

Protein binding of valproic acid in Japanese pediatric and adult patients with epilepsy

YASUO KODAMA, HIROFUMI KODAMA, MASAE KURANARI, KIMIKO TSUTSUMI, SHUNSUKE ONO, TAKUHIRO YAMAGUCHI, AND AKIO FUJIMURA

Valproic acid, a branched-chain fatty acid that is structurally unrelated to any other antiepileptic drugs, is valuable for the treatment of primary generalized epilepsy, especially tonic-clonic fits, absence seizures, and myoclonus in adult patients.¹ In pediatric patients with seizure disorders, valproic acid is effective treatment for generalized tonic-clonic seizures, generalized absences, and myoclonic epilepsy.² However, the drug's mechanism of action is not fully understood, although blockade of voltage-dependent sodium channels and potentiation of GABAergic transmission are postulated.³ More than 90% of valproic acid binds to plasma proteins, mainly albumin,⁴ and this concentration-dependent protein binding occurs near or within the usual therapeutic range of concentrations.⁵ A determination of the characteristics of the valproic acid-plasma protein inter-

Abstract: The binding of valproic acid to serum proteins in pediatric and adult patients was studied.

Serum samples were obtained from 48 Japanese pediatric patients with epilepsy (group A) and 48 Japanese adult patients with epilepsy (group B) receiving valproic acid monotherapy. The patients' age ranged from 1 to 15 years for the pediatric patients and from 18 to 44 years (group B—younger) and 45 to 63 years (group B—older) for the adult patients. The serum concentrations of total and unbound valproic acid were measured by fluorescence polarization immunoassay, and the unbound serum fraction of valproic acid was analyzed by ultrafiltration.

The mean association constant, K , and total concentration of binding sites, $n(P)$, were as follows: group A, $K = 0.016$ L/ μ mol, $n(P) = 1077$ μ M; group B, $K = 0.011$ L/ μ mol, $n(P) = 1365$ μ M; group B—younger, $K = 0.013$ L/ μ mol, $n(P) = 1291$ μ M; and group B—older, $K = 0.006$ L/ μ mol, $n(P) = 1827$ μ M. Significant differences between groups A and B were observed in the serum free fatty acid concentration and the serum concen-

tration ratio of free fatty acids to albumin. However, no significant differences between the two groups were observed in the binding of valproic acid to serum proteins. Group A's serum concentration ratio of free fatty acids to albumin was significantly lower than in group B—older and was lower than in group B—younger. However, there were no significant differences in binding between group A and groups B—younger and B—older. The serum concentration of albumin was significantly higher in group B—younger than in group B—older. Consequently, there was a significant difference in binding between groups B—younger and B—older.

The serum protein binding of valproic acid was similar in pediatric and adult patients with epilepsy, but binding characteristics differed between younger and older adults.

Index terms: Age; Anticonvulsants; Binding; Blood levels; Epilepsy; Japanese; Pediatrics; Valproic acid

Am J Health-Syst Pharm. 2002; 59:835-40

YASUO KODAMA, PH.D., is Clinical Research Pharmacist, Department of Clinical Pharmacology, Jichi Medical School (JMS), Tochigi, Japan. HIROFUMI KODAMA, PH.D., is Vice Director of Pharmacy, Miyazaki Medical College, Miyazaki, Japan, and Clinical Research Pharmacist, Department of Clinical Pharmacology, JMS. MASAE KURANARI, B.S.PHARM., is Clinical Research Pharmacist, Department of Clinical Pharmacy, Oita Medical University (OMU), Oita, Japan. KIMIKO TSUTSUMI, PH.D., is Clinical Research Pharmacist, Department of Clinical Pharmacology and Therapeutics, OMU. SHUNSUKE ONO, PH.D., is Chief Reviewer, Pharmaceuticals and Medical Devices Evaluation Center, National Institute of Health Sciences, Ministry of

Health, Labor, and Welfare, Tokyo, Japan. TAKUHIRO YAMAGUCHI is Biostatistician, Department of Biostatistics/Epidemiology and Preventive Health Sciences, University of Tokyo. AKIO FUJIMURA, M.D., PH.D., is Professor, Department of Clinical Pharmacology, JMS.

Address correspondence to Dr. Yasuo Kodama at the Department of Clinical Pharmacology, Jichi Medical School, Minamikawachimachi, Tochigi 329-0498, Japan (kodama@kiko.go.jp).

Copyright © 2002, American Society of Health-System Pharmacists, Inc. All rights reserved. 1079-2082/02/0501-0835\$06.00.

action would be useful in clinical practice, because the concentration of unbound plasma valproic acid reflects the level in cerebrospinal fluid.⁶

A few detailed studies of the *in vivo* binding of valproic acid to serum and plasma proteins have been conducted in pediatric or adult patients receiving valproic acid,⁷⁻⁹ but comparative information between these two age groups is limited. The binding of valproic acid in epileptic children receiving valproic acid monotherapy or combination therapy⁷ seems to be slightly different from that in epileptic adults⁸ and in adults receiving the drug prophylactically after traumatic head injuries.⁹ In view of the potential effect of serum protein binding on drug disposition, it is important that a possible age-related alteration in the binding characteristics of valproic acid be evaluated.

