	61	162	104/63	63	23.24
36	12/12/255	M	42	80	165	172/11	105	29.38
37	9/1/2551	M	39	80	165	153/10	129	29.38
38	12/12/255	M	19	65	180	130/92	77	20.06
39	12/12/255	F	22	47	160	84/54	73	18.36
40	12/12/255	M	18	82	160	124/70	91	32.03
41	8/1/2551	M	27	63	160	112/72	91	24.61
42	9/1/2551	M	31	98	165	130/98	79	36.00
43	9/1/2551	M	34	84	165	112/59	83	30.85
44	30/1/2551	M	29	66	165	116/75	91	24.24
45	8/4/2551	M	41	66	165	144/90	94	24.24

No of patients	Date of sampling	Gender	Age (years)	Weight (kg)	Height (cm)	BP (mmHg)	HR time	BMI ($kg/m^{2)}$
46	8/4/2551	M	46	52	160	127/80	85	20.31
47	22/4/2552	M	31	72	162	106/57	65	27.43
48	27/4/2552	F	28	60	160	111/75	106	23.44
49	28/4/2552	M	45	84	165	116/84	91	30.85
50	28/4/2552	F	53	54	150	107/78	78	24.00
51	28/4/2552	M	27	50	165	129/79	80	18.37
52	29/4/2552	F	46	65	155	130/70	91	27.06
53	29/4/2552	F	37	43	155	127/85	82	17.90
54	29/4/2552	M	28	59	165	109/77	76	21.67
55	29/4/2552	F	55	58.3	157	126/77	96	23.65
56	29/4/2552	M	40	64	160	129/79	79	25.00
57	1/5/2552	M	38	65	160	125/78	71	25.39
58	22/4/2552	F	31	44	150	117/73	91	19.56
59	1/5/2552	F	42	77	160	134/83	96	30.08
60	1/5/2552	F	52	49	150	126/74	76	21.78
61	1/5/2552	M	42	104	175	131/87	103	33.96
62	1/5/2552	M	34	77	170	111/66	80	26.64
63	3/5/2552	F	41	61	150	100/74	82	27.11
64	4/5/2552	M	50	74	165	121/84	84	27.18
65	4/5/2552	F	41	68	152	98/69	89	29.43
66	7/5/2552	F	59	48	150	143/87	107	21.33
67	7/5/2552	F	44	87	155	132/84	87	36.21
68	23/4/2552	F	59	65	155	111/72	70	27.06
69	7/5/2552	F	34	72	160	93/56	62	28.13
70	7/5/2552	F	30	48	155	99/63	74	19.98
71	7/5/2552	M	38	58	160	114/78	92	22.66
72	13/5/2552	F	35	59.2	150	99/81	80	26.31
73	13/5/2552	M	55	72	160	122/73	73	28.13
74	13/5/2552	M	36	88	172	119/88	85	29.75
75	13/5/2552	M	47	75	175	128/89	90	24.49
76	13/5/2552	M	32	50	155	131/92	76	20.81
77	15/5/2552	M	50	69	170	119/67	86	23.88
78	15/5/2552	F	41	66	162	93/63	79	25.15
79	23/4/2552	M	30	70	170	102/66	89	24.22
80	18/5/2552	M	33	88	179	129/73	79	27.46
81	18/5/2552	M	26	130	179	120/89	111	40.57
82	18/5/2552	F	30	58.5	165	103/72	108	21.49
83	18/5/2552	F	37	66	160	110/73	79	25.78
84	18/5/2552	M	20	49	178	112/85	72	15.47
85	18/5/2552	M	44	63	165	111/80	67	23.14
86	19/5/2552	F	43	52.5	150	121/89	111	23.33
87	19/5/2552	M	19	59.5	170	137/76	111	20.59
88	19/5/2552	M	40	65.5	162	125/80	123	24.96
89	19/5/2552	F	52	67	160	104/72	74	26.17
90	23/4/2552	F	54	58	155	128/81	68	24.14
91	19/5/2552	M	26	69	170	126/68	90	23.88
92	20/5/2552	M	39	54	157	120/79	92	21.91

No of patients	Date of sampling	Gender	Age (years)	Weight (kg)	Height (cm)	BP (mmHg)	HR time	BMI ($kg/m^{2)}$
93	20/5/2552	M	32	66.5	172	130/83	89	22.48
94	29/5/2552	F	34	44	154	119/72	101	18.55
95	29/5/2552	F	49	82	151	136/78	83	35.96
96	24/4/2552	F	60	43	152	111/70	73	18.61
97	24/4/2552	M	26	107	180	123/86	91	33.02
98	27/4/2552	M	50	94	167	115/82	106	33.71
99	27/4/2552	M	42	67	165	108/79	84	24.61

No of patients	Coffee consuming	Smoking	VPA dose (mg/day)	VPA dose (mg/kg/day)	Frequency	Time of last dose at	Sampling time at	Time from last dose (hr)
1	Yes	No	500	9.80	1	19.00	7.45	12.75
2	Yes	No	500	9.80	1	19.00	13.30	18.50
3	No	No	1000	17.86	2	8.00	8.00	12.16
4	No	Yes	1000	13.33	1	21.00	9.00	12.00
5	No	Yes	1000	13.33	1	21.00	11.00	14.00
6	Yes	No	1000	17.54	2	22.00	7.50	9.83
7	Yes	No	1000	17.54	2	22.00	10.00	13.00
8	No	No	1500	33.33	3	7.00	9.30	2.50
9	No	No	1500	33.33	3	7.00	15.30	8.50
10	No	No	1000	9.35	2	6.00	9.50	3.83
11	Yes	No	500	8.20	1	21.00	7.40	10.66
12	Yes	No	500	8.20	1	21.00	9.45	12.00
13	No	No	1000	16.67	2	9.00	10.10	1.17
14	No	Yes	1000	18.18	2	7.00	15.00	8.00
15	No	Yes	1000	18.18	2	16.00	6.00	14.00
16	No	Yes	1000	12.20	1	19.00	8.00	13.00
17	No	Yes	1000	12.20	1	19.00	10.00	15.00
18	Yes	No	1000	14.71	1	20.00	10.00	14.00
19	No	No	600	9.09	2	22.00	9.50	11.83
20	No	Yes	400	7.02	1	20.00	10.00	14.00
21	No	No	600	13.04	3	18.00	9.20	9.33
22	No	Yes	1000	15.87	1	17.00	10.30	17.50
23	No	No	1000	13.89	2	6.00	9.40	2.60
24	No	No	1000	14.29	2	18.00	8.00	14.00
25	No	No	1500	24.19	2	17.00	9.15	16.25
26	No	No	1000	13.89	2	22.00	8.30	10.50
27	No	Yes	600	8.96	1	21.00	9.00	12.00
28	No	No	1000	16.26	2	19.00	8.00	13.00
29	No	Yes	500	7.46	1	20.00	10.50	14.83
30	No	No	1500	23.08	2	17.00	6.00	9.00
31	No	Yes	1000	18.87	2	6.00	10.10	4.17
32	Yes	No	500	5.95	1	20.00	10.00	14.00
33	No	Yes	1000	17.24	1	21.00	10.30	13.50
34	No	No	1250	19.23	2	19.00	9.30	14.50
35	Yes	No	800	13.11	2	18.00	10.25	16.42
36	No	Yes	500	6.25	1	23.00	11.00	12.00
37	Yes	No	1000	12.50	2	7.00	11.20	4.33

