Free and Total Serum Valproate Concentrations: Their Relationship to Seizure Control, Liver Enzymes and Plasma Ammonia in Children

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ABSTRACT: The relationships between total and free serum valproate (VPA) concentrations and seizure control, serum liver enzyme activity and plasma ammonia concentration were studied in 61 epileptic children. Enzyme-immunoassay (EMIT)^{R} methods gave higher values of total VPA concentration than gas-liquid chromatography (GLC) methods. In over 80% of children with complete seizure control the ranges of total VPA concentration were 140-420 umol/L with GLC methods and 210-560 umol/L with EMIT methods. The range of free VPA concentrations in 78% of children with complete seizure control was 8.8-26.4 umol/L. Increased liver enzyme activity was observed in 6 of the 61 children and raised plasma ammonia concentration in 11 of 50 children. Plasma ammonia concentration was related to total serum VPA but was not related to free serum VPA. Increased serum liver enzyme activity was related to VPA dose per kg but not to free or total serum VPA concentration. Thus free VPA concentrations do not appear to be more useful than total VPA concentrations in predicting seizure control and do not correlate with liver enzyme activity or plasma ammonia concentration.

RÉSUMÉ: Rapport entre la concentration sérique de valproate libre et total et le contrôle des crises convulsives, le taux des enzymes hépatiques et de l'ammoniaque plasmatique chez l'enfant. Nous avons étudié la relation entre la concentration sérique de valproate (VPA) libre et total et le contrôle des crises convulsives, l'activité des enzymes hépatiques du sérum et la concentration de l'ammoniaque dans le plasma, chez 61 enfants épileptiques. Les valeurs obtenues pour la concentration totale de VPA étaient supérieures par la technique immuno-enzymatique (EMIT)^{R} que par la technique de chromatographie gaz-liquide (CGL). Chez plus de 80% des enfants dont les crises convulsives étaient parfaitement contrôlées, l'intervalle de la concentration totale de VPA était de 140-420 umol/L avec la technique de CGL, alors qu'il était de 210-560 umol/L avec la technique EMIT. Chez 78% des enfants dont les crises convulsives étaient parfaitement contrôlées, l'intervalle pour la concentration de VPA libre était de 8.8-26.4 umol/L. Nous avons observé une augmentation de l'activité des enzymes hépatiques chez 6 enfants parmi les 61 enfants étudiés et une augmentation de la concentration plasmatique de l'ammoniaque chez 11 enfants parmi les 50 étudiés. La concentration plasmatique de l'ammoniaque était en relation avec le VPA sérique total, mais n'était pas reliée au VPA sérique libre. L'augmentation de l'activité des enzymes hépatiques dans le sérum était en relation avec la dose de VPA par kg, mais n'était pas reliée à la concentration sérique de VPA libre ou totale. Donc, il ne semble pas que la concentration de VPA libre soit plus utile que la concentration totale de VPA pour la prédiction du contrôle des crises convulsives et qu'il n'y a aucune corrélation avec l'activité des enzymes hépatiques ou la concentration plasmatique de l'ammoniaque.

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The therapeutic range for total serum valproate (VPA) is often reported as 350 to 700 umol/L.[1,2] However, in some patients seizures may be controlled at concentrations of 210 umol/L while in others VPA concentrations in excess of 700 umol/L are required.[3,4] The relationship between side effects and serum total VPA concentrations is even less well established.[5] Thus, although raised liver enzyme activity has been reported in 3-66% of patients on VPA and occurs more commonly at higher VPA dosages,[6,7] liver enzyme activity does not correlate with total VPA concentration. Furthermore, although

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hyperammonemia has been reported in 53-73% of patients taking VPA,[8-12] only two studies have shown a correlation between plasma ammonia and total VPA concentration.[11,12]

Valproate is highly bound to plasma protein and the extent of this binding is affected by a wide variety of endogenous and exogenous factors.[13,14] Because only the unbound drug reaches the brain, the clinical effect of VPA may correlate better with the free rather than the total VPA concentration in the blood. Thus, we determined the ranges of total VPA and free VPA concentration in the serum of children in whom seizures were controlled. In addition, we examined the relationship between serum glutamic oxaloacetic transaminase (SGOT) and ammonia with total and free valproate concentrations.

