EVALUATING THE PHARMACOKINETICS OF VALPROATE IN THE ELDERLY:
DOSE-RELATED CHANGES AND THEIR INFLUENCE ON EFFECTS

by

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A thesis submitted in conformity with the requirements
for the degree of Master of Science
Graduate Department of Pharmaceutical Sciences
University of Toronto

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ABSTRACT

Evaluating the Pharmacokinetics of Valproate in the Elderly: Dose-Related Changes and their Influence on Effects. Susy Felix, MSc 2001, Department of Pharmaceutical Sciences, University of Toronto

Valproate exhibits concentration-dependent protein binding and clearance that has not been assessed in the elderly at higher doses. Side effect severity has also not been evaluated across dosages. Ninety steady state free/total valproate concentrations were collected in an observational manner from 41 mood disorder patients (14-85 years). The elderly had higher free fractions (10.3% vs. 9.7%) at lower valproate concentrations (≤350μmol/L) with a similar trend at higher concentrations, compared to the young. A single blind within subject study in 6 healthy elderly volunteers (65-76 years), assessed steady state total/free valproate concentrations at 3 doses: 500, 1000 and 1500mg/day. As concentrations increased, the free fraction (10 to 17.4%) and total clearance (4.8 to 6.7ml/hr/kg) increased significantly. Unbound clearance decreased (49 to 39ml/hr/kg) with increasing concentrations. CNS and nausea severity correlated with total and free valproate levels. This study demonstrates valproate dose-related pharmacokinetic changes, and side effect severity in the elderly.
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I  Statement of Problem

Valproate exhibits a complex pharmacokinetic profile because of its concentration dependent protein binding as well as a concentration dependent clearance. It is known that the protein binding of valproate decreases as the serum concentration increases in a younger population, and that protein binding is decreased in the elderly compared to younger adults at lower concentrations. The extent of valproate protein binding at higher concentrations in the elderly is not yet known. Likewise, studies conducted in the elderly have found valproate unbound clearance to be decreased compared to younger adults, although the change in clearance of the unbound fraction has not been evaluated at higher concentrations in the elderly. Therefore we have evaluated the pharmacokinetics of valproate (protein binding and clearance) across a dosage range in the elderly and the impact of this on the pharmacodynamics (side-effects) using 2 different studies. We conducted both an observational study in patients taking valproate for a mood disorder as well as a single blind within subject study in elderly volunteers taking valproate at 3 different doses.
Background

2.1 Bipolar Disorder

Bipolar disorder affects every race, nationality, educational and social class (1) and affects approximately 1% of the population (2). The onset of the disorder often occurs during late adolescence into early adulthood. One of the most important factors that determines the development of this disorder is genetic predisposition, with organic and neurological brain syndromes also playing a role in late onset of first manic episodes in the elderly population (3). It is known that approximately 80-90% of patients with bipolar disorder (1) have a biological relative with a mood disorder. Bipolar disorder is characterized by recurring episodes of mania or of mania and depression. The American Psychiatric Association’s DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, fourth edition) divides bipolar disorder into 4 subtypes: Bipolar I (consisting of manic or mixed episodes ± major depressive episodes), Bipolar II (consisting of major depressive episodes + hypomanic episodes), cyclothymic disorder (chronic fluctuations between subsyndromal depressive and hypomanic episodes – 2 years for adults and 1 year for children and adolescents), and bipolar disorder not otherwise specified (mood states that do not meet criteria for any specific bipolar disorder) (1). A mixed episode is defined as a simultaneous occurrence of mania and depression, nearly everyday for at least a one week period (1). Rapid cycling bipolar disorder is defined as four major depressive or manic episodes (manic, mixed, or hypomanic) occurring in a 12 month period (1). This disorder can have damaging long-term effects because of the deterioration in social and occupational functioning of these patients. There is a high risk of suicide in untreated, depressed or mixed state bipolar patients, where approximately 1 in 4-5 patients commits suicide (1). A lack of judgement and
appropriate insight is often seen in acutely manic patients and may put them at risk and in need of protection.

Bipolar disorder is also fairly common and presents a risk in the elderly population. It is estimated that bipolar disorder affects approximately 0.4% of people older than 65 years (4). Patients with bipolar disorder constitute about 5% to 19% of the elderly presenting for treatment of mood disorders (5,6). The genetic predisposition of bipolar disorder in the elderly is fairly high where about 50% of elderly manic patients (4) have a biological relative with a mood disorder.

2.2 Valproate in the Treatment of Bipolar Disorder

Fortunately there is efficacious pharmacotherapy for bipolar disorder. The three most commonly used mood stabilizers for patients with bipolar disorder are lithium, valproate and carbamazepine. They are used for acute and maintenance treatment in this disorder. Valproate was initially marketed for use in epilepsy as sole or adjunctive therapy in treating simple or complex absence seizures as well as primary generalized seizures with tonic-clonic manifestations (7). It is also now approved for use in the treatment of manic episodes in bipolar disorder (7). The mechanism of action of valproate in mood disorders is not exactly known but it is thought to be related to the inhibition of gamma-aminobutyric acid (GABA) metabolism, stimulation of GABA synthesis and release, and augmentation of the post-synaptic inhibitory effect of GABA (1). Recent research also suggests that valproate may modulate CNS signal transduction pathways, more specifically through the Protein Kinase C signaling pathway (8), as well as potentially having an effect on gene expression. Valproate has been shown to be effective in patients who did not respond to lithium, relapsed on lithium, or could not tolerate
lithium (9-13). Valproate has been shown to have similar response rates in patients with mixed mania compared to pure mania (11), and has been efficacious in adults with rapid cycling (14). Therefore, valproate is recommended for the treatment of classical and mixed mania and for those with rapid cycling (15) although no specific recommendations are available with respect to its use in the elderly.

The therapeutic range for valproate in the treatment of mood disorders is not known. In one study, valproate concentrations in the range of 45 and 125μg/ml (312-870 μmol/L) were more likely to produce efficacious responses in acutely manic patients (n=65) than higher or lower levels of valproate (16). A retrospective study conducted in an elderly population (n=29) reported that the therapeutic window in these elderly manic patients was 450-625μmol/L (17).

2.2.1 Side-effects

The side-effects of valproate can be divided into dose related and idiosyncratic (not related to dose). The dose related effects of valproate, which usually subside with a dose reduction or withdrawal of valproate, include gastrointestinal symptoms: nausea (18) and vomiting (in about 9-16 % of adults) (19) and CNS effects: ataxia, tremor and sedation (20). Rash, alopecia, anorexia, stimulation of appetite, reduced platelet count and elevation of liver enzymes have been occasionally observed but their relation to dose is not clear (20,21). Valproate's idiosyncratic effects include: hepatic failure, pancreatitis and agranulocytosis (21). Clinical reports indicate that valproate may be teratogenic (21). Side-effects have been cited as a determinant of noncompliance with mood stabilizers (22).
In one study, the first occurrence of sedation was found in 1.5% of patients with valproate levels less than 180μmol/L, 4.6% of patients with levels of 180-350μmol/L, 3.3% of patients with levels of 350-525μmol/L, 3.8% of patients with levels of 525-700μmol/L, 8.6% of patients with levels of 700-870μmol/L, and 18.2% of patients with levels greater than 870μmol/L (16). Sedation has also been reported in elderly patients treated with valproate (23). A study conducted in elderly patients found that sedation occurred in 2 of the 21 patients, with valproate levels of 460μmol/L and 625μmol/L (13).

Nausea was found by Bowden et. al 1996, to first occur in 6% of patients treated with divalproex sodium with concentrations of less than 180μmol/L, 1.5% in patients with levels of 180-350μmol/L, 4.9% of patients with levels of 350-525μmol/L, 7.5% of patients with levels of 525-700μmol/L, 2.9% of patients with levels of 700-870μmol/L and in 27.3% of patients with levels greater than 870μmol/L (16). In another study, nausea occurred in 4.5% of pediatric patients at a mean valproate level of 270μmol/L reported in the clinical record and in 6.8% of patients with a mean valproate level of 440μmol/L using a questionnaire (24).

Tremor has been reported to occur with valproate at levels ranging from 555 to 1040μmol/L in epileptic patients (over 16 years old) (25). Herranz et. al. found that tremor occurred at a mean valproate level of 470μmol/L in 2.3% of pediatric patients using reports from a clinical record and in 1.1% of patients with a mean valproate level of 330μmol/L using a questionnaire (24). Cramer et al (26) reported that tremor most commonly occurred with free (not bound to plasma proteins) valproate levels ranging from 7-24μmol/L in epileptic patients (mean ages 23-29 years). Valproate induced tremors have been known to occur in the elderly (23).
There is very little information available regarding the relationship between valproate concentration (free or total) and side-effects in the elderly.

2.3 Valproate Pharmacokinetics

2.3.1 Products & Drug Administration

Valproate was first released onto the market in France in 1967 (27) and in Canada in 1978 as the valproic acid form (28). It is available in a variety of formulations and products around the world: capsules or enteric-coated tablets, syrup, sodium salt (sodium valproate) and divalproex sodium (7,29). Valproic Acid (Depakene® Abbott laboratories), shown in figure 1.a., is a branched fatty acid chain and is available orally as 250mg and 500mg capsules as well as a 250mg/5ml syrup formulation (7,30). Its recommended administration is 15mg/kg/day increasing by 5-10mg/kg/day every 7 days until therapeutic effect or until further increases are hindered by increasing side effects. The total daily dosage should not exceed 60mg/kg/day and when it exceeds 250mg should be given in a divided regime (7,30). Various generic brands are available for valproic acid.

Valproate is also available intravenously as sodium valproate (Epiject® I.V.-Abbott), its sodium salt analog (see figure 1.b.), for patients for whom oral administration is not possible. Each ml of intravenous solution contains sodium valproate, which is equivalent to 100mg valproic acid. It should be administered as a 60 minute infusion (not exceeding 20mg/min) with the same frequency as the oral preparations (7,30).
Divalproex sodium (Epival® -Abbott), in figure 1.c., consists of valproic acid and sodium valproate in a 1:1 ratio, and dissociates to the valproate ion in the gastrointestinal tract (7,30,31). It was released onto the Canadian market in 1984 and is widely used in Canada, appearing in the top 200 most prescribed drugs in Canada in 2000 (32). It is supplied in 3 oral dosage strengths: 125mg, 250mg and 500mg tablets (each tablet being equivalent in mg strength to valproic acid – i.e. 125 mg of divalproex sodium is equal to 125 mg valproic acid) and its administration is as per valproic acid mentioned above. Various generic brands are available for divalproex sodium.

Figure 1: Chemical structures of different valproate products

Figure 1: Chemical structures of different valproate products

2.3.2 Bioavailability

The bioavailability of valproic acid, sodium valproate and divalproex sodium oral preparations are virtually complete. It ranges from 90-100% in studies conducted in healthy volunteers and therefore undergoes relatively little first pass metabolism by the liver (33,34). The rate of absorption differs between the valproic acid preparations and the enteric-coated divalproex sodium formulation. Valproic acid has been shown to have peak plasma levels in most cases occurring between 1 to 4 hours (7,33-35) with an absorption rate constant of approximately
0.6hr⁻¹ (36). In contrast, the time to peak concentration of divalproex sodium is usually 3-4 hours (7,37).

From hereinafter, the general term valproate will be used to refer specifically to the ion that is absorbed into the systemic circulation when describing the subsequent pharmacokinetic properties from all available products mentioned above.

2.3.3 Distribution

The distribution properties of valproate give it a rather complex profile. It has been found to have a low volume of distribution ranging from 0.1-0.4L/kg indicating that the drug is mainly restricted to the circulation (33,34). The volume of distribution at steady-state (Vss) calculated by Pollack et al (38), with sampling prolonged over 1 week after the last dose, was 0.22 ± 0.86L/kg. The tissue concentration of valproate in human brains ranges from 7-28% of that found in plasma, and CSF levels fall within this range as well (39). Studies have described the pharmacokinetics of valproate using either a linear 1 (34,37) or 2 (33,40) compartment model. The use of a linear 2 compartment model to describe the plasma valproate concentration versus time curve in the above studies was due to the appearance of a relatively fast distributive phase, which was not found in the other studies.

A critical property of valproate is its high protein binding of about 90% to plasma proteins (i.e. albumin), because it is the free unbound drug that can diffuse out of the vascular system to the site of action (41). Valproate’s protein binding has been shown to be concentration dependent (42,43) and therefore total plasma levels may not be a constant reflection of the free active levels
Attempts have been made to correlate total valproate concentration and free concentration. Bowdle et al (43) found that in normal adult subjects, when valproate concentrations increased from 325 to 660 µmol/L, the free fraction increased 44% and did not remain constant. Cramer et al found a curvilinear relationship between total (480- 735µmol/L) and free valproate concentration in young adults with seizure disorder using a second degree polynomial equation. In this study, binding averaged 93% at 50µg/ml (350µmol/L), 91% at 75µg/ml (520µmol/L), 85% at 100µg/ml (700µmol/L), 78% at 125µg/ml (870µmol/L) and 70% at 150µg/mL (1040µmol/L) of valproate (26). They attributed the high variability between free/total relationship at high valproate levels to saturation of albumin sites and to the possible competition of binding sites by free fatty acids (26). A linear relationship was found between free fraction and total valproate concentration in young adults (18), and in children and adolescents (44) in the concentration range of 200-800µmol/L, where free fraction increased with increasing valproate levels. Another study in 9 healthy adults (aged 20-35 years), also found that the valproate free fraction increased as the total level increased, with the changes in free fraction becoming significantly different at total drug concentrations above 700µmol/L (45).

In vitro studies have also shown that free fraction of valproate increases from 13% at 27µg/ml (190µmol/L) to 49% at 103µg/ml (715µmol/L) (42). In vitro, Cramer et al found that in normal human plasma free fraction of valproate is 5% at 20-60 µg/ml (140-415µmol/L), significantly increasing to 8% at 80 µg/ml (550µmol/L), and increasing to 20% at 145µg/ml (1005µmol/L) (46).

Renal insufficiency may have an effect and alter protein binding of valproate in the presence of normal total plasma protein or plasma albumin concentration. This may be due to a qualitative
change in the serum albumin or a displacement from binding sites by endogenous substances accumulating in renal failure such as free fatty acids (47).

2.3.4 Metabolism and Elimination

In healthy volunteers, valproate's elimination half-life is approximately 12 hours (33,34). Less than 3% of valproate is excreted unchanged in the urine (48). Three metabolic pathways have been reported for valproate. About 50% is metabolized by conjugation, about 40% by beta oxidation in the mitochondria, and about 10% undergoes cytochrome P450 oxidation (48). The specific P450 enzymes that are involved in the metabolism of valproate have not been fully determined, however, they may include: CYP2A6, 2C9, 2C19, 2B6 4A and 4B (49-51). A study conducted by Pollack et al in 5 healthy adult men examined the kinetics of valproate and its active metabolites (38). The accumulation of the oxidative metabolites was found to be slower compared to valproate. Also, the elimination half-lives of the metabolites were much longer than that of the parent drug (table 1) indicating that the elimination of the metabolites is not rate-limited by the elimination of the parent drug. This is important because the 2-ene-valproic acid and 4-ene valproic acid metabolites have been found to have anticonvulsant potency (38) (60-100% of parent drug in mice (52)). In addition, 4-ene-valproic acid is thought to be linked to valproate's hepatotoxicity (39).

Valproate may affect other metabolic pathways. It exhibits inhibitory effects on epoxide hydrolase and glucuronyltransferase and therefore can cause an increase in concentration for drugs metabolized through these pathways (50,53,54). It has also been theorized that plasma concentrations of valproate may be altered in renal insufficiency as a result of accumulation of
valproate glucuronide, followed by systemic hydrolysis of the glucuronide, regenerating the parent compound (55).

Table.1: Terminal Elimination half-lives of Valproate Metabolites (38)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>3-oxo-valproic acid</th>
<th>4-OH-valproic acid</th>
<th>5-OH-valproic acid</th>
<th>2-ene-valproic acid</th>
<th>3-ene-valproic acid</th>
<th>4-ene-valproic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Half-life (hrs)</td>
<td>28.0 ± 3.6</td>
<td>37.6 ± 17.3</td>
<td>30.3 ± - *</td>
<td>43.4 ± 18.4</td>
<td>75.1 ± - *</td>
<td>50.7 ± 28.7</td>
</tr>
</tbody>
</table>

* standard deviations not reported by study (not enough data for determination)

Clearance

The total plasma clearance of valproate is approximately 7-9mL/min (33,34,43,56,57) or ~6-8mL/hr/kg (43,56,57). Diurnal variations have been observed in the clearance of valproate with the total and free level plasma concentrations being lower during the evening dose compared to the morning dose (57). Part of the diurnal variability was thought to be meal related (57). Valproate is a low hepatic extraction drug, with an extraction ratio (ER) of ~zero (F=1-ER), therefore its hepatic clearance is determined by the intrinsic clearance and the unbound fraction

\[ CL_h = CL_{int} \times f_u \]

and is not dependent on hepatic blood flow (58).