No of patients	Coffee consuming	Smoking	VPA dose (mg/day)	VPA dose (mg/kg/day)	Frequency	Time of last dose at	Sampling time at	Time from last dose (hr)
38	No	No	1000	15.38	1	20.00	10.00	14.00
39	No	No	1000	21.28	1	22.00	11.00	13.00
40	No	No	1000	12.20	2	18.00	10.30	14.50
41	No	Yes	400	6.35	2	18.15	10.30	15.25
42	No	No	800	8.16	2	19.30	10.15	15.25
43	No	Yes	1000	11.90	1	21.00	11.00	14.00
44	Yes	No	1000	15.15	2	18.30	9.15	14.75
45	No	No	500	7.58	1	18.00	10.00	16.00
46	No	No	500	9.62	1	18.00	11.00	17.00
47	Yes	No	400	5.56	1	23.00	11.00	12.00
48	No	No	500	8.33	1	22.00	11.20	13.33
49	Yes	No	500	5.95	1	18.00	9.35	15.58
50	No	No	250	4.63	1	19.00	10.00	15.00
51	Yes	No	500	10.00	1	21.00	11.00	14.00
52	Yes	No	1000	15.38	2	21.00	9.00	12.00
53	No	No	750	17.44	1	21.00	9.40	12.66
54	No	No	1000	16.95	2	22.00	11.30	13.50
55	No	Yes	1000	17.15	1	20.00	11.15	15.25
56	No	No	750	11.72	2	20.30	12.00	16.50
57	Yes	No	400	6.15	2	19.00	8.45	12.75
58	No	No	600	13.64	3	6.00	11.10	5.17
59	No	No	500	6.49	1	22.00	9.50	11.83
60	Yes	No	500	10.20	1	21.00	11.15	14.25
61	No	Yes	1000	9.62	2	7.30	10.44	3.23
62	No	No	1000	12.99	4	22.00	8.30	10.50
63	No	No	500	8.20	1	19.00	9.00	14.00
64	Yes	No	500	6.76	1	19.00	10.15	15.25
65	Yes	No	500	7.35	1	21.00	11.00	14.00
66	Yes	No	750	15.63	2	8.00	8.45	0.75
67	No	No	400	4.60	1	22.00	9.30	11.50
68	No	No	1500	23.08	1	23.00	9.00	10.00
69	No	No	2000	27.78	3	7.00	10.30	3.50
70	No	No	600	12.50	3	22.00	11.00	13.00
71	No	Yes	500	8.62	1	20.00	11.00	15.00
72	Yes	No	1500	25.34	2	20.00	8.00	12.00
73	Yes	No	1000	13.89	2	2.00	10.10	8.17
74	No	Yes	800	9.09	2	9.30	10.00	0.50
75	Yes	Yes	500	6.67	1	20.00	11.00	15.00
76	No	Yes	1000	20.00	2	8.00	11.40	3.66
77	No	Yes	1000	14.49	2	20.00	8.50	12.83
78	No	No	750	11.36	1	20.00	8.00	12.00
79	Yes	Yes	500	7.14	1	20.00	11.00	15.00
80	No	Yes	500	5.68	1	21.00	9.30	12.50
81	Yes	No	500	3.85	1	19.00	9.45	14.75
82	No	No	1000	17.09	2	21.00	10.15	13.25
83	No	No	500	7.58	1	18.00	11.10	17.16

No of patients	Coffee consuming	Smoking	VPA dose (mg/day)	VPA dose (mg/kg/day)	Frequency	Time of last dose at	Sampling time at	Time from last dose (hr)
84	Yes	Yes	500	10.20	1	21.30	11.45	14.25
85	No	No	200	3.17	1	22.00	9.45	11.75
86	No	No	1000	19.05	1	15.30	9.45	18.25
87	Yes	No	500	8.40	1	21.00	10.30	13.50
88	Yes	Yes	250	3.82	1	20.00	11.30	15.50
89	No	No	500	7.46	1	21.00	11.15	14.25
90	No	No	1000	17.24	2	8.00	12.00	4.00
91	Yes	Yes	500	7.25	1	20.00	10.10	14.16
92	No	Yes	250	4.63	1	23.00	11.30	12.50
93	No	Yes	1000	15.04	2	21.00	9.25	12.42
94	Yes	No	1000	22.73	2	9.00	11.00	2.00
95	Yes	No	1000	12.20	2	21.00	10.05	13.00
96	No	No	200	4.65	1	18.00	10.00	16.00
97	No	Yes	750	7.01	1	20.00	9.30	13.50
98	No	Yes	500	5.32	1	19.30	9.45	14.25
99	No	Yes	1000	14.93	1	22.00	10.30	12.50

No of patients	C_{total} (mg/l)/(mg/kg)	C_{free} (mg/l)/(mg/kg)	C_{total} (mg/l)	C_{free} (mg/l)	Fu	% free VPA	CL_{total} (l/h)	CL_{free} (l/h)
1	4.99	1.08	48.95	10.63	0.22	21.72	0.37	1.69
2	4.78	0.56	46.85	5.44	0.12	11.61	0.38	3.29
3	1.93	0.15	34.44	2.75	0.08	7.98	1.04	13.03
4	3.56	0.68	47.39	9.11	0.19	19.22	0.76	3.93
5	4.50	0.51	59.94	6.78	0.11	11.31	0.6	5.29
6	4.47	0.55	78.49	9.58	0.12	12.21	0.46	3.74
7	3.41	0.38	59.82	6.63	0.11	11.08	0.6	5.4
8	2.48	0.38	82.6	12.63	0.15	15.29	0.65	4.26
9	2.39	0.40	79.65	13.34	0.17	16.75	0.67	4.03
10	8.39	0.76	78.42	7.14	0.09	9.1	0.46	5.02
11	3.96	0.32	32.48	2.59	0.08	7.97	0.55	6.92
12	3.65	0.23	29.96	1.87	0.06	6.24	0.6	9.58
13	3.73	0.36	62.21	6.04	0.1	9.71	0.58	5.93
14	4.09	0.57	74.38	10.34	0.14	13.9	0.48	3.47
15	3.45	0.50	62.69	9.04	0.14	14.42	0.57	3.96
16	6.21	0.77	75.82	9.35	0.12	12.33	0.47	3.83
17	5.87	0.63	71.58	7.64	0.11	10.67	0.5	4.69
18	5.38	0.80	79.09	11.72	0.15	14.82	0.45	3.06
19	6.00	0.54	54.58	4.87	0.09	8.92	0.39	4.41
20	6.66	0.47	46.74	3.29	0.07	7.04	0.31	4.36
21	4.03	0.35	52.51	4.5	0.09	8.57	0.41	4.78
22	3.42	0.54	54.23	8.52	0.16	15.71	0.66	4.21
23	4.62	0.33	64.12	4.62	0.07	7.21	0.56	7.76
24	4.72	0.57	67.39	8.17	0.12	12.12	0.53	4.39

No of patients	C_{total} (mg/l)/(mg/kg)	C_{free} (mg/l)/(mg/kg)	C_{total} (mg/l)	C_{free} (mg/l)	Fu	% free VPA	CL_{total} (l/h)	CL_{free} (l/h)
25	3.60	0.47	87.18	11.48	0.13	13.17	0.62	4.68
26	6.82	0.92	94.71	12.78	0.13	13.49	0.38	2.8
27	7.00	0.67	62.69	6.02	0.1	9.6	0.34	3.57
28	3.34	0.33	54.29	5.39	0.1	9.93	0.66	6.65
29	7.00	0.57	52.21	4.28	0.08	8.2	0.34	4.19
30	1.66	0.20	38.27	4.69	0.12	12.26	1.4	11.46
31	5.11	0.48	96.52	9.14	0.09	9.47	0.37	3.92
32	8.17	0.60	48.64	3.59	0.07	7.38	0.37	4.99
33	3.30	0.24	56.82	4.12	0.07	7.25	0.63	8.7
34	4.77	0.22	91.67	4.26	0.05	4.65	0.49	10.51
35	4.66	0.73	61.03	9.54	0.16	15.63	0.47	3
36	2.56	0.22	15.98	1.4	0.09	8.76	1.12	12.8
37	4.83	0.41	60.35	5.17	0.09	8.57	0.59	6.93
38	7.98	0.88	122.77	13.6	0.11	11.08	0.29	2.63
39	5.27	0.64	112.24	13.6	0.12	12.12	0.32	2.63
40	2.65	0.23	32.28	2.82	0.09	8.74	1.11	12.71
41	3.78	0.22	23.99	1.42	0.06	5.92	0.6	10.09
42	3.12	0.21	25.44	1.74	0.07	6.84	1.13	16.48
43	2.45	0.18	29.18	2.18	0.07	7.47	1.23	16.44
44	2.99	0.23	45.31	3.51	0.08	7.75	0.79	10.21
45	6.28	0.40	47.62	3.02	0.06	6.34	0.38	5.93
46	3.54	0.18	34.05	1.76	0.05	5.17	0.53	10.18
47	7.55	0.49	41.99	2.72	0.06	6.48	0.34	5.27
48	5.59	0.36	46.53	3.02	0.06	6.49	0.39	5.93
49	5.31	0.41	31.61	2.42	0.08	7.66	0.57	7.4
50	7.31	0.42	33.83	1.95	0.06	5.76	0.26	4.59
51	7.68	0.62	76.75	6.18	0.08	8.05	0.23	2.9
52	4.09	0.58	62.96	8.87	0.14	14.09	0.57	4.04
53	5.94	0.73	103.54	12.72	0.12	12.29	0.26	2.11
54	4.69	0.43	79.54	7.34	0.09	9.23	0.45	4.88
55	3.50	0.31	60.08	5.35	0.09	8.9	0.6	6.7
56	2.55	0.14	29.91	1.6	0.05	5.35	0.9	16.8
57	3.12	0.25	19.21	1.52	0.08	7.91	0.75	9.43
58	6.13	0.43	83.67	5.89	0.07	7.04	0.26	3.65
59	9.37	0.74	60.83	4.8	0.08	7.89	0.29	3.73
60	5.04	0.42	51.42	4.28	0.08	8.32	0.35	4.19
61	5.56	0.42	53.51	4.07	0.08	7.61	0.67	8.8
62	5.62	0.58	73.04	7.54	0.1	10.32	0.49	4.75
63	3.89	0.27	31.87	2.25	0.07	7.06	0.56	7.96
64	12.02	1.20	81.25	8.11	0.1	9.98	0.22	2.21
65	10.76	1.19	79.11	8.75	0.11	11.06	0.23	2.05
66	3.83	0.45	59.8	7.02	0.12	11.74	0.45	3.83