Methods

The study population comprised 61 children receiving a non-enteric coated formulation of VPA and 25 children who were receiving a different anticonvulsant. Thirty of the children on VPA were also receiving carbamazepine, phenytoin, ethosuximide, phenobarbital or acetazolamide. The children were 9 months to 18 years in age and were being seen regularly by a neurologist in the Seizure Clinic. They had no known renal, gastroenterological, hepatic or recent intercurrent illness and their medication had not been changed in the previous two weeks. A fasting blood sample was obtained before the morning dose and approximately 12 hours after the evening dose. All patients receiving VPA had total VPA concentrations measured by enzyme immnoassay techniques (EMIT)^{R} on the day of sampling. In 50 patients, an aliquot was stored at −18°C until total and free VPA concentrations were measured within three months by gas-liquid chromatography (GLC).[15] Plasma ammonia was measured in 50 patients receiving VPA and in 25 patients who were receiving a different anticonvulsant. Plasma samples were centrifuged immediately and an aliquot frozen until the ammonia level was measured within three days.[16] Serum glutamic oxaloacetic transaminase, BUN and creatinine were measured in all patients by standard methods with an Abbott Bi-Chromatic Analyser. In addition, the GLC and EMIT^{R} methods for measurement of total VPA were compared on 155 paired samples from children and adults who were receiving VPA.

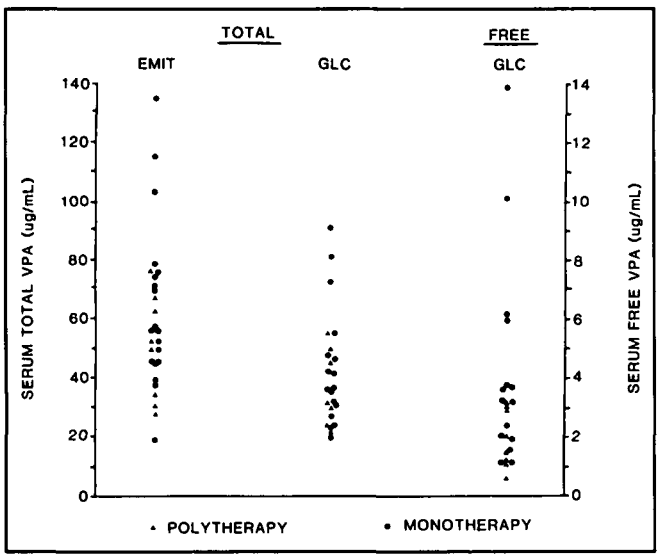

Figure 1 — Serum total and free valproate concentration measured by gas-liquid chromatography (GLC) and enzyme-immunoassay (EMIT) methods in children whose seizures were controlled.

Results

The free and total VPA concentrations in 27 children who had been seizure-free for a period of at least six months are displayed in figure 1. The seizure types in the patients whose seizures were controlled included absence seizures (9), tonic-clonic seizures (10), partial seizures (5), myoclonic seizures (3) and atonic seizures (4).

There was good correlation between free and total serum VPA concentrations (GLC assay) in patients on monotherapy ($r = 0.8068$, $p < 0.00001$) and polytherapy ($r = 0.8572$, $p < 0.00001$). In 27 children on monotherapy there was correlation between VPA dose/kg/day and both total VPA concentration (GLC assay) ($r = 0.4058$, $p < 0.04$), and free VPA concentration ($r = 0.4805$, $p = 0.008$). However, in 15 children on polytherapy there was no correlation between VPA dose/kg/day and either total or free serum VPA concentrations. There was good correlation between total VPA concentrations, measured by the GLC method in the research laboratory ($r = 0.8606$, $p < 0.0001$). However, the EMIT method tended to produce higher values. A similar trend was demonstrated in a further 155 samples which were measured by both methods in the research laboratory (Table 1).

The children on VPA polytherapy but not those in VPA monotherapy had a higher mean fasting plasma ammonia concentration than the control group (Table 2). Four patients on monotherapy and 7 patients on polytherapy had plasma ammonia concentrations above 40 umol/L, the upper limit of normal in our laboratory. However, the highest concentration was only 53 umol/L. The plasma ammonia concentration correlated with the total serum VPA concentration ($r = 0.6606$, $p < 0.007$). There was no correlation between plasma ammonia concentration and serum free VPA concentration or VPA dose/kg/day.