Previous studies have shown valproate clearance to be concentration dependent. In 6 normal adult subjects, total plasma clearance decreased 20% (8.33mL/hr/kg to 6.67mL/hr/kg) in a 250mg single dose study compared to 500mg/day in a multiple dose (steady state) experiment (43). Clearance did not change with a further increase in dose to 1000mg/day. This was attributed to a decrease (~20%) in the intrinsic clearance (perhaps valproate auto-inhibition), counterbalancing the increase in fraction unbound. Clearance increased 21% (to 8.2mL/hr/kg) at 1500mg/day, which was attributed to a further increase in free fraction (43). Gidal et al also found that at
doses of 10-50 mg/kg at steady-state (total concentration ~70-1180μmol/L) the unbound clearance decreased as the dose increased. The total clearance was found to increase- which was attributed to a greater increase in free fraction that more than offset the decrease in intrinsic clearance (59). Gomez Bellver et al found statistically significant increases in clearance of valproate when the dose was increased from 1000mg to 3000mg (single doses) in the concentration range of approximately 140μmol/L to 1390μmol/L. No significant changes were found in the unbound valproate clearance, despite its tendency to decrease with an increase in dose (45).

2.4 Valproate Pharmacokinetics in the Elderly

Three studies have been conducted to evaluate the pharmacokinetics of VPA in the elderly (56,57,60)

Bryson et. al (60) studied the pharmacokinetics of a single, intravenous, 400mg dose in 7 healthy male volunteers aged 20-35 years, and in 6 elderly male patients aged 75-87 years. Blood samples were collected for 48 hours. The median elimination half live of VPA was significantly different between the 2 groups (7.2 hours in the young subjects and 14.9 hours in the elderly). This difference was attributed to a significantly larger volume of distribution that was found in the elderly (0.19L/kg) compared to the younger subjects (0.13L/kg). This study however, did not find any significant changes in clearance between the 2 groups (0.58L/hr in the elderly vs. 0.69L/hr in young), and free valproate levels were not measured.

Perucca et al (56) compared the pharmacokinetics of valproic acid in six young healthy volunteers (age 24 to 26 years) and six healthy elderly volunteers (age 68-89 years) after a single oral dose of 800mg. Blood samples were collected for 32 hours. No significant differences were
observed between groups for the half life (13 hours in the young, 15.3 hours in the elderly), the volume of distribution (0.14L/kg in the young, 0.16L/kg in the elderly) or the total valproate clearance (7.7ml/hr/kg in the young, 7.5ml/hr/kg in the elderly). They did, however, find a significantly higher free fraction, (9.5% vs. 6.6%) in the elderly subjects. This single dose study achieved a $C_{\text{max}}$ of 500µmol/L. They also found a significant decrease in unbound clearance in the elderly (78ml/hr/kg) compared to young adults (127ml/hr/kg). The 2 groups differed significantly in plasma albumin levels with the elderly having lower levels.

The third study by Bauer et. al. (57) studied the steady state kinetics of valproate (250mg twice daily x 5 days) in 6 healthy young volunteers (aged 22-25 years) and 6 healthy elderly volunteers (aged 60-88 years). On the fifth day of dosing, blood samples were drawn over each dosage interval (12 hours). Morning and evening total clearance, $C_{\text{ss}}$ (steady-state concentration), $C_{\text{max}}$ (maximum concentration) and $t_{\text{max}}$ (time of maximum concentration) did not differ between the 2 groups. The unbound fraction of valproate was found to be higher in the elderly, both during the day (10.7%) and in the evening (9.7%) compared to the younger adults (morning-6.4%, evening-6.1%). Also, the unbound clearance was lower in the elderly both during the day (64ml/hr/kg) and in the evening (75ml/hr/kg) compared to younger adults (morning-106ml/hr/kg, evening-123ml/hr/kg). Due to these differences in unbound clearance, the elderly had much higher unbound steady-state concentrations than the younger adults during the day (33µmol/L and 19µmol/L respectively) and evening (28µmol/L and 17µmol/L respectively), although their total concentrations were similar (~300µmol/L during the day, ~280µmol/L in the evening) (57).
Although it is clear that valproate protein binding decreases as the concentration increases in a younger population, and that protein binding is decreased at lower concentrations in an elderly population, it is not yet known what changes in the extent of protein binding occurs at higher concentrations in the elderly. Likewise, although studies conducted in the elderly have found unbound clearance to be decreased compared to younger adults at lower concentrations, the change in clearance of the unbound fraction has not been evaluated at higher concentrations in the elderly. Understanding the changes in these parameters across a dosing range in the elderly provides important information for the interpretation of valproate plasma levels and how these levels may impact the occurrence of side-effects in this population. Therefore, we have evaluated the dose related changes in the steady-state pharmacokinetics of valproate in the elderly and their influence on pharmacodynamic effects.
Hypothesis

We hypothesize that an increase in free fraction and decrease in the unbound clearance of valproate at high total valproate concentrations in the elderly will result in higher free valproate concentrations than expected. Also, elevated concentrations of free valproate in the elderly across a dosage range may be associated with an increasing severity in side-effects.

Objectives

1. To compare valproate free fraction and side-effect profiles in patients taking valproate for a mood disorder, in different age groups and at different concentrations, using data obtained in an observational manner.

2. To evaluate the steady-state pharmacokinetics and pharmacodynamics of valproate in healthy, elderly volunteers at 3 different doses (500mg, 1000, 1500mg daily) in a within subject study.
5 Methods

5.1 Study Design 1 – Observational Study

Patients taking valproate (any brand: e.g., valproic acid (Depakene®), divalproex sodium (Epival®)) for a mood disorder participated in 1 to 5 study sessions during which steady-state valproate concentrations (free and total) were determined. The patients were also interviewed to determine their side-effect profile. Patients were recruited as part of a larger study (The Mood Stabilizer Study), for which detailed information related to their course of illness and response to mood stabilizer therapy was systematically collected.

5.1.1 Inclusion and Exclusion Criteria

Inclusion criteria for the study consisted of patients of any age who were receiving or about to receive valproate for the treatment of a mood disorder and provided written consent to participate in the study. In the event that a patient was not able to provide consent (<16 years of age), consent was sought from their caregivers or guardians.

Inpatients and outpatients were recruited from the Department of Psychiatry at Sunnybrook & Women’s College Health Sciences Centre. Outpatients were identified by their treating psychiatrist, they were then approached, the study was explained, and they were asked to participate. Patients either signed the consent form (appendix 1) or declined to participate and this information was recorded. Their first and subsequent sessions were coordinated to occur either before or after their appointments with their psychiatrists.
For inpatient recruitment, the inpatient units in psychiatry at Sunnybrook Health & Women’s Health Sciences Centre, Sunnybrook site, were checked everyday for patients who were taking valproate for a mood disorder. The treating psychiatrist was consulted and, if the patient met study criteria, they were approached for consent to participate. If the patient signed the consent form, a session was then scheduled as soon as possible.

5.1.2 Sample Size

The sample size needed to detect at least a 100% difference (e.g. from 10% free fraction to 20%) in the free fraction of valproate (with a power of 80%, an alpha of 0.05, and assuming a standard deviation equivalent to the difference) we would need at least 13 subjects in each group. The 2 independent groups would consist of: the elderly free fraction at high and low concentrations, and also the elderly free fraction compared to the younger adult free fraction.

5.1.3 Study Session Procedures

Patients attended one to five study sessions in the Human Psychopharmacology Laboratory at Sunnybrook and Women’s College Health Sciences Centre. Each study Session would take place either before or after the patient’s psychiatry appointment.

At each session, a blood draw (as close to trough as possible) was performed to obtain the patient’s serum valproate levels. The patient’s height and weight was also taken. During the session the patient would answer questions and fill out forms regarding their valproate therapy. The interview duration was approximately 1 to 2 hours depending on the patient. Information
regarding the patient's valproate therapy that was pertinent to this study was extracted from the following forms used during the interview process:

1. Demographic Information- This form contains information on the patient's age.

2. Current Drug List- This form listed any current prescription and over-the-counter medications the patient was taking. The product, reason for taking the medication, daily dosage, frequency, route, duration at current dose and total duration were also recorded.

3. Mood Stabilizer Assessment - The patient indicated the perceived efficacy and side effects of their valproate therapy on visual analog scales. One visual analog scale (VAS) rated the degree to which side effects of their mood stabilizer medication were interfering with their functioning. This VAS was anchored at one end with "not interfering at all" and "markedly interfering with functioning" at the other end. Subjects were also asked to list any side effects they feel were due to valproate therapy and to mark on a VAS the degree of bothering by this side effect. The anchors included "not bothered at all" to "extremely bothered".

4. Valproic Acid Pharmacokinetic Data Form- the product brand, dosage, duration on present dosing schedule, time of the last three doses of valproate were recorded here. Also recorded were weight, height of the patient. This form was also used to record information about lithium or carbamazepine if the patient was taking any one of these medications concurrently with valproate.

5. General Health Status (SF-36) (61) which consists of 11 questions asking the patients their views about their general, mental and physical health. Two summary scores were calculated from this questionnaire to represent physical health, Physical Composite Score (PCS), and mental health, Mental Composite Score (MCS). The PCS and MCS incorporate means, standard deviations and factor coefficients from the general US population so that both have
a mean of 50 and SD of 10. Therefore for the norm-based interpretation, all scores below or above 50 are above and below the mean respectively.

At the end of the study session a DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) diagnostic form was given to the treating psychiatrist to be filled out. The DSM-IV Diagnosis form included the Global Assessment of Functioning (GAF) of the patient as rated by the psychiatrist.

5.1.4 Drug Assay

All blood samples were drawn into two 10mL red-top clot activator vacutainer tubes, one of them used for total valproate analysis at the Biochemistry lab at Sunnybrook Hospital as well as TSH level, and the other used for free valproate determination at The Hospital for Sick Children Toxicology laboratory. One 6mL green-top sodium heparin vacutainer tube was also collected to test for serum creatinine, blood urea nitrogen, albumin, total protein and bilirubin. The three tubes were then taken directly to the lab at Sunnybrook and Women's College Health Sciences Centre, Sunnybrook site. The free and total valproate determination were organized to be analyzed at Sunnybrook laboratory but they in turn analyzed total valproate levels and sent out the samples for the free levels to be done at The Hospital for Sick Children. The samples analyzed for total valproate were performed on the Hitachi 917 (Boehringer Mannheim Canada Ltd.) by recombinant DNA technology utilizing fragments of the enzyme B-galactosidase which recombine to form the catalytically active enzyme. The amount of B-galactosidase formation is monitored by the hydrolysis of chlorophenol-red-B-D-galactopyranoside (CPRG) and is directly proportional to the concentration of valproic acid in the sample (62,63) The hydrolysis
of rate of CPRG was measured at 570nm. The coefficient of variation for this assay was 7.5% and the lowest level of detection was 21 µmol/L.

Free valproate, analyzed at The Hospital for Sick Children Laboratory, was first separated from the protein-bound drug by the method of ultrafiltration using a Centrifree® filter by AMICON (64). Analysis of free valproate levels was determined using the enzyme multiplied immunoassay technique (EMIT by CIVA) on the Monarch 2000 analyzer for samples that were collected between December 1998- January 2000. This method was carried out on the Monarch 2000 and is based on the competition between drug in the sample and the drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PD) for antibody binding sites. The enzyme activity decreases when it binds to the antibody and so drug concentration is measured in terms of enzyme activity at 340nm. The coefficient of variation and limit of detection for this method was 5% and 7µmol/L respectively (65).

Valproate samples that were analyzed from January 2000 onward, were separated by ultrafiltration with a Centrifree® filter and then analyzed using Fluorescence Polarization Immunoassay (FPIA) technology on the Abbott TDxFLx® System (64). The coefficient of variation for the assay was 5% with a limit of detection of 7µmol/L.

5.1.5 Ethical Considerations

Written informed consent was obtained from each participant of the study. If for some reason the patient was unable to provide consent (i.e. children under the age of 16, or those that were too ill), consent was sought by the patients’ caregiver. All data is confidential and was recorded in research records separate from hospital records. The data files, both paper and computerized,
were identified by patient initials and patient number, not by patient name. The laboratory information obtained was provided to the treating psychiatrist for use in the care of their patient according to regular clinical practice.

**Blood Sampling**

A total of 1 blood sample (3 vacutainer tubes, ~26mL total) was taken per study session by a departmental research assistant or a nurse at the Specimen Collection Laboratory at Sunnybrook and Women’s Health Sciences Centre, Sunnybrook site. The research assistant was trained in venipuncture in accordance with standard training protocol at Sunnybrook and Women’s College Health Sciences Centre. The risks associated with blood draws involved little and brief pain. There may have been a slight chance of bruising or inflammation, but this procedure presented very low risk.

5.1.6 Data Analysis

All statistical analyses were performed using the statistical package SPSS 10.0 and Excel 97 for Windows. The pharmacokinetic parameters of interest were free and total valproate concentration and the free fraction of valproate. The pharmacodynamic parameters of interest were: interference of functioning due to side-effects measured by the patient, average and maximum bothering of side-effects reported, and the number of side-effects reported by each patient. Correlations between valproate free fraction versus age, valproate dose, valproate total concentration and albumin were conducted. Correlations were also conducted between age versus total valproate concentration, free valproate concentration, daily dose and albumin. These correlations were also conducted within the elderly group. Correlation for the
pharmacodynamics included correlating each dynamic variable with age, total and free valproate concentration, valproate free fraction and daily dose.

Multiple regression was also performed using valproate free fraction, total and free concentration each separately as the dependent measures. Regression used 2/3 of the data as a training set and 1/3 as a test set. The independent variables for each predictive model included a combination of: valproate dose, patient age, weight, albumin concentration, time since last dose, total and free valproate concentration.

Hypothesis testing was also conducted. The patients were divided into two arbitrary age groups, group 1: ≤ 45 years (younger adults) old and group 2: ≥ 65 years old (elderly group). First a student's t-test was used to test for any difference in valproate free fraction between the two groups. Student's t-tests were used to test if the free fraction of valproate differed between the 2 age groups at different total valproate concentration levels. The same hypothesis testing was done with the interference of functioning due to side-effects measured by the patient, average and maximum bothering of side-effects reported, and the number of side-effects as the variable of interest.

**Fuzzy Logic**

Data analysis in medical science is usually approached using probability statistics. An alternative method for analyzing data has been developed that is based on fuzzy set theory and fuzzy logic inference. Fuzzy logic modeling is a knowledge based approach that elicits, analyzes and interprets knowledge for solving a problem much like human reasoning through the use of
Fuzzy sets allow memberships to sets that are partial rather than restricted to the classical all or none (i.e., 0 or 1) membership. The knowledge base (rulebase) is made up of fuzzy rules, in the “if”…”then” form, and these rules can be derived from an expert’s qualitative knowledge of that system (66) or constructed using input/output numerical data without a priori knowledge of the system (67). Fuzzy logic modeling has been successful in many areas, however, its usefulness in analyzing pharmacological data has not been fully evaluated. Due to the variability, complexity and subjectivity associated with psychopharmacological data, a fuzzy logic approach for analyzing this information may offer benefits over more traditional approaches (68,69).

As a secondary analysis, fuzzy logic modeling was used for predicting free fraction of valproate. In developing the rulebase for the fuzzy model, first the values of the output variable were clustered to form fuzzy output sets. Next the relationships of the input variables, represented as $n$-dimensional input data points, to the fuzzy output sets were determined by assigning membership degrees to their fuzzy sets. The proportional contribution of each input variable to the performance of the model was then determined based on a local heuristic search approach with the objective of minimizing the modeling training error. The inference process, which is the last step, involves determining the degree of firing for each rule for a particular set of input variables, and then inferring a crisp output value.

The fuzzy model used 2/3 of the data as a training set and the remaining 1/3 as a test set. The input variables for the fuzzy model included the pertinent patient variables such as age, weight, albumin concentration, dosage of valproate, time since last dose, total and free valproate concentration with the resulting output being either valproate free fraction total valproate concentration or free valproate concentration.
The predictive performances of the regression and fuzzy logic models were assessed by comparing the output predictions for our test data to the known outputs (i.e., prediction error = predicted – actual). This was done using the method by Sheiner and Beal (70) which determines the precision and bias of the model. The precision (the size of the ‘miss’ of the predictions) was calculated using the root mean squared prediction errors (RMSE) and the bias (the degree to which the predictions are too high or too low) associated with the predictions was calculated as the mean of the prediction errors (ME).