No of patients	C_{total} (mg/l)/(mg/kg)	C_{free} (mg/l)/(mg/kg)	C_{total} (mg/l)	C_{free} (mg/l)	Fu	% free VPA	CL_{total} (l/h)	CL_{free} (l/h)
67	7.54	0.60	34.7	2.77	0.08	7.98	0.41	5.17
68	6.22	1.41	143.59	32.62	0.23	22.72	0.37	1.65
69	4.29	0.73	119.31	20.34	0.17	17.05	0.6	3.52
70	5.00	0.34	62.48	4.29	0.07	6.87	0.34	5.01
71	7.17	0.63	61.84	5.45	0.09	8.81	0.29	3.29
72	4.06	0.48	102.91	12.28	0.12	11.93	0.52	4.38
73	5.13	0.58	71.28	8.01	0.11	11.24	0.5	4.47
74	7.87	0.68	71.53	6.2	0.09	8.67	0.4	4.62
75	5.02	0.38	33.47	2.55	0.08	7.62	0.54	7.03
76	3.22	0.30	64.41	5.93	0.09	9.21	0.56	6.04
77	2.91	0.20	42.12	2.9	0.07	6.89	0.85	12.36
78	5.49	0.50	62.34	5.64	0.09	9.05	0.43	4.77
79	5.66	0.36	40.39	2.59	0.06	6.41	0.44	6.92
80	8.30	0.58	47.15	3.31	0.07	7.02	0.38	5.41
81	11.42	0.76	43.97	2.92	0.07	6.64	0.41	6.14
82	6.96	1.11	118.9	18.92	0.16	15.91	0.3	1.89
83	7.04	0.63	53.34	4.75	0.09	8.91	0.34	3.77
84	4.30	0.33	43.82	3.34	0.08	7.62	0.41	5.36
85	6.04	0.46	19.14	1.45	0.08	7.58	0.37	4.94
86	3.84	0.37	73.06	6.99	0.1	9.57	0.49	5.13
87	8.47	0.60	71.14	5.02	0.07	7.06	0.25	3.57
88	7.89	0.56	30.14	2.13	0.07	7.07	0.3	4.21
89	8.51	0.54	63.46	4.04	0.06	6.37	0.28	4.43
90	3.99	0.34	68.81	5.94	0.09	8.63	0.52	6.03
91	7.93	0.58	57.5	4.24	0.07	7.37	0.31	4.23
92	4.05	0.29	18.74	1.34	0.07	7.15	0.48	6.69
93	3.75	0.40	56.33	5.96	0.11	10.58	0.64	6.01
94	2.37	0.22	53.8	4.96	0.09	9.22	0.67	7.22
95	6.21	0.63	75.77	7.72	0.1	10.19	0.47	4.64
96	7.32	0.52	34.06	2.43	0.07	7.13	0.21	2.95
97	8.10	0.61	56.77	4.29	0.08	7.56	0.47	6.26
98	13.22	1.06	70.33	5.63	0.08	8.01	0.25	3.18
99	4.13	0.33	61.62	4.9	0.08	7.95	0.58	7.31

No of patients	CL_{total} (l/kg/h)	CL_{free} (l/kg/h)	CL_{total} (ml/kg/h)	CL_{free} (ml/kg/h)	C_{saliva} (mg/l)	Albumin (gm/dl)
1	0.0072	0.0331	7.1769	33.0487	NA	4.20
2	0.0075	0.0646	7.4986	64.5785	NA	4.20
3	0.0186	0.2327	18.5796	232.6840	NA	3.80
4	0.0101	0.0525	10.0818	52.4454	NA	4.90
5	0.0080	0.0705	7.9709	70.4687	NA	4.90
6	0.0080	0.0656	8.0094	65.6216	NA	4.10
7	0.0105	0.0948	10.5091	94.8198	NA	4.10
8	0.0145	0.0946	14.4606	94.5720	NA	4.00
9	0.0150	0.0895	14.9962	89.5386	NA	4.00
10	0.0043	0.0469	4.2705	46.9035	NA	4.50
11	0.0090	0.1134	9.0430	113.4038	NA	4.40
12	0.0098	0.1571	9.8036	157.0673	NA	4.40
13	0.0096	0.0989	9.6001	98.8779	NA	3.90
14	0.0088	0.0630	8.7593	63.0092	NA	3.60
15	0.0104	0.0721	10.3927	72.0703	NA	3.60
16	0.0058	0.0467	5.7635	46.7371	NA	4.80
17	0.0061	0.0572	6.1049	57.1979	NA	4.80
18	0.0067	0.0450	6.6628	44.9625	NA	3.70
19	0.0060	0.0669	5.9684	66.8907	NA	4.50
20	0.0054	0.0764	5.3800	76.4322	NA	4.10
21	0.0089	0.1039	8.9010	103.8647	NA	4.10
22	0.0105	0.0668	10.4884	66.7586	NA	4.20
23	0.0078	0.1077	7.7618	107.7241	NA	4.20
24	0.0076	0.0627	7.5962	62.6566	NA	4.70
25	0.0099	0.0755	9.9442	75.5170	NA	4.70
26	0.0053	0.0389	5.2548	38.9425	NA	4.90
27	0.0051	0.0533	5.1188	53.3049	NA	4.60
28	0.0107	0.1081	10.7323	108.0994	NA	4.10
29	0.0051	0.0625	5.1219	62.4797	NA	5.00
30	0.0216	0.1763	21.6076	176.3162	NA	4.40
31	0.0070	0.0740	7.0048	73.9716	NA	4.00
32	0.0044	0.0594	4.3852	59.4133	NA	3.90
33	0.0109	0.1500	10.8732	149.9554	NA	4.20
34	0.0075	0.1618	7.5172	161.7612	NA	4.40
35	0.0077	0.0493	7.7002	49.2605	NA	4.10
36	0.0140	0.1600	14.0149	159.9702	0.36	5.10
37	0.0074	0.0866	7.4220	86.6377	NA	4.90
38	0.0045	0.0405	4.4904	40.5354	3.71	4.70
39	0.0068	0.0561	6.7927	56.0597	1.72	3.70
40	0.0135	0.1550	13.5375	154.9617	NA	4.40
41	0.0095	0.1602	9.4837	160.2206	0.13	5.30
42	0.0115	0.1681	11.4983	168.1132	0.1	4.90
43	0.0146	0.1957	14.6192	195.6823	0.13	4.60
44	0.0120	0.1547	11.9826	154.6807	0.43	4.20
45	0.0057	0.0899	5.7006	89.8890	NA	4.60
46	0.0101	0.1958	10.1190	195.7678	NA	4.20
47	0.0047	0.0732	4.7410	73.1890	NA	4.90

