CHAPTER V

DISCUSSION

1. Demographic data

The data were obtained from 29 pediatric patients (girls 45%, boys 55%) using valproic acid as monotherapy during steady state. Their ages ranged from 3 to 15 years with mean age of 6.38±2.34 years. In this study the seizures were not satisfactory controlled in one-fifth of the patients as shown in Table VII. Among these patients, two were diagnosed as Lennox-Gastaut syndrome (LGS), which is a childhood epileptic syndrome that is generally medically intractable and unresponsive to monotherapy of standard antiepileptic drugs (Wallace 1990)(12,31). However, in the United State, valproic acid is still one of the most commonly used antiepileptic drugs in the treatment of LGS. There were four patients who were diagnosed as secondarily generalized seizure (2^{nd} GZ) and complex partial seizure (CPS) with 2^{nd} GZ. One of these was classified into idiopathic, however she was uncontrolled with the high dose of valproic acid. The later investigation will be necessary in this case. Three of these patients were classified as symptomatic epilepsy which has been considered to be more difficult to control of the seizure in comparison to those with idiopathic epilepsy (12). Therefore, achievement of seizure control in majority of the patients enrolled in this study should not be difficult and was confirmed by the result of the study that 79.3 % of the patients were seizure-free.

Most of the laboratory data were within normal ranges except for the alkaline phosphatase and creatinine levels. Alkaline phosphatase was higher than normal range which is quite common among children and is considered to be normal if other liver enzymes are within normal limits. Low creatinine levels might be partly explained by the lesser amount of muscle mass in small children.(32)

Valproic acid may disturb function of the liver. Its effects ranged from an asymptomatic elevation of liver enzymes to fatal hepatotoxicity. According to Anon 1992 and Powell-Jackson 1984's reports, there were approximate 11 % of patients who received valproic acid had transient alteration of liver enzyme activities. These included elevation of alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase.(5) Elevations of these enzymes are generally dosage-related, since all patients in this study received the recommended dosage of valproic acid, therefore none had elevated liver enzymes in this study. Although most adverse effects of valproic acid observed in the patients with epilepsy were not severe and most of the patients would be able to tolerate these reversible effects. Occasionally, idiosyncratic reactions could be serious or even life threatening. It is controversial whether routine laboratory monitoring actually can identify the patient at risk for serious reactions (5,16,17). Willmore et al. 1991 reported that the fulminant and irreversible hepatic failure occurred during valproic acid therapy was not reliably predicted by laboratory monitoring. In addition, some researchers had reported that the routine laboratory screening was of doubtful value. Most pharmaceutical companies recommend periodic laboratory tests during anticonvulsant therapy. There is also a belief that it is prudent to obtain routine laboratory monitoring during treatment with valproic acid.(16) Hence, performing routine laboratory tests of serum chemistries and complete blood count to identify a patient at risk of developing adverse effects is still practiced.

However there are specific issue which must be considered in monitoring blood chemistries and analysis concentration of valproic acid. These included children who are younger than 2 years and patient with associated illness. Children younger than 2 years are universally accepted that they have higher risk for hepatotoxicity from valproic acid than older children.(16) Risk of having adverse effect is also increased in those who had presumptive metabolic disorders, neurodegenerative disease, or history of previous adverse drug experiences.(5,16) Any child who developed any symptom suggesting adverse

reaction must be promptly evaluated. These clinical symptoms included nausea, vomiting, anorexia, malaise, edema, lethargy or unexplainable recurrent seizures. These symptoms often occur prior to the alteration of liver functions and appear to be the reliable indicators of developing hepatotoxicity. (5)

Regarding serum lipid, there were reports that lipid might be altered during chronic valproic acid therapy. The concentration of lipid observed in the studied patients, were all within normal ranges. This finding was similar to the results from many studies revealed normal lipid levels in patients who took valproic acid from three to six months. (34-36)