5.2 Study Design II- Experimental Study

A single blind, within subject, study was conducted in healthy elderly volunteers ages 65 years and older. The pharmacokinetics and pharmacodynamics of valproate (divalproex sodium, Epival®, Abbott Laboratories Inc., Canada) were assessed at steady-state at 3 doses (500mg/day, 1000mg/day, and 1500mg/day). A single blind study was chosen to avoid extending the length of the study in order to accommodate titrating up to higher doses. The length of the study for each subject was 22 days on the drug, in which subjects attended 1 practice session for the computer tasks and 3 full study sessions. Subjects took valproate 250mg b.i.d on days 1-8, 500mg b.i.d on days 9-15, and 750mg b.i.d on days 16-22. In previous studies (56,60) the half-life of valproic acid was at most 16.9 hours in the elderly, but these studies only used lower therapeutic doses. In the study conducted by Bowdle et.al., the half-life of valproate in adults was as high as 24 hours with 1500mg/day. Since the pharmacokinetics of valproate in the elderly
may be altered, we assumed steady-state conditions would be reached after a minimum of 6 days. A diagram of the study design is shown in the appendix 2.

Subjects were recruited by placing advertisements on bulletin boards at Sunnybrook & Women’s College Health Sciences Center, and with permission at other hospital notice boards, community centers, Toronto public libraries, senior’s centers and residences and by personal contact.

5.2.1 Inclusion Criteria

Table 2: Inclusion and exclusion criteria for subject recruitment in experimental study

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
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<tbody>
<tr>
<td>Healthy elderly volunteers aged 65 and over</td>
<td>Use of potent metabolic enzyme inhibitors or inducers</td>
</tr>
<tr>
<td>No consumption of alcohol, caffeine 12 hours before and during the study sessions</td>
<td>Current hepatic disease or renal failure</td>
</tr>
<tr>
<td>Written informed consent</td>
<td>Known contraindications or hypersensitivity to valproic acid</td>
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<td></td>
<td>Abnormal biochemical or hematological laboratory tests as judged by the study physician</td>
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<tr>
<td></td>
<td>Concomitant use of drugs metabolized by CYP450: 2A6, 2C9, 2C19, 2B6, 4A</td>
</tr>
<tr>
<td></td>
<td>Use of other highly protein bound drugs (≥ 90%)</td>
</tr>
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<td></td>
<td>Abnormal albumin levels</td>
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Healthy subjects 65 years of age or older were determined eligible for a study assessment after a brief telephone screening. During the assessment, after providing written informed consent (appendix 3) (approved by the Research Ethics Board at Sunnybrook), subjects filled out a medical history questionnaire, and had a blood sample taken for routine clinical biochemistry and hematological tests. The hematological and biochemical lab tests included determination of: albumin levels, the liver profile (ALP, ALT, AST and bilirubin), GGT, renal profile (creatinine,
urea), electrolytes, glucose (random), total protein, and a complete blood count (including differential). In addition a urine screen was performed to test for any interfering drugs. Subjects who were using any drugs that are known potent inhibitors or inducers of hepatic enzymes involved in valproate's metabolism were excluded because of potential interference of valproate's pharmacokinetics. Subjects taking any drugs that were highly protein bound (≥90%) to plasma albumin (e.g. phenytoin) were excluded because of the possible displacement of valproate from its albumin binding sites during concomitant therapy which may have influenced our pharmacokinetic analysis. Subjects who had abnormal albumin levels were also excluded because they may have had abnormally increased/decreased free fraction of valproate (46). Renal insufficiency may alter the binding of valproic acid and these subjects were also excluded (47). Subjects were excluded if they were presently taking any mood stabilizer medications or if they had taken any in the past.

5.2.2 Sample Size

Sample size calculations were based on the primary pharmacokinetic objective. In order to detect at least a doubling (e.g. 10% to 20% fraction unbound) in free fraction of valproate within subjects (with power of 80%, alpha of 0.05, and assuming the coefficient of variation is 70%) we would need 6 subjects for this study. In a previous study, the mean free fraction in the elderly was 10.7 with a standard deviation of 1.6 at a steady-state concentration of ~300µmol/L (57). The coefficient of variation in this study was very small (15%). We have proposed a larger one to be on the cautious side, since the variability may increase at higher concentrations.
5.2.3 Study Session Procedures

Subjects attended 1 assessment, 1 computer tasks practice session and 3 full study day sessions over a period of at least 22 days in the Human Psychopharmacology Laboratory at Sunnybrook & Women’s Health Sciences Centre, Sunnybrook site. The practice session (day 1) was used to familiarize the subject with the dynamic tests that were to be used in the subsequent full study sessions. The subjects were trained in each test at a certain level until their performance plateaued (same score on the test 3 consecutive times) in order to reduce learning effects over the course of the study.

During the study a dose of the drug was taken at 8:00am and 8:00pm everyday for at least 7 days in order to achieve steady-state levels, starting with the lowest dose. Divalproex sodium 250mg b.i.d was administered on days 1-8 (day 1 - the first dose was taken in the evening after the training session, day 8 - only the morning), 500mg b.i.d on days 8-15 (the first dose was taken immediately after the first study session on day 8, and on day 15 only in the morning), and 750mg b.i.d on days 15-22 (on day 15 the first 1000mg dose was taken in the evening after the second study session was done, and on day 22 only the morning dose was taken in order to do the study session). Each subject took 4 tablets twice daily with a different number of placebo tablets at each dose. For example, at the 250mg b.i.d dose, each subject took one active tablet and 3 placebo tablets during each dosing interval to maintain the blind. Placebo Epival® tablets were provided by Abbott Laboratories Inc, Canada. The weekly medication for each subject was packaged in blistered dosettes to enhance compliance. Twice daily dosing was used since a divided regimen is recommended for daily doses greater than 250mg (71). Subjects were also asked to record daily the time of valproate administration and the type and severity of side-effects they experienced throughout the study.
The study session days proceeded from approximately 8:00am to 8:00pm, in order to obtain valproate concentrations over the entire 12 hour dosing interval. The subjects were asked to fast overnight from 11:00pm until 8:00am and to try to get at least 8 hours of sleep before the start of each study session day. Twelve hours before and during the study sessions, consumption of alcohol or caffeine containing food or beverages were prohibited because of the possible interference with the pharmacodynamic assessments of valproate. Urine samples for drug screening were obtained and a breathalyzer test was administered before each study session. Light meals were given at 2, 5 and 9 hours post dose.

Valproate was administered at 8:00am on the morning of the study session. Peak plasma concentrations were expected between 3 to 4 hours (71) and therefore blood collection times were scheduled at baseline (0hr), 1, 2, 3, 4, 6, 8, 10, and 12 hours after dosing. The valproate concentrations at these time points were used to determine the pharmacokinetic parameters and to relate these concentrations to the results of the pharmacodynamic assessments. An intravenous catheter was inserted into the subjects’ forearm cephalic vein at the start of each study session for the collection of the blood samples (approximately 20mL/sample) from baseline to 12 hours. Immediately after blood samples at time 0, 2, 4, and 8, subjects rated their current level of sedation, mood, nausea, performed the psychomotor tests and were rated for their level of tremor (described below). Therefore, after 8 hours the subject was kept comfortable and was not required to perform further tests. After the 12 hour blood sample, the catheter was removed and the subject was finished for the day. Subjects were assessed at the end of the study session, and if necessary were taken to their residence by taxi and/or accompanied by study personnel.
5.2.4 Pharmacodynamic Assessments

Subjects were evaluated for sedation, nausea, tremor and psychomotor performance at baseline, 2, 4, and 8, hours after the administration of the divalproex sodium dose. Assessments were performed using the following scales and tests:

**Manual Tracking Test (MTT):** This is a test of psychomotor performance. It is a manual tracking test which measures the subject’s ability to keep a computer image of a “plane” in the center as it scrolls down a winding sigmoid-shaped roadway, using a mouse. The computer calculates the distance in centimeters between the plane and the center of the road, and the proportion of time the plane spends over the road. Three trials are run, with each trial lasting 50 seconds with a 5 second break between them. The 3 trials are averaged to determine a final score. There has been extensive experience with the use of this test by our group to assess psychomotor performance (72-75).

**Visual Analogue Scale (VAS):** Sedation was rated subjectively using 12 computerized 0-100mm visual analog scales that contain left and right anchor statements (see appendix 4) from the Tufts University Benzodiazepine Scale (76). In previous studies, this scale was found to be reliable and sensitive in measuring sedation (74,75) A pencil-paper visual analog scale was also used to rate nausea anchored with “Not at all” at one end and “A Lot” at the other.

**Digit Symbol Substitution Task (DSST):** This is a computerized test in which subjects are asked to make as many correct symbol-for-digit substitutions on screen as possible within 90 seconds using a computer mouse (see appendix 4). The DSST has been used in previous studies as a

**Trails Making Test:** This test measures psychomotor and cognitive performance. The paper/pencil Trail-Making Test consists of 2 parts: part A and B. Part A consists of 25 circles on a sheet with the numbers 1-25 printed inside. The subject is required to connect the circles starting with the number 1 in numerical sequence, as quickly as possible. Part B consists of 25 circles with either a number (1-13) or a letter (A-L). The subject is required to connect the circles in sequence alternating between number and letters (1, A, 2, B...) as quickly as possible. The scores are recorded as the time in seconds required to complete each part. The Trails Making Test has been previously used in other studies to assess psychomotor and cognitive performance (79,80).

**Tremor Scale:** The tremor part of the revised Clinical Institute Withdrawal Assessment for Alcohol scale (CIWA-Ar) (81) was adapted into a visual analog scale and used to assess the presence and severity of tremor in subjects. Tremor was objectively rated by study personnel in which they asked the subject to extend their arms with fingers apart to observe any tremor. This scale has also been used in other studies to measure tremor severity (82).

5.2.5 Medication Compliance

A study log was given to each subject in which they recorded the time the study medication was taken, as well as the time and amount of any prescription or over-the-counter medication taken throughout the study. The study medication was packaged in blister packs containing a weeks supply of medication to make it easier for the subjects to keep track of when to take their medications. The study medication was given as twice daily dosing and subjects were asked to
take them at 8:00am and 8:00pm in order to get blood samples as close to trough as possible during the full study day sessions.

5.2.6 Side-effect Monitoring

Subjects were given a side-effect log in which they were asked to subjectively record the frequency and severity of any side-effects they may have experienced in the last 24 hours for each day they were on medication. The form contained a list of common side-effects that may be expected with valproate therapy (7). For each side effect there were 2 visual analog scales, one measuring frequency with which the side effect has occurred in the last 24 hours from “never” to “constantly”. The second visual analog scale measure the severity of each side effect from “mild” to “severe”. Subjects were also able to report any other side-effects that were not included on the list.

5.2.7 Drug Assay

Blood samples taken during the full study days were drawn into two 7mL glass royal blue-top additive- free vaccutainer tubes. These were left at room temperature for approximately 45-60 minutes and then centrifuged at 3000rpm for 10 minutes. The serum was then separated and stored in polypropylene cryo tubes with screw on tops at -20°C for no longer than 6 months. Determinations of the free and total valproate levels were made at Sunnybrook and Women’s Health Sciences Centre, Department of Clinical Pathology, Sunnybrook site using the Roche Modular system (Boehringer Mannheim Systems). This system uses recombinant DNA technology in a homogeneous enzyme immunoassay based on the bacterial enzyme β-galactosidase (63,83). In this assay, the formation of enzyme causes an absorbance change that
is directly proportional to the amount of analyte present in the sample. The sensitivity of the assay (limit of detection) is 20.8μmol/L and has an intra-assay coefficient of variation of 2.3% ± 0.5 and an inter-assay coefficient of variation of 3.9% ± 0.9 at the lowest concentrations. For the free levels, a protein-free filtrate was prepared by centrifuging using an Amicon Centrifree® filter and analyzed using the above method.

5.2.8 Ethical Considerations

Safety of Drug Doses

Subjects were informed of the potential side-effects before signing consent to participate in the study, and were told that they could withdraw from the study at any time. All data was kept strictly confidential and was identified by subject number and initials, and not by subject name.

In previous retrospective and case studies conducted, divalproex and valproate doses ranging from 250 to 3000mg/day were generally well tolerated in elderly patients (9,12,13). Side-effects were minimal and included sedation (6% of patients (12) and 10% of patients (13)), nausea (6% of patients (12)) and confusion (3% of patients (12)) being reported and therefore these doses posed minimal risk to our volunteers. In one 1990 study, there were no adverse events reported that could be attributed to divalproex (9). In a recent case report of 6 elderly patients who received divalproex in the range of 250-1000mg/day, only in one patient was divalproex discontinued because of agitation and insomnia and fears of death, although it was possibly due to the course of his refractory illness (84). The treatment-emergent adverse events with an incidence ≥5% in short term placebo-controlled trials with oral divalproex sodium in the treatment of acute mania that were greater than placebo were: accidental injury, asthenia (lack of
appetite), abdominal pain, nausea, vomiting, dyspepsia (indigestion), somnolence, dizziness and rash. In elderly patients (65 years or older) accidental injury, infection, pain, somnolence and tremor were more frequently reported compared to patients 18-65 years old (71). Rare but fatal hepatic failure has occurred in patients (1 in 10 000) receiving valproate but this incidence has decreased (~1 in 50,000) due to increased physician awareness (85). Children under the age of 2 are at greatest risk especially those taking other medications. No deaths were reported in patients over the age of 10 years on valproate alone (71).

Safety of Blood Sampling
A total of 9 blood samples (~150ml total) were taken per study session. These were taken via a catheter inserted into the subject’s forearm cephalic vein by a nurse. This procedure involved little pain and allowed for the majority of blood samples to be collected with only one puncture, during a short duration (12 hours). The assessment session involved one blood sample taken by venipuncture by a departmental research assistant or a nurse and might have involved little and brief pain. The research assistant was trained in venipuncture in accordance with standard training protocol at Sunnybrook and Women’s College Health Sciences Centre. There may have been a slight chance of bruising or inflammation, but this procedure presented very low risk.

5.2.9 Data Analysis
All statistical analyses were performed using the statistical package SPSS 10.0 and Excel 97 for Windows. The pharmacokinetic parameters of interest were calculated based on the concentration-time curve for the 12 hour dosage interval. These parameters were: free fraction, maximum concentration ($C_{\text{max}}$) for total and free valproate, time of maximum concentration ($t_{\text{max}}$) for total and free concentrations, total and unbound clearance, and average total and free
concentration at steady-state ($C_{ss \, \text{avg}}$). Total and unbound valproate concentration area under the curve ($\text{AUC}_T$ and $\text{AUC}_U$ respectively) were calculated for each dosing interval using the trapezoidal rule. Total and unbound clearances were calculated by dividing the dose by the appropriate AUC. The average total and unbound steady state concentrations were calculated by dividing the corresponding AUC by the dosage interval. The average free fraction for each subject at each dose was calculated by dividing the unbound AUC by the total valproate AUC. Since during the study sessions the baseline blood samples were taken 15 to 64 minutes prior to the morning valproate dose, trough concentrations for the AUC calculations were obtained by calculating an elimination rate constant ($k_e$) for the last 3 points on the curve, (i.e., the linear portion on the semi log graph curve). We then used the equation $C_n = C_0 e^{k_e t}$, solving for $C_n$, using our original baseline value as $C_0$, our $k_e$ value obtained from the curve and the time difference between our original baseline value and 0hr. $C_{\text{max}}$ and $t_{\text{max}}$ for both total and free were obtained by inspection of the kinetic curves. For each subject, at each dose, all the above parameters were analyzed by analysis of variance for repeated measures with dosage as the condition.

Correlations were also conducted between the kinetic variables: between free fraction and dose, total valproate concentration, albumin level, total clearance and unbound clearance; between total clearance and dose, total and free valproate concentration and unbound clearance; and between unbound clearance and albumin concentration, dose, free fraction and total and free valproate concentrations.

For the pharmacodynamic parameters of interest described below a change from baseline (0hr) score was calculated from the relevant time points (2, 4, and 8 hr). A sedation summary score was determined from the visual analog scales 1-12 in the TUBS. A raw score for tremor and nausea were manually measured from the paper visual analog scales. Psychomotor performance
was determined from the MTT, DSST and the Trails Making Test. In the MTT the percentage of
time the airplane spent over the centre of the road was calculated. In the DSST the number of
correctly matched patterns in 90 seconds served to measure performance where as in the Trails
Making Test (A and B), the amount of time to complete the test was used.