No of patients	CL_{total} (l/kg/h)	CL_{free} (l/kg/h)	CL_{total} (ml/kg/h)	CL_{free} (ml/kg/h)	C_{saliva} (mg/l)	Albumin (gm/dl)
48	0.0064	0.0989	6.4176	98.8779	NA	4.60
49	0.0068	0.0881	6.7477	88.1379	NA	4.50
50	0.0049	0.0851	4.9038	85.0744	NA	4.80
51	0.0047	0.0580	4.6688	57.9827	NA	4.70
52	0.0088	0.0622	8.7561	62.1513	NA	3.80
53	0.0060	0.0491	6.0363	49.1352	NA	4.30
54	0.0076	0.0827	7.6357	82.7445	NA	4.30
55	0.0102	0.1149	10.2303	114.8854	NA	4.60
56	0.0140	0.2625	14.0395	262.4512	NA	5.30
57	0.0115	0.1451	11.4791	145.0742	NA	4.60
58	0.0058	0.0830	5.8400	82.9603	NA	4.80
59	0.0038	0.0485	3.8252	48.4758	NA	4.40
60	0.0071	0.0854	7.1110	85.4314	NA	4.30
61	0.0064	0.0847	6.4390	84.6563	NA	4.40
62	0.0064	0.0617	6.3714	61.7199	NA	4.60
63	0.0092	0.1305	9.2161	130.5404	NA	4.60
64	0.0030	0.0299	2.9799	29.8542	1.65	4.50
65	0.0033	0.0301	3.3306	30.1120	0.3	4.20
66	0.0094	0.0798	9.3628	79.7572	0.76	4.60
67	0.0048	0.0595	4.7479	59.4769	0.41	4.20
68	0.0058	0.0254	5.7589	25.3502	NA	4.00
69	0.0083	0.0489	8.3427	48.9366	1.4	4.20
70	0.0072	0.1044	7.1690	104.4095	NA	4.50
71	0.0050	0.0567	4.9953	56.6804	0.8	3.70
72	0.0088	0.0739	8.8227	73.9364	NA	4.60
73	0.0070	0.0621	6.9821	62.1330	0.61	4.30
74	0.0046	0.0525	4.5541	52.5415	1.67	4.60
75	0.0071	0.0937	7.1374	93.6819	0.44	4.90
76	0.0111	0.1209	11.1266	120.8544	1.39	4.10
77	0.0123	0.1791	12.3296	179.0771	NA	4.50
78	0.0065	0.0722	6.5319	72.1980	NA	4.30
79	0.0063	0.0988	6.3370	98.8233	NA	4.30
80	0.0043	0.0615	4.3181	61.5101	NA	4.60
81	0.0031	0.0472	3.1344	47.1988	NA	4.70
82	0.0052	0.0324	5.1517	32.3750	NA	4.00
83	0.0051	0.0572	5.0893	57.1505	NA	4.30
84	0.0083	0.1095	8.3443	109.4749	NA	4.20
85	0.0059	0.0785	5.9434	78.4528	NA	4.90
86	0.0093	0.0977	9.3422	97.6452	0.48	4.60
87	0.0042	0.0600	4.2328	59.9842	1.25	4.80
88	0.0045	0.0642	4.5378	64.2105	1.00	4.20
89	0.0042	0.0662	4.2139	66.1913	NA	4.80
90	0.0090	0.1040	8.9786	104.0094	NA	4.10
91	0.0045	0.0612	4.5159	61.2410	1.57	4.51
92	0.0089	0.1238	8.8525	123.8023	0.36	4.40
93	0.0096	0.0904	9.5659	90.4106	NA	4.70

No of patients	CL_{total} (l/kg/h)	CL_{free} (l/kg/h)	CL_{total} (ml/kg/h)	CL_{free} (ml/kg/h)	C_{saliva} (mg/l)	Albumin (gm/dl)
94	0.0151	0.1642	15.1374	164.1923	NA	4.00
95	0.0058	0.0566	5.7674	56.6052	NA	4.20
96	0.0049	0.0686	4.8933	68.5871	NA	4.00
97	0.0044	0.0586	4.4243	58.5474	NA	4.50
98	0.0027	0.0339	2.7101	33.8549	0.16	5.00
99	0.0087	0.1092	8.6794	109.1481	0.35	4.50

APPENDIX B : The effects of gender and excess weight on VPA pharmacokinetics performed using Two-way ANOVA.

Focusing on both gender and excess weight influence sodium valproate pharmacokinetics in adults patients is not known. The purpose of this section of analysis was to determine their effects on valproate pharmacokinetics performed using two way ANOVA. Due to highly variation in some group and the assumption of Two way did not met, so the results were placed in the appendix B.

Results

A total of 99 patients with neuro-psychiatric were classified according to gender and BMI into four subgroups. Two way ANOVA testing for both effects of gender and excess weight on VPA pharmacokinetic parameters.

Dependent variable (Y) continuous type	Independent variables (X) : category type
C_{total} (mg/l)	Factor 1: gender • female • male
C_{total} (mg/l per mg/kg)	
C_{free} (mg/l)	
C_{free} (mg/l per mg/kg)	
Fu	
%free VPA	Factor 2: excess weight • normal weight • OW/OB
CL_{total} (l/h)	
CL_{total} (l/ kg/h)	
CL_{free} (l/h)	
CL_{free} (l/ kg/h)	

Step 1 Assumption test

1. The distribution of dependent variables of each group must have normal distribution.
2. The Variance of dependent variables of each group should equally.
3. No association between Dependent variable (DV) and Independent variable (IV)

Number of subjects in each cell (n=99)

Factors		Excess weight	
		Normal weight group (BMI<25 kg/m^2) n=58	OW/OB group (BMI≥25 kg/m^2) n=41
Gender	Female; n=35	n=23	n=12
	Male; n=64	n=35	n=29

One-Sample Kolmogorov-Smirnov Test (n=99)

Dependent variables	Normal Parameters		Kolmogorov-Smirnov Z	Asymp. Sig. (2-tailed)	Normal distribution was assumed
	Mean	Std. Deviation			
Age; years	39.25	10.88	.670	.760	Yes
Height; cm	162.07	7.38	1.351	.052	Yes
Weight; kg	66.21	15.41	1.253	.087	Yes
BMI; kg/m2	25.10	4.95	1.046	.224	Yes
Albumin; gm/dl	4.41	0.37	.889	.408	Yes
Dosage; mg/day	793.43	340.14	2.120	.000	No
Dosage; mg/kg/day	12.56	6.21	1.022	.248	Yes
C_{total}; mg/l	59.86	24.48	.925	.359	Yes
C_{total}; mg/l per mg/kg	5.36	2.24	.954	.323	Yes
C_{free}; mg/l	6.27	4.62	1.514	.020	No
C_{free}; mg/l per mg/kg	.51	.25	1.037	.233	Yes
Fu	.10	.03	1.956	.001	No
Percentage Free VPA	9.71	3.46	1.600	.012	No
CL_{total}; l/h	.51	.22	1.408	.038	No
CL_{total}; ml/kg/h	7.911	3.42	.931	.352	Yes
CL_{free}; l/h	5.781	3.19	1.818	.003	No
CL_{free}; ml/kg/h	88.89	46.17	1.326	.059	Yes

Norrmality test of each subgroups by using Kolmogorov-Smirnov Test

Factors / Dependent variables	Gender	Normal weight group (BMI<25 kg/m^2)		OW/OB group (BMI≥25 kg/m^2)	
		p-value	Normal distribution assumed	p-value	Normal distribution assumed
Age; years	Female	.057	Yes	.200*	Yes
	Male	.200*	Yes	.150	Yes
Height; cm	Female	.042	No	.050	No
	Male	.001	No	.002	No
Weight; kg	Female	.114	Yes	.014	No
	Male	.200*	Yes	.007	No
BMI; kg/m2	Female	.130	Yes	.087	Yes
	Male	.190	Yes	.016	No
Albumin; gm/dl	Female	.200*	Yes	.200*	Yes
	Male	.091	Yes	.200*	Yes
Dosage; mg/day dl	Female	.000	No	.016	No
	Male	.000	No	.000	No
Dosage; mg/kg/day	Female	.060	Yes	.063	Yes
	Male	.151	Yes	.189	Yes

*. This is a lower bound of the true significance

OW/OB-overweight/obese.