Besides the laboratory monitoring, clinicians should aware of chronic adverse affects of valproic acid such as weight gain, tremor and alopecia. Body weight should be monitored because weight gain has been frequently reported in many studies. Increase in body weight was reported to be associated with an increase in fatty tissue due to the increase of appetite, the reduction of facultative thermogenesis and the increase in availability of long chain fatty acid caused by competition between valproic acid to serum albumin. This untoward weight gain can sometime be controlled by reducing caloric intake. (5)

In this study, there were approximately one-third of the patients who had history of febrile convulsions. Febrile convulsions occurred between the age of 1 to 3 years. There were only small number of children with febrile seizures who would develop epilepsy. (37) There were two children who had febrile convulsions and relatives with febrile seizure. However, there is no evidence that febrile convulsion is associated to epilepsy. There were reports of genetic preponderance in children with febrile convulsion. In Thailand it is still not possible to identify the genetic cause of febrile convulsion at present. Therefore, association between the febrile convulsions and epilepsy in these two patients could not be able to be concluded. However, valproic acid is one of the antiepileptic drugs, which showed good efficacy in treatment of febrile convulsions. (37)

2. Total, unbound concentration and free fraction

2.1 Therapeutic concentration

The unbound valproic acid concentrations were classified into over therapeutic in three patients whose had total concentration in therapeutic range. They weren't showed any adverse effects but the clinician will be aware with them. Although valproic acid levels observed in some patients were above the therapeutic level but none of the patients in this study showed any adverse effect. There were six patients whose required total valproic acid concentration above 100 μg/mL for seizure-control. There was one patient (patient # 16) whose trough total valproic acid level was above 120 μg/mL (unbound concentration > 15μg/mL) to achieve seizure-control, no adverse effect was observed. On the other hand, there was another patient (patient # 25) who still had seizure despite his total valproic level was above 140 μg/mL (unbound concentration > 20 μg/mL). This implied that not all patients taking valproic acid will be seizure-free. At the same time, there was no adverse effect observed in any of these two patients. For this later patient, measurement of valproic acid level would be beneficial for further decision making in alteration of the treatment regimen or to other AEDs. In some occasions, high concentration above the recommended level is necessary to achieve seizure-control. In Thai children the total concentration may be achieved to 120 μg/mL (unbound concentration > 15 μg/mL) for seizure control with no adverse effect that required dosage reduction. (2,38) On the other hand, there were five patients whose total trough levels were sub-therapeutic and one of them could not control her seizures. Considering her unbound concentration were very low (2.03 μg/mL). In addition, therapeutic ranges were a statistical average of the concentrations, which were based on relatively small number of patients. These data were mainly obtained from adults. There were also limited numbers of studies in children. In general, children require higher doses of AEDs to achieve the same serum concentrations as adults because of faster metabolism (5,7,20) and may require higher concentration to control their seizures. (5,7)

Besides the age of the patient, other variables such as variable in protein binding and the severity of the underlying seizure disorder must be considered.

2.2 Relationship of total and unbound concentration

Optimal dosage is mainly determined by seizure control.(6) Measurement of plasma valproic concentration is indicated in many situations. For instance, in any patient who had recurrent seizures despite of taking high dosage of valproic acid, determination of the drug concentration may be a tool in monitoring patient's compliance.(11) In case where there is suspicion of the efficacy of the drug, high concentration obtained in patient presenting seizure will provide more information for the physician to add second AEDs or change to other AEDs than to change the dosage regimen. In general, pharmacological effect and biotransformation are known to be more closely related to free drug concentration rather than to the total level usually determined.(39) Guyot et al in 1982 (40) found that the correlation between free plasma valproic acid and total CSF concentration was higher than that between total plasma valproic concentration and total CSF concentration. Thus it is essential, whenever possible to estimate the free level of valproic acid instead of the total concentrations. In this study there was high degree of linear relationship either when the total concentration ranged from 32.96 to 147.09 μg/mL, the unbound concentration ranged from 2.03 to 29.3 μg/mL. The reason could be due to the binding capacity of valproic acid had not quite reach the saturation at these concentrations. Therefore, the graph obtained in this study did not change into non-linear fashion, as it should be at higher concentrations. However, the increment of free fraction trended to have markedly increase at higher concentrations. This finding was similar to that described in previous studies by Hall et al. 1985, (41) Herngren and Nergardh A. 1988. (42) However, there was a report by Scheyer et al. 1990(39) who conducted a study in thirty-seven patients who took chronic valproic acid therapy depicted the nonlinear relationship between total related to unbound drug concentration. For optimal

