Free Valproic Acid: Steady-State Pharmacokinetics in Patients with Intractable Epilepsy

N. Otten, K. Hall, J. Irvine-Meek, M. Leroux, D. Budnik, S. Seshia

ABSTRACT: Free and total valproic acid (VPA) pharmacokinetic evaluation was carried out at steady state in six young epileptics who were also receiving other anticonvulsants. Subjects received their usual morning dose of VPA after an overnight fast. Blood samples for free and total VPA were taken prior to the dose and frequently thereafter for 12 hours. The calculated pharmacokinetic parameters for total VPA and free VPA were: half-lives of 7.5 ± 1.6 hours and 5.0 ± 1.5 hours, volumes of distribution of 0.189 ± 0.038 l/kg and 1.51 ± 0.98 l/kg, and clearances of 0.30 ± 0.06 and 3.6 ± 2.0 ml/min/kg., respectively. There was a strong correlation between percent free VPA and total VPA (r = 0.81) but marked inter- and intra-subject variations were seen. Studies attempting to correlate VPA levels to clinical response must take such data into account.

RÉSUMÉ: L'évaluation pharmacokinetique de l'acide valproïque (AVP) libre et total a été étudiée à l'état constant chez six jeunes témoins épileptiques qui aussi prenaient d'autres anticonvulsivants. Les témoins reçurent leur dose habituelle le matin d'AVP après un jeûne nocturne. Des échantillons de sang pour la mesure de l'AVP libre et total ont été prélevés pris avant la dose et fréquemment par la suite pendant 12 heures. Les paramètres pharmacokinetiques calculés de l'AVP total et libre réspectivement étaient: demi-vie de 7,5±1,6 heures et 5,0±1,5 heures; volumes de distribution à 0,189±0,038 L/kg et 1,51±0,98 L/kg; et clairance à 0,30±0,06 et 3,6±2,0 mL/min/kg. Il y avait une bonne correlation entre le pourcentage d'AVP libre et total (r=0,81), mais nous avons remarqué beaucoup de variation entre et intra-sujets. Des études qui tentent de mettre en corrélation les niveaux d'AVP avec la réponse clinique doivent tenir compte de tels renseignements.

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There is a poor correlation between total valproic acid (VPA) serum concentration and pharmacological effect. The pharmacological properties of VPA suggest that free VPA concentrations may correlate better with clinical response than do total concentrations (Levy 1980; Perucca 1984). The pharmacokinetic behavior of free VPA has to be characterized before one can begin to use free VPA levels for therapeutic drug monitoring. Although the pharmacokinetics of free VPA have been studied in healthy volunteers and in a few epileptics older than 21 years, there is little data for those under this age. In this study, we report on six patients in the latter category.

Material & Methods

The ages of the six patients in this study ranged from 5.5 years to 21 years. All had intractable epilepsy and their renal and hepatic functions and serum albumin levels were normal. They were receiving VPA, in amounts of 7.5 mg/kg/dose to 17.7 mg/kg/dose taken on an 8 hourly schedule and were also on other anticonvulsant drugs (Table 1). Compliance was evaluated by interview, serum concentration determinations and by pill count technique.

Outpatients were admitted to the Clinical Investigation Unit of the Children's Hospital, Winnipeg, for 12 to 24 hours. VPA had been administered for at least 8 weeks prior to the admission and no alteration in any medication was made during the fortnight prior to the study. Patients were fasted overnight and for two hours after the morning dose. VPA was administered in capsule or syrup form. Blood samples were collected just prior to the dose (time 0), and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 hours thereafter. Samples were centrifuged immediately and the plasma separated. A portion of the plasma was used for free VPA analysis. Plasma water containing free VPA was prepared using an ultra-filtration technique (Ultra-Free anticonvulsant

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Table 1: List of comedications taken by subjects

Patient	Comedications
1	Phenytoin, Carbamazepine
2	Phenytoin
3	Phenytoin, Methsuximide
4	Phenytoin
5	Methsuximide
6	Primidone, Diazepam, Ethosuximide

drug filters, Worthington Diagnostics, Freehold, New Jersey). VPA concentration was determined on the ultra-filtrate by gas — liquid chromatography (GLC) (Leroux et al., 1981). The GLC method was modified to measure the lower concentrations by diluting VPA standard 1/10 and internal standard 1/5 with 0.05 N NaOH and by running the Sigma III gas chromatograph (GC) at a lower attenuation ($10^{2nd-4th}$). The coefficient of variation in the G.C. ranged from 2.6% at 13.8 mg/l to 10.5% at 1.2 mg/l (Table 2).