Norrmality test of each subgroups by using Kolmogorov-Smirnov Test (cont)

Factors / Dependent variables	Gender	Normal weight group (BMI<25 kg/m^2)		OW/OB group (BMI≥25 kg/m^2)	
		p-value	Normal distribution assumed	p-value	Normal distribution assumed
Ctotal; mg/l	Female	.077	Yes	.137	Yes
	Male	.200*	Yes	.200*	Yes
Ctotal; mg/l per mg/kg	Female	.176	Yes	.200*	Yes
	Male	.043	No	.105	Yes
Cfree; mg/l	Female	.003	No	.089	Yes
	Male	.027	No	.200*	Yes
Cfree; mg/l per mg/kg	Female	.004	No	.113	Yes
	Male	.200*	Yes	.200*	Yes
Fu	Female	.029	No	.200*	Yes
	Male	.000	No	.022	No
Percentage of Free VPA	Female	.138	Yes	.200*	Yes
	Male	.001	No	.046	No
CLtotal; l/h	Female	.200*	Yes	.200*	Yes
	Male	.200*	Yes	.001	No
CLtotal; ml/kg/h	Female	.200*	Yes	.200*	Yes
	Male	.200*	Yes	.002	No
CLfree; l/h	Female	.200*	Yes	.200*	Yes
	Male	.005	No	.001	No
CLfree; ml/kg/h	Female	.200*	Yes	.120	Yes
	Male	.019	No	.000	No

*. This is a lower bound of the true significance.

Levene's Test of Equality of Error Variances

Dependent variables	F	df1	df2	Sig.	Equally variance assume
Age; years	4.111	3	95	.009	No
Height; cm	.448	3	95	.719	Yes
Weight; kg	3.862	3	95	.012	No
BMI; kg/m2	4.308	3	95	.007	No
Albumin; gm/dl	2.068	3	95	.110	Yes
Dosage; mg/day	4.566	3	95	.005	No
Dosage; mg/kg/day	3.640	3	95	.016	No
C_{total}; mg/l	1.159	3	95	.330	Yes
C_{total}; mg/l per mg/kg	4.140	3	95	.008	No
C_{free}; mg/l	5.984	3	95	.001	No
C_{free}; mg/l per mg/kg	.983	3	95	.404	Yes
Fu	2.526	3	95	.062	Yes
Percentage Free VPA	2.157	3	95	.098	Yes
CL_{total}; l/h	5.969	3	95	.001	No
CL_{total}; ml/kg/h	3.104	3	95	.030	No
CL_{free}; l/h	5.201	3	95	.002	No
CL_{free}; ml/kg/h	3.059	3	95	.032	No

Tests the null hypothesis that the error variance of the dependent variable is equal across groups. a. Design: Intercept + gender + BMIgr2 + gender * BMIgr2

Conclusion of Assumption test for Two way ANOVA (n=99)

Dependent variables	One-Sample Kolmogorov-Smirnov Test		Levene's Test		Two way ANOVA	Friedman test
	Sig. (2-tailed)	Normal distribution assume	Sig. (2-tailed)	Equally variance assume		
Age; years	.760	Yes	.009	No		Yes
Height; cm	.052	Yes	.719	Yes	Yes	
Weight; kg	.087	Yes	.012	No		Yes
BMI; kg/m2	.224	Yes	.007	No		Yes
Albumin; gm/dl	.408	Yes	.110	Yes	Yes	
Dosage; mg/day	.000	No	.005	No		Yes
Dosage; mg/kg/day	.248	Yes	.016	No		Yes
C_{total}; mg/l	.359	Yes	.330	Yes	Yes	
C_{total}; mg/l per mg/kg	.323	Yes	.008	No		Yes
C_{free}; mg/l	.020	No	.001	No		Yes
C_{free}; mg/l per mg/kg	.233	Yes	.404	Yes	Yes	
Fu	.001	No	.062	Yes		Yes
Percentage Free VPA	.012	No	.098	Yes		Yes
CL_{total}; l/h	.038	No	.001	No		Yes
CL_{total}; ml/kg/h	.352	Yes	.030	No		Yes
CL_{free}; l/h	.003	No	.002	No		Yes
CL_{free}; ml/kg/h	.059	Yes	.032	No		Yes

Means of dependent variables (characteristics of patients) in each cells.				
Factors / Dependent variables	Gender	Normal weight group (BMI<25 kg/m^2)	OW/OB group (BMI≥25 kg/m^2)	Total
		Mean±SD	Mean±SD	Mean±SD
Age; years	Female	40.70±13.31	43.50±7.31	41.66±11.56
	Male	36.09±9.83	40.17±10.69	37.94±10.35
	Total	37.91±11.45	41.15±9.85	39.25±10.88
Height; cm	Female	155.83±4.96	155.75±4.54	155.80±4.75
	Male	165.63±5.80	165.34±6.80	165.50±6.22
	Total	161.74±7.28	162.54±7.59	162.07±7.38
Weight; kg	Female	52.88±6.77	68.43±7.44	58.21±10.18
	Male	60.40±7.63	82.86±15.00	70.58±16.08
	Total	57.42±8.13	78.64±14.73	66.21±15.41
BMI; kg/m^2	Female	21.74±2.19	28.23±3.01	23.96±3.98
	Male	22.01±2.45	30.20±4.32	25.72±5.33
	Total	21.90±2.34	29.62±4.05	25.10±4.95
Albumin; gm/dl	Female	4.29±0.29	4.26±0.32	4.28±0.30
	Male	4.36±0.41	4.63±0.30	4.48±0.39
	Total	4.33±0.37	4.52±0.35	4.41±0.37
Dosage; mg/day	Female	739.13±286.42	887.50±524.03	790.00±383.44
	Male	782.86±354.99	810.34±270.05	795.31±317.19
	Total	765.52±327.57	832.93±357.53	793.43±340.14
Dosage; mg/kg/day	Female	13.98±5.16	13.28±8.04	13.74±6.19
	Male	13.40±7.20	10.12±4.09	11.92±6.18
	Total	13.63±6.43	11.04±5.62	12.56±6.21
Ctotal; mg/l	Female	63.70±24.87	74.46±33.12	67.39±27.96
	Male	58.02±23.18	52.99±19.29	55.74±21.49
	Total	60.28±23.81	59.27±25.69	59.86±24.48

Means of dependent variables (pharmacokinetic parameters) in each cells

Factors / Dependent variables	Gender	Normal weight group (BMI<25 kg/m^2)	OW/OB group (BMI≥25 kg/m^2)	Total
		Mean±SD	Mean±SD	Mean±SD
Ctotal; mg/l per mg/kg	Female	4.78±1.30	6.39±2.29	5.33±1.84
	Male	4.93±1.89	5.91±2.94	5.37±2.45
	Total	4.87±1.67	6.05±2.74	5.36±2.24
Cfree; mg/l	Female	6.47±4.16	9.90±8.80	7.65±6.24
	Male	5.92±3.73	5.01±2.52	5.51±3.24
	Total	6.14±3.88	6.44±5.55	6.27±4.62
Cfree; mg/l per mg/kg	Female	0.47±0.24	0.71±0.31	0.55±0.29
	Male	0.45±0.19	0.53±0.25	0.49±0.22
	Total	0.46±0.21	0.58±0.28	0.51±0.25
Fu	Female	0.10±0.04	0.12±0.05	0.10±0.04
	Male	0.09±0.03	0.09±0.03	0.09±0.03
	Total	0.10±0.03	0.10±0.04	0.10±0.03
Percentage of Free VPA	Female	9.73±3.72	11.58±4.85	10.36±4.17
	Male	9.51±3.22	9.16±2.69	9.35±2.98
	Total	9.59±3.40	9.87±3.58	9.71±3.46
CLtotal; l/h	Female	0.43±0.13	0.42±0.12	0.43±0.13
	Male	0.50±0.18	0.62±0.31	0.56±0.25
	Total	0.47±0.17	0.56±0.28	0.51±0.22
CLtotal; ml/kg/h	Female	8.06±2.31	6.27±2.10	7.45±2.37
	Male	8.44±3.42	7.84±4.39	8.17±3.87
	Total	8.29±3.01	7.38±3.90	7.91±3.42
CLfree; l/h	Female	4.94±2.27	4.04±1.61	4.64±2.09
	Male	5.70±2.72	7.26±4.16	6.41±3.51
	Total	5.40±2.56	6.32±3.88	5.78±3.19
CLfree; ml/kg/h	Female	92.02±35.89	59.96±26.88	81.03±36.14
	Male	95.26±45.46	90.68±56.87	93.19±50.58
	Total	93.98±41.63	81.68±51.60	88.89±46.17

Two way ANOVA tests of patient' s characteristics.