The mean SGOT activity was similar in patients on VPA monotherapy, polytherapy, and controls (Table 2). Six of 61 children on VPA had an SGOT above 40 IU/L, the upper limit of normal in our laboratory. The SGOT correlated with VPA

Table 1: Comparison of Gas Liquid Chromatography (GLC) and Enzyme Immunoassay (EMIT) Methods Measuring Valproic Acid

	Monotherapy and Polytherapy	Monotherapy	Polytherapy
Number of Samples	155	70	85
Mean VPA concentration by EMIT	398.24*	454.27	352.09
Mean VPA concentration by GLC	348.26	399.06	306.43
Correlation (r)	0.947	0.934	0.956
Ratio of EMIT:GLC	1.143	1.138	1.149

*Significantly greater than by GLC - $p < 0.001$ (Student's t test)

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Table 2: Plasma Ammonia Concentration and SGOT Activity in Children Receiving Valproic Acid

	PLASMA AMMONIA (umol/L)				SGOT LEVELS (I.U./L)			
	Patient	Mean	(SD)	Range	Patient	Mean	(SD)	Range
Valproate Monotherapy	31	30.2	(9.3)	12-52	35	25.4	(9.4)	7-43
Valproate Polytherapy	19	34.9*	(9.0)	22-53	26	25.8	(10.1)	14-61
Other Monotherapy	25	29.8	(10.8)	6-53	23	23.2	(6.4)	9-38

*$P < 0.05$ compared with other monotherapy (Student's t test)

dose/kg/day ($r = 0.4677$, $p < 0.0001$) but not with total or free serum VPA concentration.

Discussion

The therapeutic range for VPA has been reported as 350-700 umol/L.[1,2] However, 21 of 24 seizure-free children in this study had total serum VPA concentrations between 140-420 umol/L using GLC methods. When EMIT methods were used, 22 of 27 seizure-free children had total free VPA concentrations between 210-560 umol/L. There have been previous reports of seizure control in children with VPA concentrations below the accepted therapeutic range. Thus Klotz[3] observed that approximately 50% of children with seizure control had total VPA concentrations less than 420 umol/L (GLC). Similarly Henrickson[17] reported seizure control in some children with VPA concentrations less than 315 umol/L. Because cognitive function is more impaired at higher serum concentrations of VPA,[18] it is important that the lowest effective dose of VPA be used. Thus we would recommend that the clinical response to a serum VPA concentration of 200 umol/L be determined and that the VPA dosage be increased only if seizures persist.

There are several possible explanations why total VPA concentrations were below the reported therapeutic range in many of our children whose seizures were controlled. First, the age of our patients may have been a factor. The types of seizure, underlying aetiology and metabolism of drugs may differ between children and adults.[19] Thus the "therapeutic" ranges for antiepileptic drugs may be different in these two age groups. A second possibility may be that some of our patients had "outgrown their epilepsy". Because only two patients had been seizure-free for greater than three years this is unlikely to be a major factor. Finally, the serum VPA concentration which is necessary to prevent seizures may depend on the severity of the seizure disorder. Thus, the anticonvulsant concentration required to suppress seizure activity in experimental animals has been shown to be dependent on the intensity of the focus or severity of the seizure process.[20] Furthermore, in a study of epileptic patients whose seizures were controlled, those with lower plasma drug concentrations tended to have clinical features usually associated with a better seizure prognosis than those with higher drug concentrations.[21] However, 18 of 27 seizure-free children in the present study received VPA only after their seizures had not been controlled with other antiepileptic drugs.

Our observation that the EMIT method gives higher values of total valproate concentration than the GLC method has been reported previously[22] and underscores the importance of comparing only those VPA measurements obtained by the same analytical method. The higher values measured by EMIT techniques may be explained on the basis of cross reactivity with a metabolite as has been reported with carbamazepine.[23]

VPA is highly bound to plasma protein and its free fraction fluctuates markedly.[2] Changes in the free fraction of a drug may have a much greater influence on the total rather than free drug concentration.[24] Thus free rather than total VPA concentration may be expected to correlate better with clinical response.[24,25] Seventy-eight percent of the seizure-free children in our study had free VPA concentrations between 8.8 and 26.4 umol/L. However, the range in free VPA concentrations in all children with complete seizure control was 4.9 to 96.5 umol/L. This was proportionately greater than the range in total VPA concentrations. Thus free VPA concentration does not appear to be more useful than total VPA concentration in determining the dosage required to achieve seizure control.