The pharmacodynamic effects were calculated in 2 ways: using the study session dynamic tests
(mentioned above) and using the side effect daily log scores. For the study session dynamic
tests, the area under the effect curve (AUE) for changes from baseline scores were calculated for
DSST, MTT, tremor scale, sedation score and nausea scale. With the side effect log, the side
effects (including only patients who experienced them) were grouped into central nervous system
(CNS: drowsy, unsteady gait, headache, dizziness, double vision), gastrointestinal (GI: decreased
appetite, stomach pains, heartburn, diarrhea and constipation), tremor (incorporated both the
study session VAS and the tremor daily log scores) and nausea (incorporated both study VAS,
distress from vomiting VAS, and nausea daily log score). CNS and GI scores were calculated by
multiplying the frequency of the side effect by the severity with a total score of 100. The tremor
scores from both the daily log and study session day were treated as separate scores out of 10 and
then a mean and max score was taken. For the nausea score, the nausea VAS score from the
daily log was added to the distress from vomiting VAS to obtain a score out of 20. The study
session nausea VAS scores were also included and a mean and maximum nausea score were
calculated. The above was done to obtain a mean and maximum score for each side effect group
at each dose for each subject. These mean and maximum scores for each side effect group and
AUE curves for study day dynamics were then plotted against the average concentration at
steady state of both total and free valproate and the correlation coefficient determined. A
repeated measures analysis of variance (ANOVA) was carried out with dosage as the condition
for the pharmacodynamic analyses to determine the effect of valproate dose on the tests.
Multiple linear regression and fuzzy logic analysis of this data was conducted. For both modeling techniques 2/3 of the data was used as a training set and the remaining 1/3 was used as a test set. For the pharmacokinetics 2 models were developed using both regression and fuzzy logic. The first model used each individual concentration point from the pharmacokinetic curves to predict free fraction. The inputs were age, weight, albumin concentration, time since last dose, valproate dosage, total valproate concentration, and the output variable: valproate free fraction. The second model was built to predict free fraction after a dosage change. The inputs here were: age, present weight, present albumin level, original total average valproate concentration at steady state, dosage change, original total valproate clearance, original valproate free fraction, and present average total valproate concentration at steady state.
6 Results

6.1 Study Design 1- Observational Study

6.1.1 Subjects

The observational data is part of a larger study (The Mood Stabilizer Study) that was in progress from September 1998- August 2000. Forty three subjects taking valproate participated in one or more sessions (figure 2.). A total of eighty-seven potential subjects taking valproate were identified through inpatient and outpatients psychiatry services at Sunnybrook & Women's College Health Sciences Centre, Sunnybrook site. Fourteen of these potential subjects were considered ineligible for the study by their treating psychiatrist due to: cognitive problems (n=4), no reason/ reason unknown (n=4), patient diagnosis (n=2), psychiatrist thought patient might not want to participate (n=1), patient mood lability (n=1), valproate being discontinued (n=1), and patient non-compliance (n=1).

Of the seventy three subjects who were eligible, fifty nine of them signed consent to participate. Fourteen patients declined consent due to: not interested (n=8), did not want blood drawn (n=2), worried about confidentiality (n=1), valproate discontinued (n=1), health reasons (n=1), and scheduling reasons (n=1). Of the fifty nine subjects that signed consent, 16 subjects dropped out before having a session due to the following reasons: not seeing psychiatrist/not followed at SWCHSC (n=3), time constraints (n=3), health reasons (n=3), not interested (n=2), discontinuing valproate (n=2), advised against participating (n=1), did not want blood drawn (n=1), and scheduling problems (n=1).
Forty-three of the subjects went on to participate in one or more sessions, of these there were 3 dropouts (did not feel like continuing (n=1), stopped seeing psychiatrist (n=1), time constraints (n=1)). A total of 109 study sessions were conducted with the 43 subjects in the study. Of these 109 sessions, 90 of them from 41 subjects had free valproate values determined and were used in the analyses.
Figure 2: Observational Study - Valproate Subject Recruitment

Potential Subjects taking valproate: 87

Ineligible subjects according to MD: 14

Total Contacts: 73

Declined Consent: 14

Signed Consent: 59

Subjects participating in 1 or more sessions: 43

Dropouts after 1 or more sessions: 3

Dropouts with no sessions: 16
Subject characteristics are shown in table 3 for all 41 subjects. The elderly and younger adult demographics are also shown separately.

Table 3: Subject Demographics

<table>
<thead>
<tr>
<th></th>
<th>All subjects</th>
<th>Young Adult Group (≤45yrs)</th>
<th>Elderly Group (≥65 yr.)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>41</td>
<td>24</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Number of sessions</td>
<td>90</td>
<td>49</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>21 (51.2%)</td>
<td>9 (37.5%)</td>
<td>7 (70.0%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20 (48.8%)</td>
<td>15 (62.5%)</td>
<td>3 (30.0%)</td>
<td>0.016</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.2 ± 22.9</td>
<td>25.0 ± 9.3</td>
<td>73.8 ± 6.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168.5 ± 10.5</td>
<td>171.7 ± 10.0</td>
<td>162.0 ± 10.8</td>
<td>0.017</td>
</tr>
<tr>
<td>Lean Body Weight (kg) *</td>
<td>63.3 ± 12.4</td>
<td>67.0 ± 11.9</td>
<td>56.6 ± 12.2</td>
<td>0.028</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.0 ± 20.0</td>
<td>82.3 ± 22.2</td>
<td>68.0 ± 11.8</td>
<td>0.066</td>
</tr>
<tr>
<td>PCS Score</td>
<td>46.1 ± 8.7</td>
<td>49.1 ± 8.0</td>
<td>43.9 ± 7.6</td>
<td>0.010</td>
</tr>
<tr>
<td>MCS Score</td>
<td>42.2 ± 12.4</td>
<td>40.1 ± 11.5</td>
<td>45.9 ± 12.6</td>
<td>0.054</td>
</tr>
<tr>
<td>Global Assessment of Functioning</td>
<td>70.8 ± 14.0</td>
<td>65.6 ± 15.5</td>
<td>77.8 ± 8.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of Illness (years)</td>
<td>14.2 ± 14.9</td>
<td>8.1 ± 8.2</td>
<td>29.6 ± 19.5</td>
<td>0.007</td>
</tr>
</tbody>
</table>

* p-value for young and elderly group comparisons

* LBW calculated by: 1)females: 100lbs/first 5ft of height PLUS 5lb for each additional inch of height 2) males: 106lbs/first 5ft of height PLUS 6 lbs. for each additional inch of height (86)

In the total subject population, there was a similar number of males and females however, the proportion of males was higher in the younger group and the proportion of females was higher in the elderly group. The average age of the subjects was 42.2 years with a wide separation between the young and elderly groups 25.0 and 73.8 years respectively. Both the young and
elderly were similar in regards to weight although there was a statistical difference in height (p=0.017).

Most patients were diagnosed with bipolar disorder including: Bipolar I Disorder (n=23), Bipolar II Disorder (n=8), Bipolar I – secondary to a general medical condition (n=3), Major Depressive Disorder (n=1), Schizoaffective Disorder- Bipolar type (n=1), General Mood Disorder- secondary to general medical condition (n=1) and 4 of the patient’s diagnoses were not reported by the treating psychiatrist. The breakdown of diagnoses into young and elderly are shown in figure 3. The average score for the Global Assessment of Functioning (GAF) from the DSM-IV for this population was 70.8 which lies in the range that states: ‘If symptoms are present, they are transient and expectable reactions to psychosocial stressors no more than slight impairment in social, occupational or school functioning’ (87). Therefore as a whole, this patient population had a mild impairment in general functioning, although the elderly group had higher functioning scores (p<0.001) compared to the younger group. The mental and physical composite scores showed that the overall group had only slightly lower than general population average scores. However, the elderly scored higher on mental functioning (p=0.054) and younger adults had higher physical functioning scores (p=0.010).
Due to the nature of an observational study, the patients were allowed to take concomitant medications and this information was recorded in table 4. Drugs that are known to be greater than 90% protein bound in each of the medication classes are also outlined, as they may have influenced the pharmacokinetics of valproate.
<table>
<thead>
<tr>
<th>Type of Medication</th>
<th>All Patients</th>
<th>Young Adult Group</th>
<th>Elderly Group</th>
<th>Drugs &gt;90% protein binding (e.g. for each group)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of cases out of: 90</td>
<td>49</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Mood Stabilizers</td>
<td>34.4</td>
<td>34.7</td>
<td>54.2</td>
<td></td>
</tr>
<tr>
<td>SSRI</td>
<td>13.3</td>
<td>6.1</td>
<td>12.5</td>
<td>Fluoxetine, paroxetine, sertraline (n=2 young, n=3 elderly)</td>
</tr>
<tr>
<td>Other anti-depressants</td>
<td>25.6</td>
<td>10.2</td>
<td>20.8</td>
<td>Clomipramine, desipramine, (n=2 young, n=1 elderly)</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>47.8</td>
<td>55.1</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td>Anticholinergics</td>
<td>17.8</td>
<td>22.4</td>
<td>0.0</td>
<td>Benztropine (n=0 young, n=0 elderly)</td>
</tr>
<tr>
<td>Anticonvulsants</td>
<td>6.7</td>
<td>2.0</td>
<td>0.0</td>
<td>Phenytoin (n=0 young, n=0 elderly)</td>
</tr>
<tr>
<td>Antipsychotics</td>
<td>40.0</td>
<td>61.2</td>
<td>8.3</td>
<td>Clozapine, olanzapine (n=10 young, n=0 elderly)</td>
</tr>
<tr>
<td>Other CNS Drugs</td>
<td>12.2</td>
<td>12.2</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>5.6</td>
<td>0.0</td>
<td>20.8</td>
<td>Fosinopril, losartan potassium (n=0 young, n=4 elderly)</td>
</tr>
<tr>
<td>Anti-adrenergics</td>
<td>14.4</td>
<td>10.2</td>
<td>25.0</td>
<td>Terazosin (n=0 young, n=2 elderly)</td>
</tr>
<tr>
<td>Calcium Channel Blockers</td>
<td>13.3</td>
<td>0.0</td>
<td>16.7</td>
<td>Amlodipine, nifedipine, verapamil (n=0 young, n=4 elderly)</td>
</tr>
<tr>
<td>Other Cardiac Drugs</td>
<td>7.8</td>
<td>0.0</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>NSAIDS</td>
<td>6.7</td>
<td>0.0</td>
<td>0.0</td>
<td>Celecoxib, (n=0 young, n=0 elderly)</td>
</tr>
<tr>
<td>Opioids</td>
<td>2.2</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Muscle Relaxants</td>
<td>1.1</td>
<td>2.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Ophthalmic</td>
<td>6.7</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Respiratory/Nasal</td>
<td>12.2</td>
<td>18.4</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>Dermatological</td>
<td>4.4</td>
<td>8.2</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Antiglycemic</td>
<td>3.3</td>
<td>4.1</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Hormone Replacement</td>
<td>33.3</td>
<td>12.2</td>
<td>66.7</td>
<td>Ethynyl estradiol (n=2 young, n=0 elderly)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>11.1</td>
<td>2.0</td>
<td>0.0</td>
<td>Omeprazole (n=1 young, n=0 elderly)</td>
</tr>
<tr>
<td>Anti-infectives</td>
<td>1.1</td>
<td>0.0</td>
<td>4.2</td>
<td></td>
</tr>
</tbody>
</table>

* Examples of drugs in these groups that are greater than 90% protein binding and the number of elderly and younger patients who were concomitantly taking one or more of these drugs.
6.1.2 Pharmacokinetics

Descriptives of the pharmacokinetic variables are shown in table 5 and were calculated using all of the sessions conducted that contained free valproate values, therefore subjects may be represented more than once at different time points in this list. The average daily dose of valproate was found to be significantly higher in the younger group (p= 0.016). Mean total and free valproate level were all found to be significantly higher in the younger group as well. Albumin was found to decrease significantly with age (p=0.002), although all the values fell within the normal range. Overall, the free fraction of valproate (9.7% vs. 10.3% respectively) was not found to be significantly different (p=0.444) between young and elderly. The elderly on average, had taken valproate for a significantly longer duration of time.
### Table 5: Descriptives of pharmacokinetic variables

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Range</th>
<th>Mean ± Std.Deviation</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Daily Dose of valproate (mg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>90</td>
<td>250 - 2500</td>
<td>1030.6 ± 443.6</td>
<td></td>
</tr>
<tr>
<td>Young Group</td>
<td>49</td>
<td>250 - 2500</td>
<td>1107.1 ± 487.5</td>
<td></td>
</tr>
<tr>
<td>Elderly Group</td>
<td>24</td>
<td>500 - 1500</td>
<td>833.33 ± 343.15</td>
<td>0.016</td>
</tr>
<tr>
<td><strong>Total valproate level (umol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>90</td>
<td>53.0 - 826.0</td>
<td>411.6 ± 166.4</td>
<td></td>
</tr>
<tr>
<td>Young Group</td>
<td>49</td>
<td>53.0 - 826.0</td>
<td>473.6 ± 167.6</td>
<td></td>
</tr>
<tr>
<td>Elderly Group</td>
<td>24</td>
<td>85.0 - 591.0</td>
<td>301.5 ± 112.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Free valproate level (umol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>90</td>
<td>2.3 - 166.5</td>
<td>44.2 ± 28.1</td>
<td></td>
</tr>
<tr>
<td>Young Group</td>
<td>49</td>
<td>2.3 - 166.5</td>
<td>49.4 ± 30.8</td>
<td></td>
</tr>
<tr>
<td>Elderly Group</td>
<td>24</td>
<td>6.0 - 78.0</td>
<td>32.7 ± 18.6</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Valproate free fraction (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>90</td>
<td>4.3 - 22.5</td>
<td>10.2 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>Young Group</td>
<td>49</td>
<td>4.3 - 20.2</td>
<td>9.7 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>Elderly Group</td>
<td>24</td>
<td>6.8 - 19.4</td>
<td>10.32 ± 3.4</td>
<td>0.444</td>
</tr>
<tr>
<td><strong>Albumin level (g/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>90</td>
<td>35.0 - 50.0</td>
<td>42.9 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>Young Group</td>
<td>49</td>
<td>36.0 - 50.0</td>
<td>43.6 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>Elderly Group</td>
<td>24</td>
<td>35.0 - 48.0</td>
<td>41.2 ± 3.0</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Total Duration on Valproate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mnths)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>89</td>
<td>0.4 - 120.0</td>
<td>33.0 ± 28.3</td>
<td></td>
</tr>
<tr>
<td>Young Group</td>
<td>49</td>
<td>0.4 - 120.0</td>
<td>28.2 ± 27.4</td>
<td></td>
</tr>
<tr>
<td>Elderly Group</td>
<td>23</td>
<td>2.5 - 99.6</td>
<td>42.5 ± 30.8</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>Dosing Schedule (hr)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>90</td>
<td>6 - 24</td>
<td>14.1 ± 4.9</td>
<td></td>
</tr>
<tr>
<td>Young Group</td>
<td>49</td>
<td>8 - 24</td>
<td>16.1 ± 5.9</td>
<td></td>
</tr>
<tr>
<td>Elderly Group</td>
<td>24</td>
<td>6 - 12</td>
<td>11.8 ± 1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Time since last dose (hr)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>90</td>
<td>4.3 - 28.7</td>
<td>13.8 ± 4.0</td>
<td></td>
</tr>
<tr>
<td>Young Group</td>
<td>49</td>
<td>4.3 - 28.7</td>
<td>13.8 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>Elderly Group</td>
<td>24</td>
<td>4.5 - 22.0</td>
<td>14.0 ± 3.7</td>
<td>0.847</td>
</tr>
</tbody>
</table>

* p-value for young and elderly comparison

The total and free valproate levels represent levels taken during the study session as close to trough as possible. The number of people taking valproate at the following dosage schedules...
were: 1) once daily: n=17 young, n=0 elderly, 2) twice daily: n=31 young, n=23 elderly, 3) three
times daily: n=1 young, n=0 elderly, 4) four times daily: n=0 young, n=1 elderly.

Correlation with age in overall patient population

There was no significant correlation between free fraction of valproate and age in all 41 subjects. As age increases the valproate dose given is smaller (r = -0.262, p=0.013), which is reflected in the negative correlations also found with total valproate level (r = -0.475, p<0.001) and free valproate level (r = -0.294, p=0.005). There was a significant, negative correlation between albumin and free fraction (r = -0.269, p=0.010), therefore, as expected, as the albumin level decreases, the free fraction increases. The free fraction of valproate significantly increased with increasing valproate daily dose (r = 0.346, p=0.001) and total valproate concentration (r = 0.394, p<0.001). When the above analyses were repeated for the elderly group similar results were found.