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	Age; year	639.937[a]	3	213.312	1.848	.144	.055	5.544	.466
	Height; cm	2130.228[c]	3	710.076	21.039	.000	.399	63.117	1.000
	Weight; kg	13367.477[d]	3	4455.826	42.783	.000	.575	128.348	1.000
	BMI; kg/m^2	1465.415[e]	3	488.472	49.729	.000	.611	149.188	1.000
	Albumin; gm/dl	2.060[f]	3	.687	5.786	.001	.154	17.357	.943
	Dosage; mg/day	186213.877[g]	3	62071.292	.529	.664	.016	1.586	.155
	Dosage; mg/kg/day	250.108[h]	3	83.369	2.245	.088	.066	6.734	.552
Intercept	Age; year	135597.976	1	135597.976	1.175E3	.000	.925	1174.838	1.000
	Height; cm	2174535.290	1	2174535.29	6.443E4	.000	.999	64430.123	1.000
	Weight; kg	368689.520	1	368689.520	3.540E3	.000	.974	3539.965	1.000
	BMI; kg/m^2	54985.094	1	54985.094	5.598E3	.000	.983	5597.795	1.000
	Albumin; gm/dl	1619.903	1	1619.903	1.365E4	.000	.993	13652.177	1.000
	Dosage; mg/day	5.460E7	1	5.460E7	465.147	.000	.830	465.147	1.000
	Dosage; mg/kg/day	13583.503	1	13583.503	365.726	.000	.794	365.726	1.000
Gender	Age; year	331.836	1	331.836	2.875	.093	.029	2.875	.389
	Height; cm	1981.693	1	1981.693	58.716	.000	.382	58.716	1.000
	Weight; kg	2536.700	1	2536.700	24.356	.000	.204	24.356	.998
	BMI; kg/m^2	26.625	1	26.625	2.711	.103	.028	2.711	.371
	Albumin; gm/dl	1.012	1	1.012	8.525	.004	.082	8.525	.824
	Dosage; mg/day	5885.538	1	5885.538	.050	.823	.001	.050	.056
	Dosage; mg/kg/day	73.443	1	73.443	1.977	.163	.020	1.977	.285
BMIgroup	Age; year	250.106	1	250.106	2.167	.144	.022	2.167	.308
	Height; cm	.682	1	.682	.020	.887	.000	.020	.052
	Weight; kg	7610.494	1	7610.494	73.072	.000	.435	73.072	1.000
	BMI; kg/m^2	1134.416	1	1134.416	115.490	.000	.549	115.490	1.000
	Albumin; gm/dl	.289	1	.289	2.437	.122	.025	2.437	.339
	Dosage; mg/day	162882.285	1	162882.285	1.388	.242	.014	1.388	.215
	Dosage; mg/kg/day	83.732	1	83.732	2.254	.137	.023	2.254	.318

Two way ANOVA tests of patient' s characteristics. (Cont)

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Gender * BMIgroup	Age; year	8.661	1	8.661	.075	.785	.001	.075	.058
	Height; cm	.227	1	.227	.007	.935	.000	.007	.051
	Weight; kg	251.581	1	251.581	2.416	.123	.025	2.416	.337
	BMI; kg/m^2	15.251	1	15.251	1.553	.216	.016	1.553	.234
	Albumin; gm/dl	.475	1	.475	4.002	.048	.040	4.002	.508
	Dosage; mg/day	76961.888	1	76961.888	.656	.420	.007	.656	.126
	Dosage; mg/kg/day	35.024	1	35.024	.943	.334	.010	.943	.161
Error	Age; year	10964.750	95	115.418					
	Height; cm	3206.278	95	33.750					
	Weight; kg	9894.308	95	104.151					
	BMI; kg/m^2	933.150	95	9.823					
	Albumin; gm/dl	11.272	95	.119					
	Dosage; mg/day	1.115E7	95	117389.668					
	Dosage; mg/kg/day	3528.413	95	37.141					
Total	Age; year	164140.000	99						
	Height; cm	2605761.000	99						
	Weight; kg	457216.030	99						
	BMI; kg/m^2	64769.187	99						
	Albumin; gm/dl	1938.870	99						
	Dosage; mg/day	7.366E7	99						
	Dosage; mg/kg/day	19399.142	99						
Corrected Total	Age; year	11604.687	98						
	Height; cm	5336.505	98						
	Weight; kg	23261.785	98						
	BMI; kg/m^2	2398.565	98						
	Albumin; gm/dl	13.332	98						
	Dosage; mg/day	1.134E7	98						
	Dosage; mg/kg/day	3778.521	98						

a. R Squared = .055 (Adjusted R Squared = .025); b. Computed using alpha = .05; c. R Squared = .399 (Adjusted R Squared = .380); d. R = .575 (Adjusted R Squared = .561); e. R Squared = .611 (Adjusted R Squared = .599); f. R Squared = .154 (Adjusted R Squared = .128); g. ed = .016 (Adjusted R Squared = -.015); h. R Squared = .066 (Adjusted R Squared = .037)

Two way ANOVA tests of pharmacokinetic parameters.

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	Ctotal; mg/l	4383.956[a]	3	1461.319	2.554	.060	.075	7.661	.613
	Ctotal; mg/l per mg/kg	35.469[c]	3	11.823	2.452	.068	.072	7.356	.594
	Cfree; mg/l	209.460[d]	3	69.820	3.525	.018	.100	10.575	.768
	Cfree; mg/l per mg/kg	.633[e]	3	.211	3.792	.013	.107	11.376	.801
	Fu	.005[f]	3	.002	1.490	.222	.045	4.469	.383
	Percentage Free VPA	52.204[g]	3	17.401	1.477	.226	.045	4.430	.380
	CLtotal; l/h	.616[h]	3	.205	4.492	.005	.124	13.476	.869
	CLtotal; ml/kg/h	42.689[i]	3	14.230	1.226	.305	.037	3.677	.319
	CLfree; l/h	115.891[j]	3	38.630	4.176	.008	.117	12.529	.841
	CLfree; ml/kg/h	11785.431[k]	3	3928.477	1.893	.136	.056	5.680	.476
Intercept	Ctotal; mg/l	327014.283	1	327014.283	571.424	.000	.857	571.424	1.000
	Ctotal; mg/l per mg/kg	2550.384	1	2550.384	528.937	.000	.848	528.937	1.000
	Cfree; mg/l	3928.333	1	3928.333	198.332	.000	.676	198.332	1.000
	Cfree; mg/l per mg/kg	24.327	1	24.327	437.139	.000	.821	437.139	1.000
	Fu	.842	1	.842	703.724	.000	.881	703.724	1.000
	Percentage Free VPA	8413.419	1	8413.419	713.965	.000	.883	713.965	1.000
	CLtotal; l/h	20.462	1	20.462	447.972	.000	.825	447.972	1.000
	CLtotal; ml/kg/h	4937.129	1	4937.129	425.209	.000	.817	425.209	1.000
	CLfree; l/h	2537.538	1	2537.538	274.341	.000	.743	274.341	1.000
	CLfree; ml/kg/h	601410.985	1	601410.985	289.857	.000	.753	289.857	1.000
Gender	Ctotal; mg/l	3881.552	1	3881.552	6.783	.011	.067	6.783	.732
	Ctotal; mg/l per mg/kg	.553	1	.553	.115	.736	.001	.115	.063
	Cfree; mg/l	156.007	1	156.007	7.876	.006	.077	7.876	.793
	Cfree; mg/l per mg/kg	.199	1	.199	3.567	.062	.036	3.567	.464
	Fu	.004	1	.004	3.309	.072	.034	3.309	.437
	Percentage Free VPA	36.813	1	36.813	3.124	.080	.032	3.124	.417
	CLtotal; l/h	.390	1	.390	8.529	.004	.082	8.529	.824
	CLtotal; ml/kg/h	19.889	1	19.889	1.713	.194	.018	1.713	.254
	CLfree; l/h	83.198	1	83.198	8.995	.003	.086	8.995	.843
	CLfree; ml/kg/h	6075.623	1	6075.623	2.928	.090	.030	2.928	.395