The mean plasma ammonia concentrations in this study were similar in children on VPA monotherapy and other anticonvulsant monotherapy. These data support a previous study in which all 38 patients receiving VPA monotherapy had normal ammonia concentrations[12] and emphasize the advantage of using VPA as monotherapy. Although patients receiving VPA polytherapy had a higher mean ammonia concentration, the highest ammonia concentration was only 53 umol/L. The lower incidence of hyperammonemia in this study may relate to the timing of the blood specimen. In other studies, blood was drawn in the nonfasting state and after the morning dose. Both of these factors may influence plasma ammonia levels, particularly when ammonia metabolism is compromised.[26,27] Thus it is now our practice to measure plasma ammonia only in those patients receiving VPA who have behavioural symptoms and to obtain the blood specimen two to three hours following the morning dose.

Our observation that fasting plasma ammonia concentration correlated with total VPA concentration is similar to two previous studies.[11,12] However, we found no correlation between plasma ammonia concentration and free VPA concentration.

The SGOT activity was increased in six of 61 children tested. Four of the six were either drowsy or were too mentally retarded to allow assessment of their higher mental function. The two asymptomatic patients had raised SGOT activities of 41 and 43 IU/L respectively. Thus significant elevation of SGOT activity was not observed in any child who did not have evidence of cognitive dysfunction. The practice of measuring SGOT activity routinely in asymptomatic patients receiving VPA appears to be of little value.[28] Although patients with dose-related elevation of liver enzymes are identified, routine monitoring does not predict those very rare patients who have life-threatening hepatotoxicity. A more practical approach to the problem of serious hepatotoxicity might be to a) avoid using VPA where

there is a family history of a severe reaction to VPA, b) use the drug with extreme caution in patients who have a neurometabolic disease or progressive myoclonic epilepsy c) stop the drug and assess the patient immediately if there are unexplained signs of lethargy, vomiting or general deterioration.[28]

The SGOT activities correlated highly with the VPA dose per kg but did not correlate with either the free or total VPA concentrations. The failure to demonstrate a correlation between VPA concentration and SGOT activity may be due to the infrequency of raised SGOT activities in our series. An alternative explanation for our findings might be that a VPA metabolite is responsible for the effect on SGOT activity. A marked elevation of some of the unsaturated VPA metabolites has been demonstrated in a patient receiving VPA who died with acute liver failure.[29] The strong correlation between SGOT activity and VPA dose per kg in our patients would also support the hypothesis that a VPA metabolite might be responsible for the increased SGOT activity.

This study shows that most children with seizure control had total VPA concentrations between 210-560 umol/L and suggests that the lower end of the therapeutic range in this age group should be 210 umol/L rather than 350 umol/L. The free VPA concentrations in children with seizure control ranged between 7-28 umol/L. Thus free VPA concentrations did not appear to be more helpful than total VPA concentrations in relation to seizure control. In addition, free VPA concentrations did not correlate with SGOT activity or plasma ammonia concentration. Thus measurement of serum free VPA concentrations appears to be of little value except, perhaps, in situations where the protein binding of VPA might be altered.