Table 2: Within-run precision of GLC assay for low serum VPA concentrations

Actual Concentration (mg/l)	Measured Concentration (Mean) (mg/l)	C.V. %	Number Of Samples
1.2	1.15	10.5	11
4.0	4.03	3.0	9
8.9	9.02	3.0	10
13.8	13.86	2.6	14

Filter to filter variability was tested before patient samples were processed for the study. One of the authors took phenytoin orally. Blood was drawn 24 hours post dose and plasma put through ten ultra-filters in parallel. Free phenytoin was determined using the EMIT^R assay. The filter to filter variation was found to be 7.5% at 0.34 mg/l. We used ultra-filters from the same batch for the entire study.

The pharmacokinetic variables were calculated from the data using standard methods. The following equations were used:

$t½_T = 0.693/Ke_T$ $\quad Vd_T = Cl_T/Ke_T$

$t½_F = 0.693/Ke_F$

$Cl_T = Dose/AUC_T$ $\quad Vd_F = Cl_{INT}/Ke_F$

$Cl_{INT} = Dose/AUC_F$

where the subscripts T, F, INT are total, free and intrinsic respectively, and t½ is the serum half-life of VPA calculated from the post-distribution elimination phase of the serum concentration-time curve; Ke is the elimination rate constant, Cl is clearance, AUC is the area under the serum concentration-time curve during the dosing interval calculated by the trapezoidal rule, and Vd is the apparent volume of distribution. Oral bioavailability was assumed to be complete (Klotz and Antonin, 1977; Perucca et al., 1978). An elimination half-life was calculated starting 1 to 3 hours post dose, depending on the presence of an alpha phase, to the final sampling time of 12 hours.

Linear and nonlinear regression analyses with statistical evaluation of the deviation from the fit of the line were used to examine the relationship between free fraction and total VPA concentration. The level of significance was set at $p < 0.05$.

Results

An example of free and total concentration-time curves is shown in Figure 1. The pharmacokinetic parameters determined in each subject for free and total VPA are shown in Table 3. Although the free t½ were considerably lower than the total t½ in 4 of 6 patients, there were no significant differences in the mean values. The Vd_F was significantly larger ($p < 0.01$) than that for total drug, with a correspondingly higher intrinsic clearance. There was a linear relationship between free and total VPA when individual data points from each patient were plotted (Fig.2). The relationship could be expressed as:

$$\% \text{ free} = 0.12 \times \text{Total VPA Concentration} + 2.18 \; (r = 0.81 \; p < 0.01).$$

The free fraction ranged from 0.039 to 0.206 over a total concentration of 20 mcg/ml to 130 mcg/ml.

Discussion

Our study differs from previous reports in two principal respects. Our patient population was younger and the twelve hour data collection period enabled us to assess alterations in VPA protein binding over a clinically relevant dosage interval. Since VPA is frequently prescribed on an 8 or 12 hour dosage schedule, data collection over shorter periods of time may not represent fluctuations in free VPA likely to occur in clinical practice.