Further Comparisons of Young and Elderly Pharmacokinetics

The young and elderly groups were compared at different total valproate levels (low= ≤ 350μmol/L, mid-low= 351-525 μmol/L, mid-high= 526-700μmol/L and high >700μmol/L) for differences in free fraction (table 6). At the lowest level the free fraction in the elderly was significantly different from the younger group (p=0.03). At the mid-low level there was a non significant trend in the same direction. There was only 1 elderly patient in the mid-high and high level groupings, therefore comparisons were not possible.
Table 6: Free fraction of Valproate in Young and Elderly at Different Total Valproate Level Groups

<table>
<thead>
<tr>
<th>All Valproate levels</th>
<th>Low total valproate group ≤350 µmol/L</th>
<th>Mid-low total valproate group 351-525 µmol/L</th>
<th>Mid-high total valproate group 526-700 µmol/L</th>
<th>High total valproate group &gt;700 µmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>9.7 (n=49)</td>
<td>7.1 (n=10)</td>
<td>9.0 (n=20)</td>
<td>11.0 (n=15)</td>
</tr>
<tr>
<td>Elderly</td>
<td>10.3 (n=24)</td>
<td>9.5 (n=16)</td>
<td>12.0 (n=7)</td>
<td>11.9 (n=1)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.444</td>
<td>0.031</td>
<td>0.108</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Predicting Valproate Free Fraction using Regression and Fuzzy Logic Models

Prediction was conducted using 2 different methods, multiple linear regression and fuzzy logic. The 41 patients were randomized into either the 2/3 train or 1/3 test set. There were 68 sessions from 31 patients in the train set and 22 sessions from another 10 patients in the test set. Predicting the free fraction of valproate included the following inputs: age, daily dose of valproate, total valproate level, albumin level and weight of the patient. The enter method was used in the regression model for prediction because it had the higher $R^2$ value ($R^2 = 0.340$) compared to the stepwise method. The average prediction error (bias) of this model indicated a small underprediction (ME = -0.5%), and the root mean square error (RMSE) was 2.6%. The fuzzy model performed with a smaller bias (ME = 0.1%), but with less precision (RMSE = 4.3%). The proportional contribution of each input variable to the fuzzy logic model are shown in table 7.
Predicting the total and free valproate concentrations included the following inputs: age, daily dose of valproate, last dose interval, albumin level and weight. Again the enter method was used for both total and free concentration predictions because of the larger $R^2$ value. Neither model predicted concentrations well (table 8).

Table 7: Proportional Contribution of Input Variables to Final Fuzzy logic Model Predicting Valproate Free Fraction

<table>
<thead>
<tr>
<th>Input variable</th>
<th>Proportional Contributions to Final Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.28</td>
</tr>
<tr>
<td>Weight</td>
<td>0.23</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.12</td>
</tr>
<tr>
<td>Daily dose of valproate</td>
<td>0.27</td>
</tr>
<tr>
<td>Total valproate concentration</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>1.00 Total</td>
</tr>
</tbody>
</table>

Table 8: Summary of Multiple Regression and Fuzzy Logic Predictive Performance Results

<table>
<thead>
<tr>
<th>Predicted Output</th>
<th>Regression</th>
<th>Fuzzy Logic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free Fraction</td>
<td>-0.5%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Bias</td>
<td>2.6%</td>
<td>4.3%</td>
</tr>
<tr>
<td>Precision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Valproate</td>
<td>30.6 μmol/L</td>
<td>45.1 μmol/L</td>
</tr>
<tr>
<td>Bias</td>
<td>132.3 μmol/L</td>
<td>147.7 μmol/L</td>
</tr>
<tr>
<td>Precision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free Valproate</td>
<td>-1.1 μmol/L</td>
<td>-2.4 μmol/L</td>
</tr>
<tr>
<td>Bias</td>
<td>24.9 μmol/L</td>
<td>28.9 μmol/L</td>
</tr>
<tr>
<td>Precision</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6.1.3 Pharmacodynamics

The summary descriptives of the side effect pharmacodynamic variables are shown in table 10 and were calculated using all 90 sessions which contained free valproate level values. The
average number of side-effects reported by patients was 1.02. Table 9 shows the type of side effects reported by all patients including both young and elderly. The degree to which the side effects were impacting the patients were quantified in 3 different ways: degree to which mood stabilizer was interfering, maximum degree bothered by side-effects and average degree bothered by side-effects. All of these measures were made on 10mm visual analog scales with means in this population of 2.4, 2.6 and 2.1 respectively. When the pharmacodynamics were compared between young adults and the elderly, the elderly reported significantly fewer side effects, lower average degree bothered by side-effects (p=<0.001), and maximum degree bothered by side-effects (p<0.001). They also reported that their mood stabilizer was interfering less with their functioning as compared to the younger group. The values for each of the dynamic variables for both the young and elderly group are shown in table 10.
Table 9: Side effects reported by patients

<table>
<thead>
<tr>
<th>Type of side effect</th>
<th>All patients</th>
<th>Young Group</th>
<th>Elderly Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of cases out of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>psychiatric</td>
<td>5.6</td>
<td>10.2</td>
<td>0</td>
</tr>
<tr>
<td>cognitive</td>
<td>1.1</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>neuromuscular</td>
<td>33.3</td>
<td>51.0</td>
<td>0</td>
</tr>
<tr>
<td>appetite change/thirst</td>
<td>16.7</td>
<td>28.6</td>
<td>0</td>
</tr>
<tr>
<td>sleep related</td>
<td>12.2</td>
<td>20.4</td>
<td>0</td>
</tr>
<tr>
<td>hematological</td>
<td>1.1</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>ocular</td>
<td>1.1</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>dry mouth</td>
<td>6.7</td>
<td>12.2</td>
<td>0</td>
</tr>
<tr>
<td>dermatological</td>
<td>4.4</td>
<td>6.1</td>
<td>4.2</td>
</tr>
<tr>
<td>sexual</td>
<td>1.1</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>gastrointestinal</td>
<td>15.6</td>
<td>24.5</td>
<td>4.2</td>
</tr>
<tr>
<td>renal</td>
<td>3.3</td>
<td>4.1</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Table 10: Descriptives of Pharmacodynamic Variables

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Range</th>
<th>Mean ± Std. Deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree to which mood stabilizer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>is interfering with functioning</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>86</td>
<td>0.0–9.8</td>
<td>2.4 ± 2.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Young Group</td>
<td>48</td>
<td>0.0–9.8</td>
<td>3.3 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>Elderly Group</td>
<td>21</td>
<td>0.0–5.6</td>
<td>1.1 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Maximum degree bothered by</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>side-effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>90</td>
<td>0.0–10.0</td>
<td>2.6 ± 3.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Young Group</td>
<td>49</td>
<td>0.0–10.0</td>
<td>4.0 ± 3.7</td>
<td></td>
</tr>
<tr>
<td>Elderly Group</td>
<td>24</td>
<td>0.0–7.3</td>
<td>0.4 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Average degree bothered by side-effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>90</td>
<td>0.0–8.9</td>
<td>2.1 ± 2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Young Group</td>
<td>49</td>
<td>0.0–8.9</td>
<td>3.3 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>Elderly Group</td>
<td>24</td>
<td>0.0–7.3</td>
<td>0.4 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Total # of side-effects reported by patient</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>All subjects</td>
<td>90</td>
<td>0.0–4.0</td>
<td>1.0 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Young Group</td>
<td>49</td>
<td>0.0–4.0</td>
<td>1.7 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Elderly Group</td>
<td>24</td>
<td>0.0–2.0</td>
<td>0.1 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>
Correlation of side effect measures in overall population

All 4 dynamic variables (degree to which mood stabilizer is interfering, average degree bothered by side-effects, maximum degree bothered by side-effects and total # of side-effects reported) were negatively and significantly correlated with age (r= -0.379, -0.435, -0.445, -0.490, p<0.001 respectively). Therefore, as age increased, less side-effects were reported and they were reported as less bothersome.

Dynamic variables were significantly correlated with total valproate concentrations: the total # of side-effects reported (r= 0.459, p<0.001), the average degree bothered by side-effects (r=0.381, p<0.001) and the maximum degree bothered by side-effects (r= 0.399, p<0.001). The only dynamic variable that did not correlate with total valproate concentration was the degree to which mood stabilizer was interfering (r=0.203, p=0.060). These same dynamic variables were also significantly correlated with free valproate level and increased as the level increased (r= 0.311, p=0.003; r= 0.279, p=0.008; r= 0.309, p=0.003 respectively). The degree to which mood stabilizer is interfering was also not significantly correlated with free valproate (r=0.142, p=0.191).

Dose was correlated with the dynamic variables: degree to which mood stabilizer is interfering (r=0.244, p=0.024), average degree bothered by side-effects (r=0.219, p=0.039), and maximum degree bothered by side-effects (r=0.277, p=0.031), although it was not associated with the number of side-effects reported by the patients (r=0.168, p=0.113). The free fraction of valproate was not found to be associated with any of the dynamic variables. When these young and elderly groups were compared at 2 different total valproate level groupings (low=
≤350μmol/L, mid-low= 351-525μmol/L) the same results were obtained. The 2 higher valproate groupings did not have enough elderly subjects to perform the calculations.

6.2 Study Design II- Experimental Study

6.2.1 Subjects

Seven subjects completed the study (figure 4). A total of 101 calls were received for this study. Of these calls, 91 were screened on the phone and 78 were ineligible mostly due to concomitant use of exclusionary medications. People who were taking any highly protein bound drugs (n=25), ≥90%, were excluded due to the potential displacement of valproate from albumin sites. The following drugs were excluded for this reason: antihypertensives (n=1 terazocin, n=1 amlodipine, n=1 nifedipine, anti-inflammatory agents (n=5 acetylsalicylic acid (88), n=2 celecoxib (89)), antihyperlipidemics (n=2 fenofibrate, n=2 atorvastatin, n=3 lovastatin (90)), biphosphonates (n=5 etidronate) (91) (92), hormonal therapy (n=1 progesterone (93), n= 1 raloxifene (94,95)) and anticonvulsants- phenytoin (n=1) (96,97).
Figure 4: Subject Recruitment (May, 2000- April 2001)

Total calls received: 101

Calls Screened: 91

Could not contact: 10

Assessments: 13

Ineligible: 78

Eligible: 9

Ineligible: 4

Subjects Completed:
1 @ 500, 1000, 2000mg
6 @ 500, 1000, 1500mg

Dropouts: 2
Other reasons for ineligibility consisted of: person's unwillingness to take the study medication or have their blood taken (n=22), smokers (n=5), study related (n=5 too much work and/or time involved), person was advised against participating (n=1 by family physician, n=1 by niece), too young (n=2), inappropriate behaviour displayed (n=1), scheduling problems (n=2).

Of the 13 people that had assessments, 4 were ineligible. One person was excluded due to a past history of pancreatitis that may have increased the risk for valproate induced pancreatitis (98). Another subject was excluded due to poor renal function that may have affected valproate's protein binding (47). Another subject dropped out after her assessment because she had no time for the study, and one person was deemed ineligible due to his inappropriate behaviour during the assessment. Of the 9 eligible subjects, 2 subjects dropped out, one due to catheter insertion problems (small veins) at the second study session (subject #3) and the other because of slight hand edema that occurred after 4 days of valproate therapy. Results from the first study session for Subject #3 (i.e., at 500mg/day) are included, where appropriate in this report.

The 8 subjects who participated in study sessions all had urine drug screens at the beginning of each study session to monitor for contraindicated medications, and these were consistently negative. Seven of the 8 subjects also had a breathalyzer test at each session and these were negative. The remaining subject had blood alcohol levels tested due to a mechanical problem with our breathalyzer machine at the time and these tests were negative as well. Four of the subjects were taking concomitant prescription medications for general medical reasons including: ramipril 10mg daily (subject #2) (7), hydrochlorothiazide 12.5mg daily (subject #2) (99), Timpilo2 ® eye drops (subject #6) (7), lisinopril 10mg daily (100), Sodium Sulamyd
10%® eye drops – 1 drop four times daily (subject #7; this was accepted because it is a topical medication and probably has limited systemic availability), Pulmicort Turbuhaler ® 2 puffs daily (subject #8) (7), and conjugated estrogens 0.625mg daily (subject#8) (93).

Some over the counter drugs and/or herbal products were also used by 5 subjects. One subject took 2 spoonfuls of sodium bicarbonate on day 6 of the study. Vitamins C 1000mg daily and E 800IU daily were taken daily by subject #2 and no pharmacokinetic interaction between these and valproate was found in the literature. Subject #3 also took Vitamin E capsules, cod liver oil capsules and a multivitamin (doses unknown) on days 9-14 of the study; and aspirin (dose unavailable) on days 11-13 of the study. One dose of loratidine (dose unavailable) was taken by subject #5 48 hours before the second study session, which would have been mostly eliminated (half life 8-11 hours) prior to the session. Saw palmetto 1500mg daily, glucosamine sulphate 1500mg daily, multivitamins, vitamins C 500mg daily and E 400IU daily were taken daily by subject #7. Subject #8 took calcium 500mg daily.

Subject demographics are shown below (table 11). Two of the subjects were female (including Subject 3 who completed only 1 session) and 6 were male. The average age was 69.3 years, average height was 166.9cm, average weight was 84.8kg and the average albumin level (42.1g/L) fell within the normal range (35-50g/L) (63).
Table 11: Subject Demographics

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Range</th>
<th>Mean ± Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>8</td>
<td>65 - 76</td>
<td>69.3 ± 4.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>8</td>
<td>155.0 - 180.0</td>
<td>166.9 ± 8.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>8</td>
<td>66.0 - 114.0</td>
<td>84.8 ± 15.3</td>
</tr>
<tr>
<td>Albumin level (g/L) (at assessment)</td>
<td>8</td>
<td>39.0 - 45.0</td>
<td>42.1 ± 2.3</td>
</tr>
</tbody>
</table>

Of the 7 subjects who completed the study, 7 of them took valproate 250 mg b.i.d on days 1-8, 500 mg b.i.d on days 9-15 and 750 mg b.i.d on days 16-22. The remaining subject (the first subject) differed in that on days 16-22 he took 1000mg b.i.d. This was the initial 3rd dosing regimen to be used in the study, but when the first subject reached steady state on this dose, he felt very unsteady on his feet and he took a slight fall at home. From this incident it was decided that for the safety of the subjects, the 3rd dose would be reduced to 750 mg b.i.d. Therefore, from the 8 subjects we have study session data under steady state conditions for the following doses: 500mg/day (n=8), 1000mg/day (n=7) 1500mg/day (n=6) and 2000mg/day (n=1).