References

1. Meinardi H, Hanke NFJ, Beveren JV. Sodium di-N-prophylacetate: estimation of effective serum levels. Pharmacol Week 1974; 109: 45-47.
2. Wilder BJ, Karas BJ. Valproate: Relation of plasma concentration to seizure control (Chap 48) *In*: Woodbury BM, Penry JK, Pippenger CE, eds. Antiepileptic drugs. New York, NY: Raven Press, 1982: 591-600.
3. Klotz U, Schweizer C. Valproic acid in childhood epilepsy: anticonvulsive efficacy in relation to its plasma levels. Int J Clin Pharm 1980; 18: 461-465.
4. Rowan AJ, Binnie CD, de Beer-Pawlikowski NKB, Goedhart DM, Gutter T, van der Geest P, Meinardi H, Meijer JWA. Sodium valproate: Serial monitoring of EEG and serum levels. Neurology 1979; 29: 1450-1459.
5. Herranz JL, Arteaga R, Armijo JA. Side effects of sodium valproate in monotherapy controlled by plasma levels: A study in 88 pediatric patients, Epilepsia 1982; 23: 203-214.
6. Covanis A, Gupta AK, Jeavons PM. Sodium valproate: Monotherapy and polytherapy. Epilepsia 1982; 23: 693-720.
7. Vining EPG, Botsford E, et al. Valproate sodium in refractory seizures. Am J Dis Child 1979; 13: 274-276.
8. Murphy JV, Marquardt K. Asymptomatic hyperammonemia in patients receiving valproic acid. Arch Neurol 1982; 39: 591-592.
9. Williams CA, Tiefenbach S, McReynold JW. Valproic acid-induced hyperammonemia in mentally retarded adults. Neurology (Ny) 1984; 34: 550-553.
10. Wyllie E, Wyllie R, Rothner AD. Valproate-induced hyperammonemia in asymptomatic children. Ann of Neurol 1982; 12: 208.
11. Ohtani Y, Endo F, Matsuda I. Carnitine deficiency and hyperammonemia associated with valproic acid therapy. J of Pediatr 1982; 101: 782-785.
12. Haidukewych D, John G, Zielinski JJ, Rodin EA. Chronic valproic acid therapy and incidence of increases in venous plasma ammonia. Therapeutic Drug Monitoring 1985; 7: 290-294.
13. Levy RH. Monitoring of free valproic acid levels. Ther Drug Monitoring 1980; 2: 199-201.
14. Orr JM, Abbott FS, Farrell K, Ferguson S, Sheppard I, Godolphin W. Interaction between valproic acid and Aspirin in epileptic children: Serum protein binding and metabolic effects. Clin Pharmacol Ther. 1982; 31: 642-649.
15. Abbott FS, Burton R, Orr J, Wladichuk D, Ferguson S, Sun T. Valproic acid analysis in saliva and serum using selected ion monitoring (Electron ionization) of the tertbutyl-dimethylsilyl derivatives. J of Chromatography 1982; 227: 433-444.
16. Verity CM, Farrell K, Applegarth DA, Kirby LT. The influence of anticonvulsants on fasting plasma ammonia and amino acids. Clin Biochem 1983; 344-345.
17. Henriksen O, Johannessen SI. Clinical and pharmacokinetic observations on sodium valproate — A 5-year follow-up study in 100 children with epilepsy. Acta Neurol Scandinav. 1982; 65: 504-523.
18. Trimble MR, Thompson PJ. Anticonvulsant Drugs, Cognitive Function, and Behaviour. Epilepsia. 1983: 24 (Suppl. 1); S55-S63.
19. Vajda FJE and Aicardi J. Reassessment of the concept of a therapeutic range of anticonvulsant plasma levels. Dev Med and Child Neurol 1983; 25: 660-671.
20. Kutt H. Phenytoin: relation of plasma concentration to seizure control. *In*: Woodbury DM, Penry JK, Pippenger CE, eds. Antiepileptic drugs. New York: Raven Press, 1982: 241-6.
21. Schmidt D, Haenel F. Therapeutic plasma levels of phenytoin, phenobarbital, and carbamazepine: Individual variation in relation to seizure frequency and type. Neurol 1984; 34: 1252-5.
22. Kumps AH, Jumps-Grandjean B, Mardens Y. Enzyme Immunoassay and gas liquid chromatography compared for determination of valproic acid in serum. Clin Chem 1981; 27: 1788-1789.
23. Monaco F, Piredda S. Carbamazepine-10,11-Epoxide determined by EMIT carbamazepine reagent. Epilepsia 1980; 21: 475-477.
24. Levy RH, Moreland TA. Rationale for monitoring free drug levels. Clin Pharmacokin 1984; 9 (Suppl. 1): 109.
25. Lockard JS, Levy RH, Congdon WC, Ducharme LL, Rhodes PH. Valproate nonreversibility: Dosing procedures critical? *In*: Levy RH et al eds. Metabolism of anti-epileptic drugs. New York, NY: Raven Press, 1984: 153-163.
26. Coulter DL, Allen RJ. Secondary hyperammonemia: A possible mechanism for valproate encephalopathy. The Lancet 1980: 1310-1311.
27. Hokanson JT, O'Brien WE, Idemotor J, Schafer IA. Carrier detection in ornithine transcarbamylase deficiency. J Pediatr 1978; 93: 75-78.
28. Green SH. Sodium valproate and routine liver function test. Arch Dis Child 1984; 59: 812-814.
29. Kochen W, Schneider A, Ritz A. Abnormal metabolism of valproic acid in fatal hepatic failure. Eur J Pediatr 1983; 141: 30-35.