dose of valproic acid, it might be appropriate to calculate from the measured unbound drug concentration.

2.3 Free fraction

The binding ability of valproic acid to albumin in vivo is a complex process. According to previous studies, other antiepileptic drugs had effect on the metabolism and binding capability of valproic acid to albumin. (4,5) Therefore we conducted this study only on the children who were taking valproic acid as the single antiepileptic drug. Table XIII showed both total and free valproic acid concentrations at the trough and at 5th hour after morning dose. The free fractions and mean were calculated and shown in Table XIII . It ranged from 0.043 to 0.198, this consistent with the result reported by Kodama et al. 1996 (39), Kodama et al. 1995(43) and Cloyd et al. 1993(9) who found free fraction ranged from 0.05 to 0.15, 0.057 to 0.16 and 0.08 to 0.17,respectively. Comparison of the mean of free fraction between those obtained from the trough concentration with those obtained from the 5th hour after morning dose concentrations showed no significant difference. However, according to previous study the levels obtained before the morning dose (trough) correlate more closely to the area under the curve (AUC) which should be the most reliable estimation of the drug exposure. (44)

Cramer et al.,1986 evaluated the albumin-binding characteristics of valproic acid in a series of 58 patients with epilepsy. According to their data, VPA-free traction was approximately 7% at 50 μg/mL, 15% at 100 μg/mL, 22% at 125 μg/mL, 30% at 150 μg/mL(45), which were similar to the data obtained in this study. The free fraction of these three groups of different concentration range: <50μg/mL, 50-100 μg/mL and >100 μg/mL were 7.1%, 9.8% and 13.46%, respectively. There were significant differences among the three groups as shown in Table XIV. Higher concentrations resulted in higher percentage of free fraction, which might imply that saturation was starting to occur in some aspect. However, in this study, there were two uncontrolled-

seizure patients whose had very low free fraction considering by total concentrations (patient #21 : 0.043 at 47.27 μg/mL and # 25 : 0.07 at 144.71 μ g/mL). This finding demonstrated that the realization of unbound concentration, which is pharmacologically active and is the form that is cleared from the circulation, would be more benefit in these cases.

The experiment by using mean that varied with total concentrations predicted free fraction. The mean prediction error and root mean square of the data from study by Kodama et al. 1995 (43) were smaller than that analyzed from the data from study reported by Scheyer et al.1990 (39), which conducted in different age from this study, therefore, may be limited in the predictive performance of free fraction. (Appendix III)

2.4 Equation to predict free fraction

From multiple regression analysis, the equation free fraction = 0.035 + 0.0008(total concentration) (r^2=0.46,$p<0.05$) were used to predict the free levels from measured total concentrations. Cloyd et al.,1993 presented the result of regression analysis of free fraction versus C_{max}: $ff = 7.8e^{0.004} \times C_{max}$, $p<0.001$ without reported correlation coefficient (r^2). (9) In the therapeutic drug monitoring practice one usually measures the trough level. According to this study, there was better correlation of free fraction versus lower valproic acid concentration (r^2= 0.411,$p<0.001$) than versus higher concentration (r^2=0.034,p=0.510). The result of this study and those reports were not consistent with the result reported by Kodama et al. 1996(33), who observed the plot of the unbound fraction and total concentrations, there was no significant relationship between them (r =0.004) or second degree (r =0.0024) polynomial regression analysis.