6.2.2 Pharmacokinetics

All subjects were compliant with study medication as determined from pill counts and study logs. All of the kinetic parameters significantly changed across the doses of 500mg/day to 1500mg/day. The only variable not found to be significantly different was total valproate $t_{\text{max}}$. We can also see from the standard deviations that there was a large interindividual variation in the pharmacokinetic parameters. The total valproate pharmacokinetic curves for each subject are...
in appendix 5. A summary of these pharmacokinetic curves is shown in figure 5. The pharmacokinetic summary for total valproate parameters are shown in table 12.
Figure 5: Summary of Total and Free valproate pharmacokinetic curves for all subjects
Table 12: Summary of Pharmacokinetic parameters for total valproate on all 3 session days

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Range</th>
<th>Mean ± Std. Deviation</th>
<th>% change*</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C(_{\text{max}}) ((\mu\text{mol/L}))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>8</td>
<td>328 - 613</td>
<td>464.6 ± 92.7</td>
<td>57.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Session 2</td>
<td>7</td>
<td>604 - 814</td>
<td>731.0 ± 86.1</td>
<td>29.8</td>
<td></td>
</tr>
<tr>
<td>Session 3</td>
<td>6</td>
<td>801 - 1281</td>
<td>948.7 ± 177.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T(_{\text{max}}) (hr)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>8</td>
<td>2.0 - 3.0</td>
<td>2.3 ± 0.5</td>
<td>0</td>
<td>0.506</td>
</tr>
<tr>
<td>Session 2</td>
<td>7</td>
<td>2.0 - 3.0</td>
<td>2.3 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 3</td>
<td>6</td>
<td>2.0 - 4.0</td>
<td>2.8 ± 0.8</td>
<td>21.7</td>
<td></td>
</tr>
<tr>
<td><strong>AUC(_{0-12\text{hr}}) ((\mu\text{mol.hr/L}))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>8</td>
<td>3005.5 - 6173.5</td>
<td>4426.2 ± 945.9</td>
<td>56.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Session 2</td>
<td>7</td>
<td>5598.0 - 7763.5</td>
<td>6932.8 ± 793.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 3</td>
<td>6</td>
<td>7448.8 - 11708.0</td>
<td>9191.1 ± 1517.7</td>
<td>32.6</td>
<td></td>
</tr>
<tr>
<td><strong>Clearance (L/hr)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>8</td>
<td>0.28 - 0.58</td>
<td>0.41 ± 0.09</td>
<td>22.0</td>
<td>0.003</td>
</tr>
<tr>
<td>Session 2</td>
<td>7</td>
<td>0.45 - 0.62</td>
<td>0.50 ± 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 3</td>
<td>6</td>
<td>0.44 - 0.70</td>
<td>0.58 ± 0.09</td>
<td>16.0</td>
<td></td>
</tr>
<tr>
<td><strong>Clearance (ml/hr/kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>8</td>
<td>4.0 - 5.9</td>
<td>4.8 ± 0.6</td>
<td>25.0</td>
<td>0.015</td>
</tr>
<tr>
<td>Session 2</td>
<td>7</td>
<td>5.2 - 7.1</td>
<td>6.0 ± 0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 3</td>
<td>6</td>
<td>5.6 - 9.0</td>
<td>6.7 ± 1.2</td>
<td>11.7</td>
<td></td>
</tr>
<tr>
<td><strong>Average steady state concentration ((\mu\text{mol/L}))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 1</td>
<td>8</td>
<td>250.5 - 514.5</td>
<td>368.8 ± 78.8</td>
<td>56.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Session 2</td>
<td>7</td>
<td>466.5 - 647.0</td>
<td>577.7 ± 66.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 3</td>
<td>6</td>
<td>620.7 - 975.7</td>
<td>765.9 ± 126.5</td>
<td>32.6</td>
<td></td>
</tr>
</tbody>
</table>

*P-value represents significance on repeated measures ANOVA performed with dose as a factor

*% change from previous dose level

For the free valproate pharmacokinetic parameters, the free fraction, C\(_{\text{max}}\), AUC\(_{0-12\text{hr}}\) hr and average steady state concentration were found to increase significantly across the dosage range. T\(_{\text{max}}\) and unbound clearance were not found to significantly change, although the unbound clearance was trending downwards. The standard deviations show that there was large interindividual variation in the free pharmacokinetic parameters as well. The free valproate
pharmacokinetic curves for each subject are in appendix 5. The summary for free valproate pharmacokinetic parameters are shown in table 13.

Table 13: Summary of Pharmacokinetic parameters for free valproate on all 3 session days

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Session 1</th>
<th>Session 2</th>
<th>Session 3</th>
<th>N</th>
<th>Range</th>
<th>Mean ± Std. Deviation</th>
<th>% change*</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free Fraction</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td></td>
<td>7.0 - 13.7</td>
<td>10.0 ± 2.3</td>
<td>30.0</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>11.5 - 14.2</td>
<td></td>
<td></td>
<td>13.0 ± 0.9</td>
<td>17.4 ± 2.4</td>
<td>33.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>14.7 - 20.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (umol/L)</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td></td>
<td>30 - 94</td>
<td>53.5 ± 19.3</td>
<td>107.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>90 - 149</td>
<td></td>
<td></td>
<td>110.9 ± 18.7</td>
<td>210.8 ± 58.4</td>
<td>90.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>150 - 311</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (hr)</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td></td>
<td>1.0 - 3.0</td>
<td>2.1 ± 0.6</td>
<td>23.8</td>
<td>0.455</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2.0 - 3.0</td>
<td></td>
<td></td>
<td>2.6 ± 0.5</td>
<td>2.8 ± 0.8</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2.0 - 4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-12hr&lt;/sub&gt; (umol.hr/L)</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td></td>
<td>290.5 - 735.0</td>
<td>453.0 ± 148.2</td>
<td>97.2</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>795.0 - 1009.5</td>
<td></td>
<td></td>
<td>893.7 ± 67.9</td>
<td>1621.1 ± 462.7</td>
<td>81.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1286.0 - 2448.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clearance (L/hr)</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td></td>
<td>2.4 - 6.0</td>
<td>4.1 ± 1.2</td>
<td>-4.9</td>
<td>0.076</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>3.4 - 4.4</td>
<td></td>
<td></td>
<td>3.9 ± 0.3</td>
<td>3.4 ± 0.8</td>
<td>-12.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2.1 - 4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clearance (ml/hr/kg)</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td></td>
<td>33.5 - 72.8</td>
<td>49.4 ± 12.7</td>
<td>-7.3</td>
<td>0.111</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>37.9 - 54.4</td>
<td></td>
<td></td>
<td>45.8 ± 5.4</td>
<td>39.4 ± 8.8</td>
<td>-14.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>30.3 - 51.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average steady state concentration (umol/L)</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td></td>
<td>24.2 - 61.3</td>
<td>37.7 ± 12.3</td>
<td>97.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>66.3 - 84.1</td>
<td></td>
<td></td>
<td>74.5 ± 5.7</td>
<td>135.1 ± 38.6</td>
<td>81.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>107.2 - 204.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P-value represents significance on repeated measures ANOVA performed with dose as a factor
* % change from previous dose level
Free fraction was found to significantly correlate with the daily dose of valproate \((r=0.857, p<0.001)\), and the average total concentration at steady state \((r=0.815, p<0.001)\). A correlation between free fraction and albumin level was not found \((r=-0.102, p=0.659)\).

The total clearance of valproate significantly correlated with the daily dose of valproate \((r=0.743, p<0.001)\), and the free fraction \((r=0.663, p=0.001)\). Total clearance was found to correlate both with total \((r=0.565, p=0.006)\) and free \((r=0.622, p=0.003)\) average concentration at steady state. Total clearance was found not to correlate to unbound clearance \((r=0.059, p=0.799)\).

The unbound clearance of valproate did not correlate to albumin \((r=-0.205, p=0.372)\) or total daily dose \((r=-0.377, p=0.092)\). Its relationship to free fraction was in the reverse direction, therefore, as the free fraction increased, free clearance was found to significantly decrease \((r=-0.660, p=0.001)\). Unbound clearance was negatively correlated both to total \((r=-0.456, p=0.038)\) and free \((r=-0.540, p=0.011)\) average concentrations at steady state. From figure 6 panel 1d, it appears that one point may be influencing the correlation. Correlation was also conducted without the outlier \((x=345.4\mu\text{mol/L}, y=72.8\text{ml/hr/kg})\) belonging to Subject #6 at session 1. The correlation using \((\text{ml/hr/kg})\) was found to be non significant with total valproate concentration \((r=-0.392, p=0.097)\) although there was still a downward trend. It was still found to be negatively and significantly correlated with free valproate \((r=-0.518, p=0.023)\). When using unbound clearance not adjusted for weight \((\text{L/hr})\), unbound clearance was found to negatively and significantly correlate with total \((r=-0.562, p=0.010)\) and free \((r=-0.584, p=0.007)\) valproate concentration.
Figure 6: Correlations of pharmacokinetic variables

(a) Relationship between total valproate concentration and daily dosage

(b) Relationship between free valproate concentration and dose (mg)

(c) Relationship between valproate unbound clearance and total concentration

(d) Relationship between valproate total concentration (umol/L) and clearance (ml/kg)

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Predicting Valproate Free Fraction using Regression and Fuzzy Logic Models

There were 2 predictive models. The first was using all individual concentration time points drawn during the study session from each subject as cases. In total there were 147 cases in the train set from 6 subjects and 53 cases from another 2 subjects in the test set. Using regression and fuzzy logic to predict free fraction of valproate included the following inputs: age, weight, albumin concentration, daily dose of valproate, time since last dose, and total valproate concentration. The enter method was used in the regression model for prediction because it had the higher $R^2$ value ($R^2 = 0.818$) compared to the stepwise method. The regression and fuzzy models were both slightly underpredictive (ME = -3.26% and -2.19%, respectively) and the fuzzy model (RMSE = 3.31%) demonstrated better precision than the regression model (RMSE = 4.56%). The proportional contribution of each input variable to the fuzzy model are shown in table 14 below.

Table 14: Proportional Contribution of Input Variables to Final Fuzzy Logic Model #1

<table>
<thead>
<tr>
<th>Input variable</th>
<th>Proportional Contributions to Final Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.21</td>
</tr>
<tr>
<td>Weight</td>
<td>0.2</td>
</tr>
<tr>
<td>Albumin</td>
<td>0</td>
</tr>
<tr>
<td>Daily dose of valproate</td>
<td>0.05</td>
</tr>
<tr>
<td>Time since last dose</td>
<td>0.01</td>
</tr>
<tr>
<td>Total valproate concen.</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td><strong>1.00 Total</strong></td>
</tr>
</tbody>
</table>

The second prediction model involved predicting valproate free fraction after a dosage change. Each of the 3 dosage changes from each subject were used as separate cases for the model.
Therefore there were 18 cases from 6 subjects for the train set and 6 cases from another 2 subjects for the test set. Both regression and the fuzzy models used the following inputs: age, present albumin concentration, present weight, dosage change (mg), past valproate free fraction, past total valproate concentration, past total valproate clearance and present total valproate concentration. The enter method was used in the regression model for prediction because it had the higher R² value (R² = 0.914) compared to the stepwise method. Again both regression and fuzzy logic underpredicted the free fraction (ME = -4.75% and -1.53% respectively) but the fuzzy model (RMSE = 2.11%) had better precision than the regression model (RMSE = 5.15%). The proportional contributions of the input variables to the fuzzy are shown in table 15 below.

Table 15: Proportional Contribution of Input Variables to Final Fuzzy Logic Model #2

<table>
<thead>
<tr>
<th>Input variable</th>
<th>Proportional Contributions to Final Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0</td>
</tr>
<tr>
<td>Present weight</td>
<td>0.10</td>
</tr>
<tr>
<td>Present albumin concentration</td>
<td>0.07</td>
</tr>
<tr>
<td>Present total valproate concentration at steady state</td>
<td>0.52</td>
</tr>
<tr>
<td>Past valproate free fraction</td>
<td>0</td>
</tr>
<tr>
<td>Past total valproate clearance</td>
<td>0.17</td>
</tr>
<tr>
<td>Past total valproate concentration at steady state</td>
<td>0.09</td>
</tr>
<tr>
<td>Dosage change</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1.00</strong></td>
</tr>
</tbody>
</table>

6.2.3 Pharmacodynamics

The subjective side effect profiles that were captured for each day on the study medication for each subject are shown in appendix 6.
Repeated measures ANOVA detected no significant differences in any of the study day AUE curve dynamic measures across the 3 different doses. Therefore there was no difference in sedation, psychomotor performance and any of the side effect scores across the doses in the study. Also none of these measures were found to be correlated to valproate dose or total and free concentration. The change from baseline summary scores for DSST correct, MTT, trails test A and B, sedation summary, nausea summary and tremor summary for each study day session at each dose are shown in figures 7-13.

The number of subjects who at any time during the study reported any of the following side effects were as follows: tremor (n=1), CNS side effects (n=7), GI side effects (n=6), nausea (n=7), although in general the severity scores were low. Some of these dynamic variables significantly correlated with valproate plasma levels. Average and maximum CNS side effect severity correlated with total (r= 0.602 and 0.538; p= 0.023 and 0.047, respectively) and free (r= 0.612 and 0.557; p= 0.026 and 0.048, respectively) valproate concentration. Maximum GI side-effect severity correlated only with total valproate level (r= 0.604, p= 0.049). There was a significant correlation between average nausea severity and both total (r= 0.639, p= 0.025) and free valproate (r=0.651, p= 0.030). Maximum nausea severity was also associated with both total (r=0.582, p=0.047) and free levels (r=0.605, p= 0.049). The correlation of average and maximum CNS and nausea with total and free valproate concentration are shown in figures 14-17.
Figure 7: DSST correct substitutions

change from baseline score

Time post dose (hr)

- 1000ng VPA (n=7)
- 500mg VPA (n=8)
- 1500mg VPA (n=6)
- 2000mg VPA (n=1)

Figure 8: MTT - % time over the center of the road

change from baseline score

Time post dose (hr)

- 500mg VPA (n=8)
- 1000mg VPA (n=7)
- 1500mg VPA (n=6)
- 2000mg VPA (n=1)
Figure 11: Trails Test A summary

Figure 12: Trails Test B Summary
Figure 13: Tremor Scale Summary

Change from baseline

Time post dose (hr)

- 500mg VPA (n=8)
- 1000mg VPA (n=7)
- 1500mg VPA (n=6)
- 2000mg VPA (n=1)
Figure 14: Average CNS side effect severity vs. Total Valproate concentration

Figure 15: Average CNS side effect severity vs. Free Valproate Concentration

Figure 16: Nausea severity vs. Total valproate

Figure 17: Nausea severity vs. Free valproate
The free fraction of valproate differed between younger adults and the elderly at lower concentration within the therapeutic range in our observational study. These elevated free valproate concentrations in the elderly were not associated with an increased number or severity of side-effects. In fact, both were reduced in the elderly compared to their younger counterparts.

Valproate free fraction also significantly increased across a dosage range (500, 1000 and 1500mg/day) in the elderly in our experimental study. The clearance of this drug was found to significantly change across the dosage range administered. The total clearance was found to increase in this population whereas there was a tendency for the unbound clearance to decrease.

There was no significant difference found in the pharmacodynamic variables when analyzed according to dosage groups, although CNS and nausea side effect severity were found to increase with increasing total and free valproate concentrations.

7.1 Pharmacokinetics

We will primarily compare our observational and experimental study results to 2 studies in the literature: Bauer et. al.,1985 (57) compared younger adults and elderly steady state pharmacokinetics at 500mg which is the low dose that we used in our experimental study; and Bowdle et al, compared steady state pharmacokinetics in younger adults at 500mg, 1000mg and 1500mg/day which are the doses we used as well (43).
The valproate serum levels achieved in the experimental study are consistent with previous studies. The average total valproate concentration at steady state achieved with the 500mg/day dose in the experimental study was 369 μmol/L. Other studies have found similar steady state valproate levels of 312μmol/L (n=6) in healthy elderly volunteers (57) and 326 μmol/L (n=6) in young healthy volunteers (43). No other studies to date have been conducted to our knowledge that look at the pharmacokinetic parameters of valproate in healthy elderly volunteers, therefore we cannot make comparisons of serum level consistency at the 2 higher dose levels used in this study (1000mg/day and 1500mg/day). There is however, data in healthy younger adults at these 2 doses. Bowdle et al 1980 reported a total valproate steady state concentration in their young subjects of 659μmol/L which is slightly higher than what was found in our elderly subjects (578μmol/L) at 1000mg/day. The total valproate concentration range achieved across the dosage range of 500-1500mg/day in the Bowdle et al study was approximately 200-1180 μmol/L which was similar to our total valproate concentration at steady state range of 250-976μmol/L.

The total valproate concentration C_{max} values achieved in this study (465μmol/L) at the 500mg/day dose were comparable to those achieved in the Bauer et al (57) study (402μmol/L) in elderly subjects. The t_{max} was also found to be similar to the Bauer et al study (2 hours). Since we were using an enteric coated formulation (divalproex sodium), our values t_{max} (2.3- 2.8 hours) are somewhat lower than the normal range found in the literature (3-4 hours) (7). A reason for this may be that our subjects were in a fasting state from the night before whereas the range form the literature did not specify if their data was obtained from fasting or fed individuals.
The average valproate free concentration level at steady state obtained in this study at the 500mg/day dose was 38μmol/L which was similar to the value obtained by Bauer et al (57) of 33μmol/L, in elderly subjects. These have been found to be higher compared to healthy younger adults at the same dose (19μmol/L) (57), possibly due to the lower unbound clearances of valproate that have been found in previous studies of the elderly. Again, because steady state pharmacokinetics at higher doses have not been tested in healthy elderly we cannot compare our results to previously published results. We were not able to compare our free valproate levels to younger adults because these values were not clearly documented in the Bowdle study.