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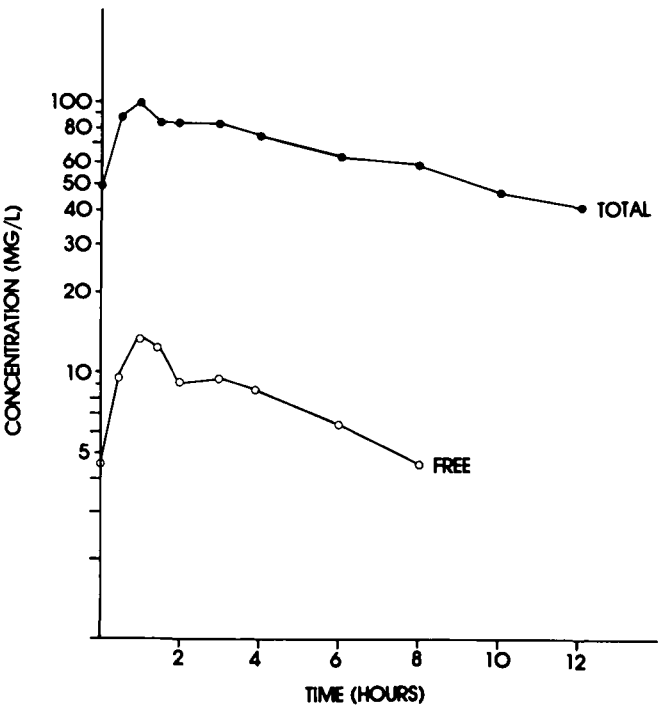

Figure 1 — Total and Free Concentration-Time Curves for one subject following administration of valproic acid.

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Table 3: Free and total VPA pharmacokinetics

Subject	Age (Yrs)	Sex	Dose mg/kg	t½ (Total) (Hrs)	t½ (Free) (Hrs)	Vd* (Total) (l/kg)	Vd (Free) (l/kg)	Clearance (Total) (ml/min/kg)	Clearance (Intrinsic) (ml/min/kg)
1	20	F	10.7	8.4	4.1	0.214	1.25	0.29	3.5
2	21	M	13.2	7.6	3.2	0.185	0.84	0.28	3.0
3	5.5	M	8.8	4.6	5.5	0.166	3.45	0.42	7.3
4	15	M	7.5	7.3	4.8	0.171	1.36	0.27	3.3
5	11	F	12.0	7.4	7.5	0.147	0.84	0.23	1.3
6	16.5	M	17.7	9.4	4.7	0.251	1.34	0.31	3.3
Mean				7.5	5.0	0.189	1.51	0.30	3.6
±				±	±	±	±	±	±
S.D.				1.6	1.5	0.038	0.98	0.06	2.0

*Vd is apparent volume of distribution.

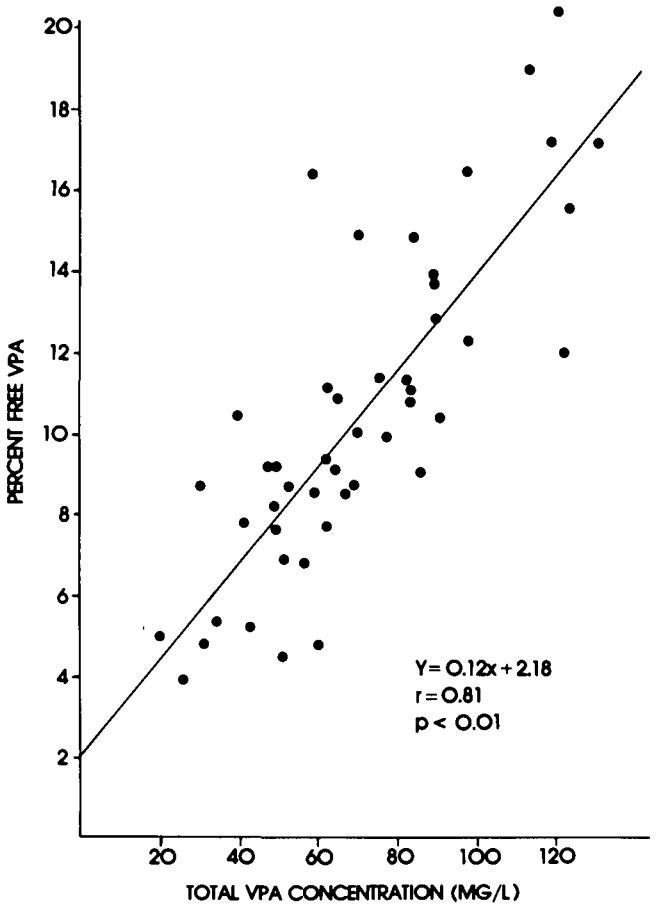

Figure 2 — Percent Free VPA vs. total VPA Concentration. Pooled data for 6 patients.