In this study, minimum relationship between albumin concentration and total concentrations was found at higher concentrations while higher relationship was found at lower concentrations. However, considering the slope

of the graph of relation, increment of free fraction by albumin at higher concentrations was higher than that at lower concentrations. Valproic acid tends to have concentration dependent kinetics, which would approach non-linear at higher concentrations. Based on the data of Patel and Levy, 1979, (46) and Scheyer et al. 1990, (39) a correlation would be expected between serum albumin concentration and the free valproic concentration. However, this correlation was not demonstrated since the concentrations obtained in this study mostly had not approach saturation and the albumin concentrations of the patients participated in this study were all within the normal range. Valproic acid trends to undergo saturated protein binding within the therapeutic range. As a result, the unbound concentration of VPA is much more variable than that of the total. This may have important implications in correlation between efficacy and/or toxicity with unbound concentrations. Patients with hepatitis, hypoalbuminemia or renal disease may have a free-fraction that was higher than patients whose do not have these conditions.

3. Pharmacokinetic parameters

3.1 Total and unbound valproic acid pharmacokinetic parameters

Elimination rate constant and clearance

Typically, children metabolize valproic acid rapidly. The means elimination rate constant of total and unbound valproic acid found in this study were 0.068±0.03 hr^{-1} and 0.103±0.08 hr^{-1}, respectively. The means of total and unbound valproic acid clearance after consumed solution dosage form were 12.37±4.16 mL/kg/hr and 101.03±33.39 mL/kg/hr, respectively. Clearance obtained from this study was inline with those obtained from previous study reported by Clold et al. 1993, (9). This study was performed in twenty-one children aged between 2 to 14 years on valproic acid monotherapy and came out with the total valproic acid clearance varied from 5.3-24.5 mL/kg/hr (14.0±4.7 mL/kg/hr) and unbound valproic acid clearance varied from 56.5-202.8

mL/kg/hr (118.7±43.7 mL/kg/hr). Chiba et al. in 1985(47) conducted a study in twenty one children who were receiving valproic acid as monotherapy to compare the steady state pharmacokinetic parameter of valproic acid with that of the children who were taking multiple antiepileptic drugs treatment. The clearance was found to be 13.0±4.7 mL/kg/mL in children who were took valproic acid monotherapy. There were three studies that reported clearances, which were differ from the clearance found in this study. Herngren and Nergardh A in 1988(42) reported the total and unbound plasma clearances of valproic acid in seven adolescents and young adults receiving valproic acid as single antiepileptic drug to be 9.22±4.82 mL/kg/hr and 125.3±69.2 mL/kg/hr respectively. Alfonso L. et al. 2000(48) reported clearance, which obtained from two neonate patients with seizures received loading doses of valproic acid to be approximately 25 mL/kg/hr. Botha J.H. et al. 1995(49) reported mean valproic acid clearance obtained from routine 52 patients measurement to be 21 mL/kg/hr (range,13-42). The first study was achieved in adolescents and young adults which may have slower valproic acid clearance than the younger children. The second study recorded from only two neonates any way the result showed younger patients metabolized VPA faster. The second and third studies were carried out from patients who received either valproic acid monotherapy or concomitant with other ADEs, which might interact and induce the clearance of valproic acid. The clearance of unbound valproic acid was approximately ten-fold of total valproic acid, the relationship was poor since free drug only was cleared from circulation. The finding of this study indicated that pediatric patients over 10 years-old had no significantly less total and/or unbound drug clearance than those of the ≤10 year-old group, which did not support the belief that clearances of less than ten year-olds children were faster than those of over ten year-old. (41) However, study by Hall K. et al. 1985(41) reported that clearance values were not age-dependent.