Two way ANOVA tests of pharmacokinetic parameters. (Cont)

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
BMIgr	Ctotal; mg/l	172.406	1	172.406	.301	.584	.003	.301	.084
	Ctotal; mg/l per mg/kg	35.109	1	35.109	7.281	.008	.071	7.281	.761
	Cfree; mg/l	33.378	1	33.378	1.685	.197	.017	1.685	.250
	Cfree; mg/l per mg/kg	.522	1	.522	9.379	.003	.090	9.379	.858
	Fu	.001	1	.001	.929	.338	.010	.929	.159
	Percentage Free VPA	11.856	1	11.856	1.006	.318	.010	1.006	.168
	CLtotal; l/h	.069	1	.069	1.519	.221	.016	1.519	.230
	CLtotal; ml/kg/h	30.135	1	30.135	2.595	.110	.027	2.595	.358
	CLfree; l/h	2.270	1	2.270	.245	.621	.003	.245	.078
	CLfree; ml/kg/h	7075.123	1	7075.123	3.410	.068	.035	3.410	.448
Gender * BMIgr	Ctotal; mg/l	1313.452	1	1313.452	2.295	.133	.024	2.295	.323
	Ctotal; mg/l per mg/kg	2.139	1	2.139	.444	.507	.005	.444	.101
	Cfree; mg/l	99.195	1	99.195	5.008	.028	.050	5.008	.601
	Cfree; mg/l per mg/kg	.147	1	.147	2.636	.108	.027	2.636	.362
	Fu	.002	1	.002	2.031	.157	.021	2.031	.292
	Percentage Free VPA	25.448	1	25.448	2.160	.145	.022	2.160	.307
	CLtotal; l/h	.090	1	.090	1.964	.164	.020	1.964	.284
	CLtotal; ml/kg/h	7.363	1	7.363	.634	.428	.007	.634	.124
	CLfree; l/h	31.747	1	31.747	3.432	.067	.035	3.432	.450
	CLfree; ml/kg/h	3975.408	1	3975.408	1.916	.170	.020	1.916	.278
Error	Ctotal; mg/l	54366.595	95	572.280					
	Ctotal; mg/l per mg/kg	458.063	95	4.822					
	Cfree; mg/l	1881.652	95	19.807					
	Cfree; mg/l per mg/kg	5.287	95	.056					
	Fu	.114	95	.001					
	Percentage Free VPA	1119.487	95	11.784					
	CLtotal; l/h	4.339	95	.046					
	CLtotal; ml/kg/h	1103.052	95	11.611					
	CLfree; l/h	878.710	95	9.250					
	CLfree; ml/kg/h	197111.350	95	2074.856					

Two way ANOVA tests of pharmacokinetic parameters. (Cont)

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Total	Ctotal; mg/l	413501.264	99						
	Ctotal; mg/l per mg/kg	3336.379	99						
	Cfree; mg/l	5978.325	99						
	Cfree; mg/l per mg/kg	31.412	99						
	Fu	1.052	99						
	Percentage Free VPA	10498.439	99						
	CLtotal; l/h	30.643	99						
	CLtotal; ml/kg/h	7346.465	99						
	CLfree; l/h	4303.536	99						
	CLfree; ml/kg/h	991079.087	99						
Corrected Total	Ctotal; mg/l	58750.552	98						
	Ctotal; mg/l per mg/kg	493.532	98						
	Cfree; mg/l	2091.112	98						
	Cfree; mg/l per mg/kg	5.920	98						
	Fu	.119	98						
	Percentage Free VPA	1171.691	98						
	CLtotal; l/h	4.955	98						
	CLtotal; ml/kg/h	1145.741	98						
	CLfree; l/h	994.602	98						
	CLfree; ml/kg/h	208896.781	98						

a. R Squared = .075 (Adjusted R Squared = .045); b. Computed using alpha = .05; c. R Squared = .072 (Adjusted R Squared = .043); d. R Squared = .100 (Adjusted R Squared = .072); e. R Squared = .107 (Adjusted R Squared = .079); f. R Squared = .045 (Adjusted R Squared = .015); g. R Squared = .045 (Adjusted R Squared = .014); h. R Squared = .124 (Adjusted R Squared = .097); i. R Squared = .037 (Adjusted R Squared = .007)

j. R Squared = .117 (Adjusted R Squared = .089); k. R Squared = .056 (Adjusted R Squared = .027)

Graphs estimate marginal means and explore the interactions of interested VPA pharmacokinetic parameters

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Normal weight group</td>
<td>X-axis represents BMI groups
Male group
Female group</td>
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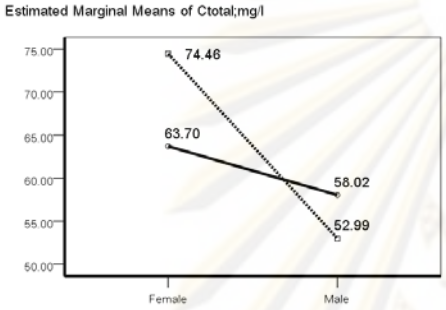
<td> 
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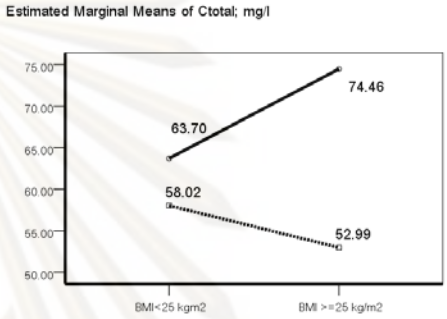
<td> 
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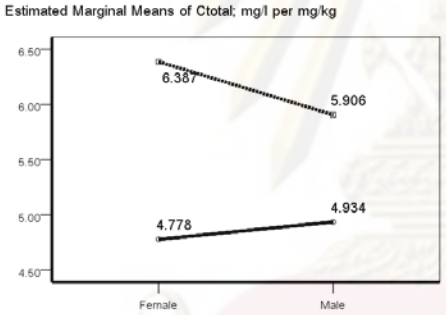
<td> 
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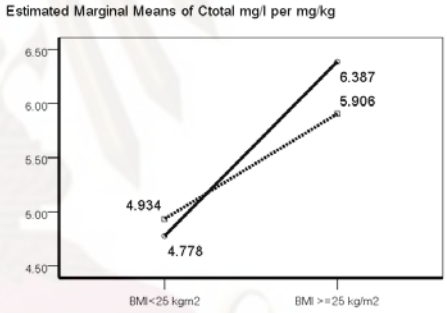
<td> 
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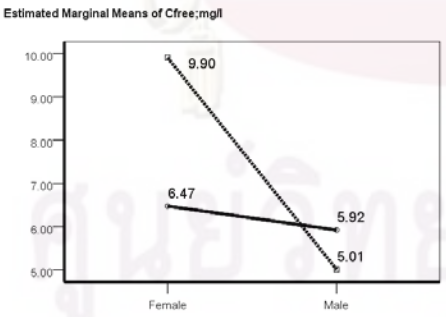
<td> 
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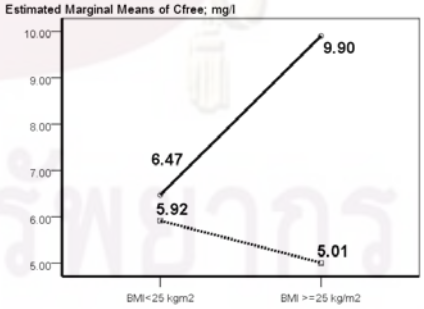
<td> 
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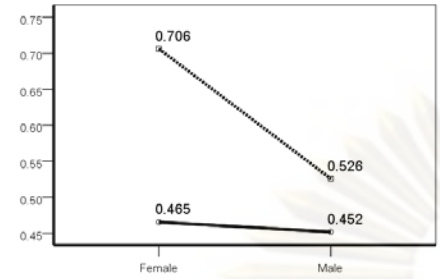
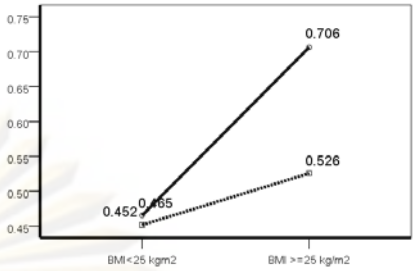
Estimated Marginal Means of Cfree; mg/l per mg/kg