We found a linear relationship between free fraction and total VPA concentration but there were marked inter- and intra-subject variations. This variability is illustrated by the results in subject 5 in whom the actual free fraction of 0.163 at a total concentration of 59 mg/l, is 81% higher than predicted.

An increase in the free fraction with increasing VPA concentrations has been found in vitro (Patel and Levy, 1979; Bruni et al., 1979) and in vivo (Bowdle et al., 1980; Roman et al., 1982). Bruni and coworkers (1979) showed a rise in free fraction from 0.042 to 0.152 when total concentration rose from 40 to 160 mg/l while Patel and Levy (1979) reported that the bound fraction decreased from 0.87 at 27 mg/l to 0.51 at 103 mg/l. In normal subjects, Bowdle et al. (1980) found a 44% increase in free fraction when the mean concentration increased from 47 mg/l to 95 mg/l. Roman et al. (1982) demonstrated a significant linear relationship between free and total concentrations. However, they noted a marked variability in free fractions at the same total concentration (ranging from 17.6 fold at 86 mg/l). They also found a nonlinear relationship suggesting a saturable process in 2 of their 4 patients. We could not substantiate this finding, possibly due to insufficient data points in the higher concentration range. Marty et al. (1982) found marked intrasubject fluctuations in free VPA concentrations over a 6 hour period when they studied 5 patients aged 18 years to 32 years who were also on other anticonvulsants. The variation in free VPA was greater than that for the total drug.

We were unable to find a relationship between dose and total or intrinsic clearance. The highest intrinsic clearance occurred in the youngest patient. The range and mean values for intrinsic clearance were larger in our group than that in normal adults (Bowdle et al., 1980). This difference may be explained by the younger age of our patients and by concurrent anticonvulsant therapy, both of which may affect clearance (Gugler and von Unruh, 1980; Cloyd et al., 1983; Hall et al., 1983).

Pippenger (1980) suggested that free drug concentrations could be quantitated accurately with Ultra-Free filters when plasma was used. Subsequently protein leakage in plasma filtrates was found to occur with some of the Ultra-Free filters (Joern 1981; Ruprah et al., 1981). We did a quality control test, using phenytoin as Joern (1981) and Ruprah et al. (1981) had done, on ten of the filters from the batch used in our study. The test did not show significant filter to filter variance. It is, however, possible that some of the variability we found in VPA levels may have resulted from protein leakage.

A number of factors such as concomitant drug therapy, poor renal and hepatic function, low albumin concentration, and free fatty acid concentration are known to influence free VPA concentrations. All our patients were on other anticonvulsants (Table 1). Phenytoin has a minimal effect on the free fraction (Monks et al., 1978; Cramer and Mattson, 1979; Bruni et al.,

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1979; Patel and Levy, 1979). Carbamazepine and phenobarbital do not affect binding (Patel and Levy, 1979; Fleitman et al., 1980; Mattson et al., 1982), while the effect of diazepam, ethosuximide, methsuximide, and primidone on VPA protein binding are unknown. Primidone and ethosuximide are unlikely to compete for binding sites because of their low protein binding. Our patients had normal renal and hepatic function as well as normal serum albumin levels. Free fatty acids were not measured.

Although there is a strong correlation between free VPA and total VPA, the marked inter- and intrasubject variations make it difficult to predict the free concentration from any given total concentration. In theory, since only the free drug is pharmacologically active, studies are needed to determine if free VPA concentrations will correlate more closely to clinical response than do total concentrations. Such studies would help to establish or refute a role for monitoring free VPA in clinical practice.

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