The mean total and free clearance from Chrono dosage form were 9.79± 2.21 mL/kg/hr and 96.44±40.25 ml/kg/hr, respectively. This study was found no significant differences of either total or unbound clearances between solution and Chrono dosage form, which was the combination of sodium valproate and valproic acid. After the drug was absorbed into circulation, the kinetic behaviors were all the same as valproic acid.

Volume of distribution

The distribution of valproic acid is controlled in part by the physicochemical properties of the drug. More than 90% of the drug is ionized at physiological pH. Valproic acid is highly bound to serum proteins. A high percentage of valproic acid is bound to albumin relative to tissue proteins and ionization of valproic acid in blood lead to the relatively small distribution volume. This consisted with reports in previous studies. Herngren L and Nergardh A. 1988(42) studied in seven adolescents and young adults reported the apparent volume of distribution (Vd) of total valproic acid to be 0.150-0.197 L/kg body weight and of free valproic acid to be 0.911-1.58 L/kg body weight. The limited distribution volume calculated from total serum valproic acid concentration has been claimed to be caused by high degree of protein binding in serum of valproic acid. In contrast, the volume of distribution from unbound levels was higher than from total levels and had low relationship with the volume of distribution of total drug. This indicates considerable distribution as well as extravascular binding of valproic acid. The assumption that high protein binding of drug in general has a restrictive influence on its distribution in the body should therefore be reconsidered. In such case the distribution volume based on the unbound plasma concentration may be better indicator of a drug's distribution in the body.

No significant differences were observed in volume of distribution of total and free drug between male and female or prepubescent (≤ 10 years) and pubescent (>10 years) patients. This finding agree with a previous research by

Kodama Y. et al. 1999(50) that suggest that there was no effect of gender and age on binding characteristic of valproic acid to serum proteins in pediatric patients with epilepsy.

Half-life

The mean half-life of valproic acid was similar to studies by Chen et al. 2000 (51) (11.71±3.48 hr), by Cloyd et al. 1993 (9) (total half-life = 11.6±3.9 hr), by Hergren L and Nergardh A. 1988 (42) (total half-life =11.9±5.9 hr and unbound half-life =6.4±3.9), Chiba et al. 1985 (total half-life = 12.3±3.1). The mean half life of free valproic acid in present study (9.20±5.34 hr) was significantly shorter ($P<0.05$) than the half life of total valproic acid (11.50± 4.05 hr.), this finding consistent with Hergren L and Nergardh A. 1988(42). The unbound concentrations showed greater decreasing between 5th hour after morning dose and trough value than did total valproic acid levels. Also, the binding in plasma varied with the total concentration of valproic acid, as seen in Table XIV. Eventhough, the total serum concentrations were not exceedingly high in the present study, a greater difference between the lowest and the highest binding value within the same dosage interval was found. This could explain the significantly shorter terminal half-life of unbound valproic acid compared with that of total valproic acid.

Half-life may be reason in the patients #21 whose still had seizures while she was received high dose. Because valproic acid half-life of patient #21 was very short, unbound drug was cleared quickly. This case will be considered to adjust the interval more than increase dose.

3.2 Equation to predict concentration and pharmacokinetic parameters

Total and unbound concentrations

Daily dose was the main predictor of both total and unbound concentrations, while including the serum albumin could be better predict the unbound concentration at 5th hour after morning dose. Since albumin is the main binding protein of valproic acid.

Elimination rate constant and Clearance

The elimination of valproic acid, a highly protein-bound drug, occurs primarily via hepatic metabolism and is classified as restrictive, which makes total clearance dependent on both free fraction and unbound clearance. Thus, factors affecting either the valproic free fraction or unbound clearance alter steady-state clearance. Total clearance in this study was found to have low significant positive correlation with unbound clearance (r^2=0.155,P<0.05) by linear. This finding was conflict with the study reported by Kodama Y. et al. 1993(52) who found high relationship between unbound clearance and total clearance (r= 0.961,p=0.001).