**Protein Binding**

The protein binding of valproate in this study at the lower dose (500mg/day) in the elderly (10.0%) was comparable to that found in the Bauer et al study using the same dose (57) in elderly subjects. The free fraction significantly increased to from 10.0% at 500mg/day to 17.4% at 1500mg/day which is a 74% increase corresponding to mean total average steady state valproate levels of 250 and 976μmol/L, which spans the therapeutic range. This supports the conclusions from in vitro (42,46) and in vivo (26,43) studies that the free fraction of valproate does not remain constant. This fact was highlighted in our regression and fuzzy models because the total valproate level was found to be an important contributor to the models for predicting free fraction even after a dosage change. Our observational study also confirmed that the free fraction of valproate is dose dependent. In the elderly patients (65-85 years) the free fraction increased from 6.8% to 19.4% within the total valproate range of 85-591μmol/L. Although the free fractions obtained in this study for the elderly are similar to that achieved in our
experimental study, the free fraction here has a wider spread of values and achieved somewhat higher free fractions within a lower total concentration range. Contributing to this could be the fact that one patient who achieved the 2 highest free fractions (15.5 and 19.2%) was taking concomitant medications (terazocin, desipramine, nifedipine and carbamazepine) (7,50) that may have caused either the displacement of valproate from protein binding sites or altered the metabolism and hence clearance of the drug. Also this patient had diabetes mellitus that has been shown to affect the protein binding of valproate due to displacement by increased free fatty acids in the blood (47,101).

When the protein binding in the elderly from our experimental study was compared to that in younger adults from the Bowdle study (43), our elderly had consistently higher free fraction of valproate compared to the younger subjects at each of their same doses (10.0, 13.0, 17.4% versus 7.0, 10.1 and 9.3% respectively). Overall, no difference in protein binding was found between younger adults and the elderly in our observational study. Free fraction was found to be higher in the elderly (9.5%) compared to the young (7.1%) when we examined only the lower total valproate range of <350μmol/L. This confirms the results by Bauer et al which found the free fraction in the elderly (9.7%) to be significantly higher than in younger adults (6.1%) at total valproate concentration levels of within this lower range. The sample size of elderly subjects at the higher levels was too small to produce significant results.

Our elderly patients received significantly lower doses as compared to their younger patients (p=0.016). Reasons for these lower doses are unclear but could have been due to a number of factors: the elderly sample size could have been so small we did not capture enough patients at the higher doses by chance, physician prescribing practice (elderly patients were only recruited
from 2 psychiatrists and this is not representative of the population) or elderly patients in the study had already been on higher doses in the past and could not tolerate them.

The influence of age on valproate free fraction was found to be important in our models used to predict free fraction. Lower protein binding in the elderly could be due to altered binding affinity to albumin. The elderly patients in our observational study had significantly lower albumin levels (41g/L) compared to the young (44g/L) although the means both fell within the normal range (35-50g/L).

**Clearance**

Clearance values are available from our experimental study only since full kinetic curves were not obtained in our observational study.

The total valproate clearance value at 500mg/day obtained in our experimental study (4.8ml/hr/kg) was lower than that achieved in the Bauer study in the elderly subjects at the same dose (6.6ml/hr/kg). Likewise, our unbound clearance was slightly lower (49.4ml/hr/kg vs. 64ml/hr/kg) at 500mg/day. There could be several reasons for these differences related to the multiple metabolic routes associated with valproate and a full metabolite analysis could have helped clarify this difference.

Repeated measures ANOVA confirmed that valproate clearance depended on the dose. Total valproate clearance was found to be significantly higher in our study as the doses increased
In younger adult populations the total valproate clearance has been found to increase across a dosage range \((43,45,59)\). Our elderly subjects had lower total clearance values when compared to the younger adults in the Bowdle study: at \(500\text{mg/day}\) was \(4.8\ \text{ml/hr/kg}\) and \(6.7\ \text{ml/hr/kg}\), respectively; at \(1000\text{mg/day}\) 6.0 and \(8.2\ \text{ml/hr/kg}\), respectively; and at \(1500\text{mg/day}\) 6.7 and \(8.2\ \text{ml/hr/kg}\), respectively. Notice that the clearance value in the Bowdle study did not increase further as the dose increased from \(1000\) to \(1500\text{mg/day}\). Reasons for this will be discussed in the next section on the relationships between protein binding and clearance.

The unbound clearance in our study was found to decrease across the dosage range and were found to significantly correlate with total \((r=-0.628, p=0.003)\) and free \((r=-0.653, p=0.002)\) valproate concentration. Previous studies have also found the unbound clearance to be dose-dependent \((43,45,59,102)\). Valproate’s dose-dependent decrease in unbound clearance has been attributed to its potential as an inhibitor of drug metabolism. Specifically, the decrease in the formation and excretion of the B-oxidation pathway has been found to account for most of the decrease in total unbound clearance \((102)\).

Valproate unbound clearance has also been shown to be decreased in the elderly compared to younger adults \((56,57,60)\). Compared to Bowdle’s younger adult subjects, our subjects had lower unbound clearances across all doses. Unbound clearances were 49.4 and \(89.2\ \text{ml/hr/kg}\) at the \(500\text{mg/day}\) dose, 45.8 and \(72.0\ \text{ml/hr/kg}\) at the \(1000\text{mg/day}\) dose and 39.4 and \(90.7\ \text{ml/hr/kg}\) at the \(1500\text{mg/day}\) dose. This may be due to differences in liver enzyme activity in the metabolism of valproate in the elderly. Of the studies that compared unbound clearance of valproate in the 2 age groups, none examined metabolite concentrations.
Interrelationship between valproate concentration, protein binding and clearance

The total clearance of valproate, which is a low hepatic extraction drug is dependent on the free fraction and the unbound clearance of valproate by the following relationship: total hepatic clearance = free fraction x unbound clearance. Since valproate is virtually eliminated solely by the liver, the total valproate clearance is the same as the total hepatic clearance. The total valproate clearance was found to increase as the doses increased in our study due to increasing free fractions (74% change) among our elderly subjects that more than offset the decrease (-20.2% change) in unbound clearance. Compared to the younger subjects in the Bowdle study, our subjects had a higher relative increase in free fraction (74% vs. 32.8%) compared to the change in unbound clearance (-20.2% vs. 1.7%). This is probably why we found a significant increase in total clearance and they did not.

The average total valproate concentration at steady state is described in the following relationship: \[ C_{avg,ss} = \frac{(\text{dose}/\text{interval})}{(\text{unbound clearance} \times \text{free fraction})} \] (58). It is dependent both on the unbound clearance of valproate as well as the free fraction if the dose stays the same. The average unbound valproate concentration at steady state, on the other hand, is dependent only on unbound clearance: \[ C_{unbound} = \frac{(\text{Dose}/\text{interval})}{\text{unbound clearance}} \]. Therefore the higher unbound valproate concentrations in our study compared to the Bauer study in younger subjects at the same dose may be due to the lower unbound clearances obtained in the elderly. The higher unbound concentrations, due to the lower unbound clearances in the elderly across the dose range, may be one of the factors that lead to the higher free fractions that were found in our elderly population compared to the young. Higher free fractions in the elderly could also indicate a lower affinity to albumin sites which was not tested in these studies.
According to the equation for total valproate levels, the total concentration values may not be very different for a young and elderly subject even though their free concentrations may vary considerably, which was demonstrated in the Bauer study. This may not be apparent if only total valproate levels are examined. Also in relation to dosage changes, depending on the relative changes between unbound clearance and free fraction, total valproate concentrations could have a smaller percent increase compared to the unbound levels which was found in our study. In our experimental study, for example, the total valproate concentration increased 108% and the unbound concentration increased 258% from the 500 to 1500mg/day doses. Therefore one cannot expect the same change in unbound valproate concentration as in total concentration across doses.

**Variation in pharmacokinetic parameters**

Considerable inter-subject variability was observed across the doses in this study. The coefficient of variations in our study were on average slightly higher than that in the elderly in the Bauer study at the 500mg/day dose. For the total valproate parameters the variation in our elderly compared to theirs was: total clearance (12.5% vs. 7.6% respectively), total average valproate concentration at steady state (21.3% vs. 19.9% respectively), $C_{\text{max}}$ (19.9% vs. 20.6% respectively). For the unbound parameters they were: unbound clearance (25.7% vs. 18.8% respectively), unbound average valproate concentration at steady state (32.6% vs. 31.9% respectively) and free fraction (23% vs. 15% respectively). When comparing degree of variation in our experimental study to that in younger adults at the same doses (500mg/day, 1000mg/day and 1500mg/day) in the Bowdle study our values were on average lower for total clearances.
(12.5, 13.3 and 17.9% vs. 18.7, 22 and 19.8%) and unbound clearances (25.7, 11.8 and 22.3% vs. 79.5, 28.9 and 19.7%).

This inter-subject variability adds to the difficulty in predicting and interpreting total valproate levels and making dosage adjustments, since there are 2 dose dependent kinetic parameters to consider (free fraction and unbound clearance) which are both influenced by interindividual variation.

**Summary Points:**

- Both valproate unbound clearance and free fraction have been shown to be concentration-dependent in our experimental study.
- The elderly were shown to have a higher free fraction as compared to younger adults at lower total valproate concentrations.
- The free fraction of valproate was shown to significantly increase across a dosage range in the elderly due to higher unbound concentrations.
- Total valproate clearance was found to significantly increase across the dosage range in the elderly due to a greater relative increase in free fraction compared to the decrease in unbound clearance.

7.2 **Pharmacodynamics**

As a secondary analysis the relationship between severity of side effects to valproate total and free concentrations was evaluated. The risk benefit ratio of valproate was not conducted in the observational study because of the time frame. Bipolar disorder is a chronic episodic illness and
patients would have to be followed for long periods of time to evaluate individual concentration-response relationships. Therefore this was not feasible in this study.

Repeated measures ANOVA on the AUE curveo-8hr measurements using all subjects detected no significant effect of valproate dose on any of the psychomotor, sedation, tremor or nausea scores across the 3 different doses. When evaluating both the average and maximum severity of a specific group of side effects, repeated measures ANOVA again found no effect of valproate dose on these scores. However, both the CNS and nausea side effect severities significantly correlated with total and free concentration, with the correlation always being slightly stronger with free valproate. The maximum GI side-effect severity was only significantly related to total valproate levels and not to free levels. The stronger association of some of these dynamic measures to free concentration supports the suggestion that free concentrations may be a better reflection of the active portion of the drug, although the clinical significance is questionable due to the small differences.

No previous studies have looked at the occurrence of side effects across a dosage range in elderly subjects therefore we have very limited data to compare our results to. The case reports and studies in the literature regarding the elderly and the occurrence of side effects are few, but the ones that are available (mostly retrospective) show valproate to be relatively safe with only minor side effects reported. Noaghiul et al (13) reported sedation to occur in 2 manic patients (n=21) at valproate levels of 457 and 624 μmol/L. Other studies do not report the specific valproate serum levels at which the side effects occur. Our subjects reported sedation at all 3 valproate dose with corresponding total average concentration at steady states of 369μmol/L at 500mg/day, 578μmol/L at 1000mg/day and 766μmol/L at 1500mg/day.
There is somewhat more information in children and younger adults regarding the side effects of valproate. A study conducted in manic patients (16) found that the number of patients reporting nausea, vomiting and sedation occurred throughout the whole range of concentrations and increased at the higher levels. The significance of this was not mentioned except to say that levels >866 μmol/L were likely to be associated with a greater frequency of side effects. This study however did not examine the relationship of free valproate with side effects. Sedation (CNS), general GI side effects and nausea were also reported by our subjects within the range of 250-976 μmol/L which is similar to the above mentioned study with respective free levels of 38 to 204 μmol/L. Other studies reported mainly descriptive measures, e.g. in one study nausea occurred in 4.5% of patients with a mean valproate level of 273 μmol/L, which does not really elaborate on how the frequency or severity of these side effects change across doses or across different levels and if it does, whether the change is significant.

In our observational study, due to the self-reported nature of the side effects we do not have them arranged into specific groups as in the experimental study. The average degree to which valproate was interfering with functioning was not found to correlate to either total or free valproate. On the other hand, the total # of side effects, average degree bothered by side effect and maximum degree bothered by side effects were found to correlate to both total and free. Although some of the correlations were found to be statistically significant, they may not be clinically significant because they were on average weak correlations. The correlations with total concentrations were similar to those with the free concentrations. This differed from our experimental study perhaps due to the fact that the patients were on concomitant medications that
may have contributed to the perceived side effects. Also perhaps a single point trough concentration to represent steady state valproate exposure was not adequate.

When comparing the occurrence and severity of side effects in younger adults and elderly patients in our observational study, t-tests significantly confirmed that the elderly reported less side-effects, that they were less bothered by them and that the side effects interfered less than reported in younger adults. This was consistent even when specific total valproate levels groupings were looked at. We are unaware of reports in the literature documenting this for valproate. This was an unexpected finding because we would assume that the older population would report more side effects, in part due to increased sensitivity to drug effects. Also, even though we asked the patients to report only the side effects they attributed to valproate, we expected the elderly to report more side effects since they were taking more concomitant medications than their younger counterparts (p=0.035). However, the younger patients were more likely to be taking other psychotropic drugs (i.e., antipsychotics) that are associated with significant side effects. Reasons for the elderly being less bothered and interfered with by side effects could be that our elderly under reported side effects or their severity so as not to be thought of as complaining about their problems. Also, their tolerance level may be different than in the young. The elderly may already be functioning at a lower level (physically) and therefore the same side effect may interfere less than in a younger person who may be more active. When the elderly were compared to the young in this observational study, they were indeed found to have significantly lower physical functioning scores.
Summary points:

- Average and maximum CNS and nausea side effects were found to significantly correlate with both total and free valproate concentration
- Elderly patients reported less side effects and on average were less bothered by them compared to younger adults
7.3 Clinical Implications for this Study

Since it is thought that it is the unbound concentration of a drug that is available to interact with drug receptors, measuring total valproate concentrations may not accurately reflect the free active drug level at these sites. Suggested criteria for measuring free levels instead of total levels for drugs are based on (103): 1) Only drugs that are highly protein bound, and 2) Only if the free fraction of the drug does not remain constant.

We observed that valproate free fraction significantly increased across a dosage range. Furthermore, we found that in the elderly subjects across this dosage range, total clearance of valproate increased while its unbound clearance decreased. Clinicians should be aware of these changes in kinetic parameters when interpreting and adjusting dosages in their patients. Using a hypothetical example, consider valproate administered at the same dose to two different patients having the same unbound clearance. Since their unbound clearances are equal, their unbound concentrations should be similar. Now assume that their free fractions differ, one is double the other (5 and 10%). According to the equation for total average concentration, the patient with the higher free fraction will have total concentrations that are twice as high. Now a clinician looking at these 2 total concentration results may be inclined to increase the dose of the patient with the lower concentration even though they have identical unbound concentrations at the active sites. When we take into account the dose dependency of valproate's unbound clearance, the situation gets more complicated. For example, if a clinician is increasing the valproate dose in a particular patient, the relative changes in the free fraction and unbound clearance, can cause the total concentration to increase, decrease or stay the same. In our experimental study, with
doses changing from 500mg/day to 1500mg/day, study subject #4 had a 132% increase in the average total concentration at steady state but had a 353% increase in free concentration. From this example it cannot be assumed that free concentration increases to the same degree as total concentration. This makes it difficult to evaluate the effect of dosage changes when trying to achieve maximal benefits for a patient who exhibits poor clinical response to drug therapy, or who is showing good clinical response but increasing side effects. It would be desirable to be able to monitor free valproate levels in the elderly, especially at higher doses. There are other factors involved in determining if monitoring of free levels are warranted including the increased economic costs associated with this monitoring. Free levels are more expensive due to the time and materials needed to separate the free from bound drug, in addition to the cost of the drug assay. It is also critical to examine the relationship between both total and free valproate serum levels to clinical response to valproate in bipolar disorder, which has not yet been fully determined.

7.4 Limitations of this Study

One of the limitations with our observational study is the small sample size obtained for our elderly population. Due to this fact we were only able to able to compare the kinetics and pharmacodynamics between young and elderly at low to mid low concentration ranges of valproate. Also the fact that our elderly sample was recruited from only 2 psychiatrists may not be very representative of the population.

The most significant limitation of our experimental study was the inability to conduct a full metabolite concentration analysis. This was not conducted due to the fact that we were not able
to find a laboratory which was equipped to perform this analysis. This may have shed some light on why there was a tendency for the unbound clearance to decrease. Also if we had included a younger adult group in this study, it have allowed us to directly compare the differences in pharmacokinetics in the age groups across the dosage range.
In our experimental study, the free fraction of valproate was found to be dose dependent in the elderly and increased 74% across the dosage range. The total clearance of valproate was also found to significantly increase across the dosage range. The unbound clearance of valproate significantly decreased as the unbound and total valproate concentration increased. These are novel findings since valproate pharmacokinetics in the elderly had not yet been evaluated across a dosage range, specifically at higher doses. Significant correlations between total and free valproate concentration versus CNS and nausea side effect severity were found.