Estimated Marginal Means of Cfree; mg/l per mg/kg



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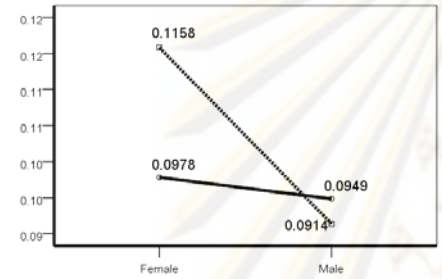

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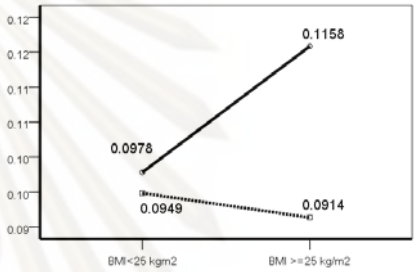
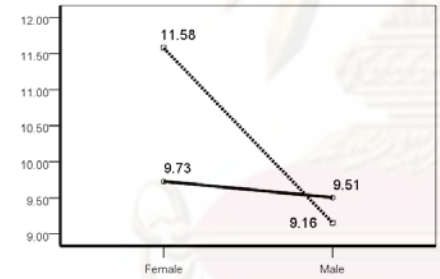
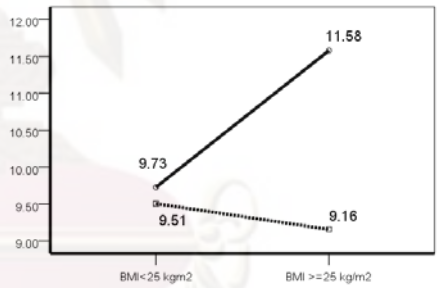


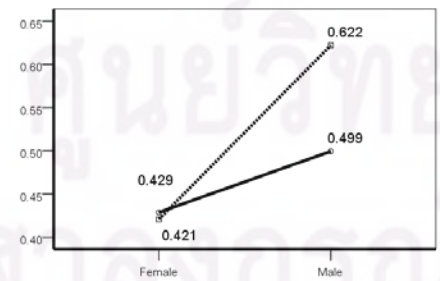
Estimated Marginal Means of Percentage of Free VPA



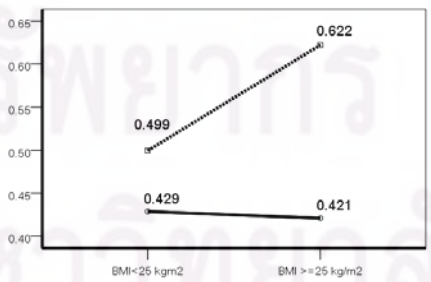
Estimated Marginal Means of Percentage of Free VPA



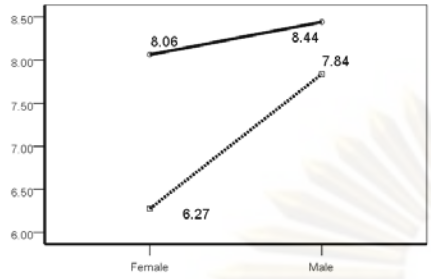
Estimated Marginal Means of CLtotal; l/h



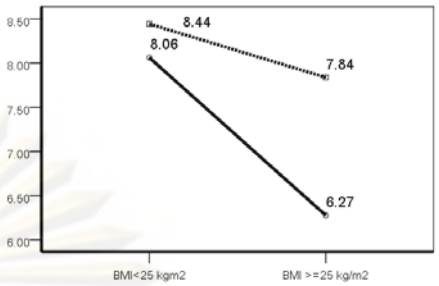
Estimated Marginal Means of CLtotal; l/h



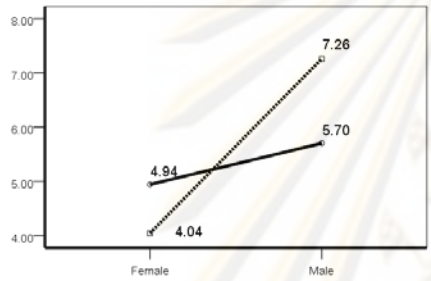
Estimated Marginal Means of CLtotal; ml/kg/h



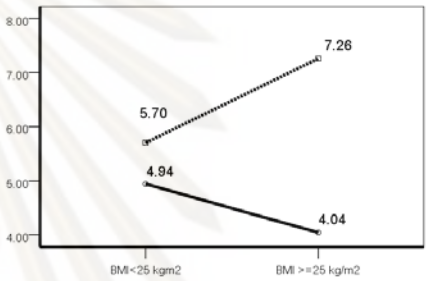
Estimated Marginal Means of CLtotal; ml/kg/h



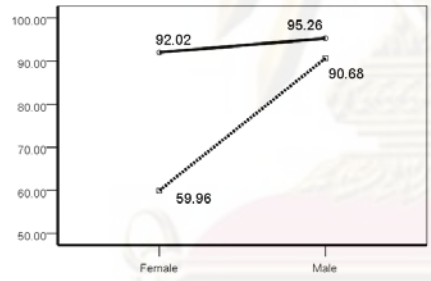
Estimated Marginal Means of CLfree; l/h



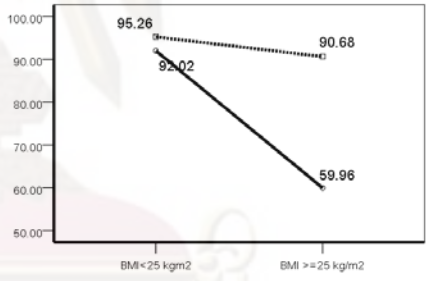
Estimated Marginal Means of CLfree; l/h



Estimated Marginal Means of CLfree; ml/kg/h



Estimated Marginal Means of CLfree; ml/kg/h



Comparison of general characteristics and pharmacokinetic parameters using Two way ANOVA (n=99)

Dependent variables	One-Sample Kolmogorov-Smirnov Test		Levene's Test		Two way ANOVA			Friedman
					Sig 2-tailed			
	Sig. (2-tailed)	Normal distribution was assumed	Sig. (2-tailed)	Equally variance was assumed	Gender	OW/OB	Interaction	Sig 2-tailed
Age; years	.760	Yes	.009	No	.093	.144	.785	.306
Height; cm	.052	Yes	.719	Yes	**.000	.887	.935	**.000
Weight; kg	.087	Yes	.012	No	**.000	**.000	.123	**.004
BMI; kg/m2	.224	Yes	.007	No	.103	**.000	.216	*.012
Albumin; gm/dl	.408	Yes	.110	Yes	**.004	.122	*.048	*.013
Dosage; mg/day	.000	No	.005	No	.823	.242	.420	.052
Dosage; mg/kg/day	.248	Yes	.016	No	.163	.137	.334	*.043
C_{total}; mg/l	.359	Yes	.330	Yes	*.011	.584	.133	.230
C_{total}; mg/l per mg/kg	.323	Yes	.008	No	.736	**.008	.507	.598
C_{free}; mg/l	.020	No	.001	No	**.006	.197	*.028	.057
C_{free}; mg/l per mg/kg	.233	Yes	.404	Yes	.062	**.003	.108	.360
Fu	.001	No	.062	Yes	.072	.338	.157	*.048
Percentage Free VPA	.012	No	.098	Yes	.080	.318	.145	*.041
CL_{total}; l/h	.038	No	.001	No	**.004	.221	.164	*.012
CL_{total}; ml/kg/h	.352	Yes	.030	No	.194	.110	.428	**.007
CL_{free}; l/h	.003	No	.002	No	**.003	.621	.067	**.013
CL_{free}; ml/kg/h	.059	Yes	.032	No	.090	.068	.170	**.022

The results met the assumption criteria.* statistically significantly difference at p-value ≤ 0.05 ;** statistically significantly difference at p-value ≤ 0.01

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- Panomvana Na Ayudhya D, Ounsaenathum S, **Sriboonruang T**. Bioequivalence study of clindamycin phaphate for intramuscular administration in healthy Thai volunteers. Chula Med J 2005;49(12):702-8
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