Using multiple regression, the correlation between total and free clearance was defined as CL_t = ff.Cl_u (r^2=0.462 P<0.05) (Appendix III). This finding agreed with the previous studies which suggested that the total clearance was determined by two independent factors: unbound fraction in serum and unbound clearance, which is defined as follows: CL_t= ff.Cl_u (ff:unbound (free) fraction).(52) So, patient whose hepatic was dysfunction or had low serum albumin should be considered more carefully.

Result of the stepwise multiple linear regression analysis for clearance of total and free drugs were shown in Table XVIII and XIX. The results revealed that the daily dose and weight were the most significant predictors of clearance of total and unbound drug. This is consistent with the results of a previous study by Cloyd et al. 1993(9). Gidal et al. 1995(53) found a positive correlation

between dose and total plasma clearance ($r=0.61, P \leq 0.001$), while an inverse correlation existed between dose and unbound valproic acid clearance ($r=-0.51, P<0.01$). Increase in clearance as the valproic acid dose increases was found by Botha et al. 1995(46) in adult patients and is consistent with the concept of concentration-dependent protein binding. However, Alcaraz et al. 1998(44) suggested an increase in clearance as the dose of valproic acid increases and a decrease in clearance as plasma concentration increases depend on the age of patient. Although the dependency of clearance to age range were not found in this study. The effect of age on valproic acid pharmacokinetics has also been investigated with varying results. According to some reports, clearance was inversely related to age (48), whereas other studies have failed to find such relationship. However, previous studies suggested that the relationship between age and valproic acid clearance was non-linear.(5,9) Small distribution of age of patient populations, and limited statistical analyses have precluded a comprehensive examination of the factors that influence valproic acid pharmacokinetics. However, age may be confound in predicted clearance models, by some of the studies suggest that there is the close correlation in the children between age and weight. Therefore the exact mechanism of any age related change in valproic acid clearances is still uncertain.

Volume of distribution

From stepwise multiple linear regression analysis volume of distribution of total and free drug were shown in Table XVIII and XIX. The results revealed that the trough concentration was the most significant predictor ($P<0.001$) of total volume of distribution. This relationship is consistent with the concept: for drugs that are highly bound to plasma proteins, distribution volume may vary when plasma concentration is alter because of changes in protein binding. The model demonstrated significant relationship between total volume of distribution and free fraction at trough ($r^2 = 0.374, P< 0.05$) and at 5^{th} hour after morning dose ($r^2= 0.342. P< 0.05$). This is consistent with the result of previous

study, which reported that the main factor contributing to predicting the volume of distribution was average free fraction.(9) Distribution volume in drug therapy is useful in calculating loading doses. The concept of loading dose is to rapidly fill up body compartments to reach a targeted plasma concentration and thus a targeted concentration at the sited of action. Little information is available about the used of valproate for acute loading of patients when a rapid increase in serum level is needed. When loading dose are calculated a volume of 0.2 L/kg is recommended in both children and adult. (16) Alfonso I. et al. 2000(48) found that each 1 mg/kg of intravenous valproate increased the 45-minute and 3-hour post-infusion serum valproic acid concentrations by approximately 4 μg/mL and 3 μg/mL, respectively and they suggested that these be used to calculate the loading dose, but the data were obtained from only two neonates who had received phenobarbital and phenytoin (enzyme inducer) before the loading infusion. The more practical implication of our finding about volume of distribution (Vd) was to use the mean apparent volume of distribution to approximate the loading dose for pediatric patients who wasn't received valproic acid before loading. For the patient who required increased concentration to stop seizure in patients that were received valproic acid, the figure from the regression model for valproic acid volume of distribution might be used to calculate the desirable loading dose of valproic acid. However adjustments should be based on response and serum concentrations.

Half-life

The predicted half-life models required trough total concentration. Half-life of total valproic acid varied with age, whereas the unbound half-life was not, it was varied by daily dose. However, the model that could more accurately predict half-life of unbound drug should have one total drug trough concentration.