The observational study found that the free fraction of valproate increases with age and concentration, as found in previous reports. The elderly had significantly higher free fraction values at low valproate concentrations (≤350μmol/L). Significance was not achieved at the higher valproate concentration groups, likely due to the small elderly sample size. Interestingly, the elderly significantly reported fewer side effects and lower interference in functioning and degree of bothering of these side effects compared to younger adults. This is contrary to the usual clinical observations that the elderly are more sensitive to side effects. This may be due to underreporting, or due to different perceptions of side effects since the elderly were functioning in general at a lower level than younger adults.
These studies were conducted to provide clinicians with valuable information for interpreting and adjusting valproate dosing in the elderly population. Due to the changes in pharmacokinetics in the elderly found in our study, specifically at higher doses of valproate, it may be important and therefore desirable for clinicians to have the ability to monitor free valproate levels in specific cases.

Further research is required to help characterize and explain the changes observed in the unbound clearance in the elderly. Since there is limited information available regarding the relationship between metabolite pharmacokinetics and dose it is difficult to infer mechanistic causes for the changes in unbound clearance and serum concentrations.

Further research should evaluate the relationship between free/total concentration and side effects, as well as clinical response in bipolar disorder patients. Larger sample sizes are required, compared to our current studies, to determine if total or free valproate are a better predictor of side effect severity and clinical response in patients. This information would help determine the importance of routine free valproate monitoring.
References


(51) Role of cDNA-expressed human CYP2B6, CYP2C9, and CYP2A6 in metabolic activation of valproic acid.: 1998.


Sunnybrook Health Sciences Center Laboratory Services. Laboratory Services. 1999.


Medical Information- Proctor and Gamble Pharmaceuticals. Protein Binding of Etidronate. 10-16-2000.

(94) Medical Information Department- Eli Lilly Canada Inc. Albumin Binding of Raloxifene. 11-27-2000.


Appendix 1: Consent Form for the Observational Study

PROJECT INFORMATION AND CONSENT FORM

Fuzzy Logic Modeling in Clinical Psychopharmacology:
Development and Evaluation of a Novel Approach
("Mood Stabilizer Project")

Background and Purpose
Mood stabilizer therapy has been used for many years in the treatment of mood disorders (for example, with drugs such as lithium, valproic acid and carbamazepine). We are still looking for ways to better predict the optimal dose of these medications for an individual patient, based on their particular illness and their body's ability to eliminate them, in order to get the maximum benefit from the therapy while minimizing the side effects. This project is designed to test whether or not a sophisticated form of computerized artificial intelligence (called "Fuzzy Logic") can help predict the best dose for a particular patient. In order to do this, we need to systematically obtain information from patients currently taking mood stabilizer medications. The investigators in this study at Sunnybrook & Women’s College Health Sciences Centre are Dr. Beth A. Sproule, Dr. Claudio A. Naranjo and Dr. Kenneth I. Shulman.

Procedures
If you agree to participate in this project, we will be recording specific information related to your use of these medications. Some of this information will be provided by your treating physician and from your hospital chart (for example, information about your diagnosis). Other information will be obtained by asking you questions, such as how you are taking your mood stabilizer medication, what other medications you are taking
and how you are feeling. We will also be taking a blood sample from you in order to determine your current mood stabilizer blood concentration and to obtain information about your kidney, thyroid and liver function. In addition, we will determine your weight and height. You may decline to answer any particular questions asked of you or refuse to participate in these specific procedures at any time.

This information will be obtained on a number of occasions (approximately 5) in conjunction with your regularly scheduled Sunnybrook visits over the course of one year.

**Risks of Harm**

The risks to you for participating in this project are low. The risks associated with blood draws include little and brief pain. Afterward, there is some chance of slight bruising or inflammation, but this is a routine procedure that presents very low risk.

**Benefits**

Since this is an observational-type study, there are no specific benefits to you. The clinical information we obtain (for example, drug concentrations) will be used by your physician as part of your regular care. The general benefits of finding a useful method for optimizing mood stabilizer dosing is significant.

**Participation**

Your participation in this study is voluntary and you may withdraw from the study at any time and for any reason. If you decide to withdraw from the study, this will not in any way affect your present or future medical care at Sunnybrook & Women’s College Health Sciences Centre.
Confidentiality

Your identity and the information obtained in this study will be kept strictly confidential and will be available only to the researchers of the study and your psychiatrist. The data will be identified by number, your initials and date of birth only, and not by your name. Published reports and presentations at scientific meetings will refer to grouped data and not to any identifiable individual.
INFORMED CONSENT

The purpose of this research, the procedures involved and the possible risks associated with the study have been fully explained to me. I have had the opportunity to ask questions and my questions have been answered satisfactorily.

I understand that I will be free to withdraw from the study at any time and this will not affect the present or future care I receive. I voluntarily consent to participate in this study.

or

I understand that the patient will be free to withdraw from the study at any time and this will not affect her/his present or future care. I agree to the patient's participation in this study.

I have been given a copy of this consent form to take home with me and I understand that I may contact Dr. Beth Sproule, Dr. Claudio Naranjo or Dr. Kenneth Shulman at the study pager number (416) 329-6796 (or phone number 416-480-6761) to ask any future questions I may have concerning the study.

________________________
Name of patient (print)

________________________
Signature of patient
Date

________________________
Name of patient representative (print)
(if applicable)

________________________
Signature of patient representative
(if applicable)
Date

________________________
Signature of investigator
Date

________________________
Signature of witness
Date
Appendix 2: Experimental Study Design

Dynamic Training

Start 500mg/day at end of training day

Study Session 1

Start 1000mg/day dose at end of session 1

Study Session 2

Start 1500mg/day at end of session 2

Study Session 3
Appendix 3: Consent Form for the Experimental Study

Evaluating the Pharmacokinetics of Valproate in the Elderly: Dose-related Changes and their Influence on Effects.

Investigators: BA Sproule, PharmD, CA Naranjo, MD, BG Hardy, PharmD
Study Sponsors: Mental Health Program and The Whitaker Foundation

Information Sheet

Background and Purpose
Bipolar disorder affects approximately 1% of the population and 0.4% in those older than 65 years of age. Mood stabilizers, such as valproic acid, have been approved for use for many years in the treatment of mood disorders, such as bipolar disorder (manic-depressive disorder). Most of the studies looking at how valproic acid is handled by the body have been conducted in young adults. We are going to be looking at how this drug is handled by the body at different doses in the elderly because the way the body handles a drug may change as people age. This information will help doctors better adjust and monitor valproic acid dosing. The investigators in this study at Sunnybrook & Women’s Health Science Center are: Dr. Beth Sproule, Dr. Claudio Naranjo, and Dr. Brian Hardy.

Procedures
In this research study we are looking for 6 healthy elderly volunteers. If you agree to participate in this study and sign a consent form, you will be assessed for any current medical or psychological conditions that may prevent your participation; we will also ask about your medical history. At this assessment you will be asked about your smoking and drinking habits, as well as any medications that you may be taking. A brief medical examination will be performed which includes routine blood tests and a urine sample to screen for drugs. At this time we will also introduce you to the testing measures that will be used during the study.
You will also attend a 1 day training session in the Human Psychopharmacology Research Laboratory at the Sunnybrook & Women’s College Health Sciences Centre, Sunnybrook site, in which you will be practicing the tests that you will be performing at the 3 study sessions. The tests include performing two computerized psychomotor tests, a pen/paper psychomotor test, and
a computerized rating of sedation. Your level of tremor, and nausea will also be assessed. This session will last approximately 2 hours. Three study day sessions will take place approximately 8, 15 and 22 days after this first training session. The total duration of the study is approximately 22 days, from the practice session until the end of the study.

Each of the 3 different doses of divalproex sodium (250, 500, 750mg) will be given at 8:00am and 8:00pm everyday for 7 days. You will be asked to record daily the time you take each dose and also any side-effects you feel the medication is causing. After 7 days on each dose a study session will be conducted. The study session days will proceed from 8:00am to 8:00pm. You will be asked to fast overnight from 11pm until 8am and to try to get at least 8 hours of sleep before the start of each study session day. Light meals will be given at 10am, 1pm, and 6pm on the study days. Twelve hours before and during the study sessions, consumption of alcohol or caffeine containing food or beverages will be prohibited. Drug screening (urine sample) and a breathalyzer test will be administered before each study session. At 8:00 a.m. an intravenous catheter will be inserted into your forearm in order to collect 9 blood samples (4 teaspoons/sample, ~180ml/study session in total) at the following times after the 8:00 a.m. medication dose: 0, 1, 2, 3, 4, 6, 8, 10, and 12 hours after dosing. This catheter will remain inserted for 12 hours each day. Immediately after blood samples at time 0, 2, 4, and 8, you will rate your current level of sedation, nausea, perform psychomotor tests; you will be rated for your level of tremor, and you can report any effects you may be experiencing. This testing procedure will take approximately 20 minutes. Therefore, after 8 hours, there are no more tests to complete and only 2 remaining blood draws. After the 12 hour study session, the catheter will be removed and you will be finished for the day. You will be assessed at the end of each study session and if necessary (for example, if you feel tired at the end of the day) you can be taken to your residence by taxi and/or accompanied home by study personnel.

**Risks and Discomforts**

Divalproex sodium is a medication that has been available and widely used for many years. The most commonly reported adverse reactions to divalproex sodium are nausea, vomiting and indigestion (6–45% of patients). Other effects that have been reported in patients include: sleepiness, drowsiness, or dizziness (1-19%); tremor (1–29%); headache (21%); accidental injury (11%); weakness (10%); decreased or increased appetite; and hair loss (1-11%). These effects
are usually transient and rarely required stopping the drug. The following effects were reported more frequently in elderly patients compared to younger patients: accidental injury, infection, pain, sedation and tremor. In studies conducted specifically in the elderly with doses of 250-3000mg/day, 6-10% of patients experienced sedation, 6% experienced nausea, and 3% experienced confusion. Rare but fatal liver failure has occurred in patients (approximately 1 in 50,000) receiving valproate. Children under the age of 2 are at greatest risk, especially those taking other medications. In some cases the liver dysfunction progressed in spite of discontinuation of the drug. No deaths were reported in patients over the age of 10 years using valproate alone. Other very rare but serious side effects that have been reported are thrombocytopenia and pancreatitis. Valproate may interact with other drugs, so it is important that you inform us of any medications (prescription, over the counter or herbal remedies) you may be taking. You may withdraw from the study at any time if you wish.

The intravenous catheter will be inserted into your forearm by someone experienced in blood collection. This procedure involves little pain and allows for collection of the majority of blood samples with only one puncture. The assessment session will involve one blood sample taken by venipuncture by an experienced person and might involve little and brief pain. There may be a slight chance of bruising inflammation or infection, but this procedure presents very low risk.

You will be asked to store drugs away from children or others for whom they are not intended.

**Benefits**
There are no specific benefits to you.

**Compensation**
In consideration for your time spent participating in the study, you will receive $400.00 upon completion of the study. If you decide to withdraw from the study early, you will receive compensation according to how much of the study you have completed up to that time point. You will also be reimbursed for travelling expenses including parking, public transportation and the use of taxis (if needed).

**Participation**
Your participation in this study is voluntary and you may withdraw from the study at any time and for any reason. If you decide to withdraw from the study, this will not in any way affect
your present or future medical care at Sunnybrook & Women's College Health Sciences Center.
You may be asked to withdraw from the study if this protocol is not followed.

Rights of the Subject
Your identity and the information obtained in this study will be kept strictly confidential and will be available only to the researchers of the study. The data will be identified by number, your initials and date of birth only, and not by your name. Published reports and presentations at scientific meetings will refer to grouped data and not to any identifiable individual. This research project has been reviewed by the Research Ethics Board at Sunnybrook & Women's College Health Sciences Centre, and in the case of any questions or concerns you may have regarding your rights in this research project, you should contact the Research Ethics Board Coordinator at 416-480-4276.
INFORMED CONSENT

The purpose of this research, the procedures involved and the possible risks associated with the study have been fully explained to me. I have had the opportunity to ask questions and my questions have been answered satisfactorily.

I understand that I will be free to withdraw from the study at any time and this will not affect the present or future care I receive. I voluntarily consent to participate in this study.

I will be given a signed copy of this consent form to take home with me and I understand that in the event of any questions, concerns, or in the event of injury that I may contact Dr. Beth Sproule at (416) 480-6100 x 3557 or at the pager # (416) 329-6796.

Name of Subject (print)

Signature of Subject __________________________ Date

Signature of Investigator _______________________ Date

Signature of Witness __________________________ Date

October 24, 2000
Appendix 4.

Sedation Measure: 100mm Visual Analog Scales

Items on this scale:
1. Anxious
2. Bloated
3. Sad
4. Fatigued
5. Thinking speeded up
6. Tense
7. Spacey
8. Seclusive
9. Elated
10. Hungry
11. Pleasant
12. Nervous
13. Excited
14. Easily Irritated
15. Contented

Please rate, by marking on the line, the severity of your nausea today:

No nausea | Extreme nausea (could not be worse)

DSST

Participants were shown a series of random box patterns, each of which correlates to a number. These patterns are changed each time the test is administered. During the test, numbers are displayed on the computer screen in a random order and subjects must use the mouse to quickly fill in a blank box with the pattern that corresponds to the displayed number.

1 2 3 4 5 6 7

4
Appendix 5: Subject pharmacokinetic curves

**Total Valproate Concentration PK curves at each dose for Subject #1**

**Free Valproate Concentration PK curves at each dose for Subject #1**
Appendix 5 cont’d: Subject Pharmacokinetic Curves

**Total valproate Concentration PK curves at each dose for subject #2**

![Graph of total valproate concentration PK curves at each dose for subject #2.]

**Free valproate Concentration PK curves at each dose for subject #2**

![Graph of free valproate concentration PK curves at each dose for subject #2.]

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Appendix 5 cont’d: Subject Pharmacokinetic Curves

Subject #3 only participated fully in session #1 and then dropped out halfway through session #2
Appendix 5 cont'd: Subject Pharmacokinetic Curves

**Total Valproate Concentration PK curves at each dose for subject #4**

- Total 250mg bid
- Total 500mg bid
- Total 750mg bid

**Free valproate concentration PK curves at each dose for subject #4**

- Free 250mg bid
- Free 500mg bid
- Free 750mg bid
Appendix 5 cont’d: Subject Pharmacokinetic Curves

**Total valproate concentration PK curves for subject #5 at each dose**

- 250mg bid
- 500mg bid
- 750mg bid

**Free valproate concentration PK curves for subject #5**

There is no free data at the lowest dose for subject #5 very low concentration outside the range detectable by the assay.
Appendix 5 cont’d: Subject Pharmacokinetic Curves

**Total valproate concentration PK curves for subject #6**

Valproate concentration (umol/L) vs. Time post dose (hr)

- 250mg bid
- 500mg bid
- 750mg bid

**Free valproate concentration PK curves for subject #6**

Valproate concentration (umol/L) vs. Time post dose (hr)

- 250mg bid
- 500mg bid
- 750mg bid
Appendix 5 cont'd: Subject Pharmacokinetic Curves

**Total valproate concentration PK curves for subject #7**

- 250mg bid
- 500mg bid
- 750mg bid

**Free valproate concentration PK curves for subject #7**

- 250mg bid
- 500mg bid
- 750mg bid
Appendix 5 cont’d: Subject Pharmacokinetic Curves

Total valproate concentration PK curves for subject #8

Free valproate concentration PK curves for subject #8

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Appendix 6: Subject side effect profiles during the experimental study

Subject #2 did not experience any side effects

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**Side-effects experienced by B1001 during the study**

- drowsy
- unsteady gait
- headache
- decreased appetite
- stomach pains
- heartburn
- diarrhea

**Side-effects experienced by J8003 during the study**

- Tremor
- drowsy
- unsteady gait
- headache
- dizziness
- decreased appetite
- stomach pains
- heartburn
- constipation
- yellow urine
- nausea
Appendix 6 cont’d

Side-effects experienced by JM004 during the study

Side-effects experienced by JS005 during the study

117
Appendix 6 cont’d.

Subject #6 did not report any side effects.

**Side-effects experienced by RJ007 during the study**

<table>
<thead>
<tr>
<th>Frequency of Side-effect in last 24 hr (10 point VAS)</th>
<th># of full days on valproate</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

- 500mg
- 1000mg
- 1500mg

**Side-effects experienced by MH008 during the study**

<table>
<thead>
<tr>
<th>Frequency of Side-effect in last 24 hr (10 point VAS)</th>
<th># of full days on valproate</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
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<tr>
<td>6</td>
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<td>4</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

- Drowsy
- Stomach Pains
- Heartburn
- Diarrhea
- Nausea