We report here the serum protein-binding characteristics of valproic acid in pediatric and adult patients with epilepsy who were undergoing monotherapy.

Methods

Subjects and collection of samples. The subjects, who were selected randomly, consisted of 48 Japanese pediatric patients with epilepsy (group A) and 48 Japanese adult patients with epilepsy (group B). We determined the concentrations of total and unbound serum valproic acid in samples obtained from these patients during routine therapeutic drug monitoring. Their age ranged from 1 to 15 years for group A (<10 years, 28 subjects; 10–15 years, 20 subjects) and from 18 to 63 years for group B (18–44 years, 24 subjects; 45–63 years, 24 subjects). To determine the influence of aging on the serum protein binding of valproic acid, the adult patients were divided into a younger group (<45 years) (group B—*younger*) and an older group (≥45 years) (group B—*older*),

in accordance with the age groups defined by Wood et al.¹⁰

All the subjects were receiving only valproic acid as their treatment for epilepsy. Biochemical screening showed their renal and hepatic functions to be normal. Each patient had been stabilized on his or her current dosage of valproic acid for more than two months, and serum valproic acid concentrations had reached steady state before the study. The patients did not habitually use any other medications. Each patient took the morning dose of valproic acid after breakfast, and a blood sample was obtained about three hours later. These samples were allowed to clot, and the serum was removed. Ninety-six samples (one per patient) at steady-state concentrations were analyzed. The study was approved by a local ethics committee.

Analysis of samples. The serum concentrations of total and unbound valproic acid were measured by fluorescence polarization immunoassay (TDx, Abbott Laboratories, Chicago, IL). The interday coefficient of variation (CV) of the assay of total valproic acid was 3.7% at 260 μM (37.5 μg/mL), 3.2% at 520 μM (75 μg/mL), and 2.6% at 867 μM (125 μg/mL). The CV of the assay of unbound valproic acid was 3.8% at 28 μM (4 μg/mL), 2.5% at 83 μM (12 μg/mL), and 2.4% at 139 μM (20 μg/mL).

Protein-binding study. The unbound serum fraction of valproic acid was analyzed by ultrafiltration with a commercially available device (MPS-3, Amicon, Tokyo, Japan) under routine laboratory conditions (temperature, 22–28 °C).¹¹ All serum samples were ultrafiltered as soon as possible after separation from blood, because pH changes affect the binding of drugs to serum proteins.¹² The degree of protein binding was calculated as the ratio of drug in the ultrafiltrate to that in the serum and was expressed as the unbound fraction.

Estimation of binding characteristics. Binding characteristics were

determined for each age group. Data analysis was performed with the SAS statistical package (SAS Institute Inc., Cary, NC).¹³ The association constant, K , and the total concentration of binding sites, $n(P)$, were estimated by iteratively reweighted least-squares regression analysis of the binding equation (equation 1) derived from the Scatchard equation for a one-site binding model^{14,15}:

$$C_f = 1/2\{C_t - n(P) - K^{-1} + [(n(P) - C_t + K^{-1})^2 + (4C_f/K)]^{1/2}\} \quad (1)$$

where C_f is unbound serum valproic acid concentration and C_t is total serum valproic acid concentration.

Calculation of theoretical minimum unbound serum fraction. The theoretical minimum unbound serum fraction, f_u , is an indicator of a drug's baseline unbound fraction in serum. It can be defined as a value of the unbound fraction when the concentration of protein-bound drug is zero and can be calculated by the following equation¹⁶:

$$f_u = 1/[1 + K \times n(P)] \quad (2)$$

where $K \times n(P)$ is the binding potential, or the capacity of serum proteins for drug-binding site interaction.

Statistical analysis. The Mann-Whitney U test was used to determine the significance of differences between pairs of age groups in total and unbound serum valproic acid concentrations, valproic acid's unbound serum fraction, serum concentrations of albumin and free fatty acids, and the ratio of the serum concentration of free fatty acids to the serum concentration of albumin. The Kruskal-Wallis test was used to evaluate differences in the same variables among groups A, B—*younger*, and B—*older*. An F test—an approximation of the likelihood ratio test—was used to evaluate differences in the binding of valproic acid between two age groups. The *a priori* level of significance was <0.05.

Results

Table 1 shows the demographics of the patients in groups A and B and their total and unbound serum valproic acid concentrations and unbound serum fractions. The serum albumin concentration was within the normal range of 435–710 μM (3.0–4.9 g/dL) in all patients. With respect to the serum concentration of free fatty acids, 46 pediatric (group A) patients were within the normal range of 150–640 μM (150–640 $\mu\text{eq/L}$) (the 2 exceptions had concentrations of 684 and 746 μM), and 42 of the adult (group B) patients were within the normal range (the 6 exceptions had concentrations of 641, 658, 678, 682, 714, and 718 μM). Significant differences were observed between the two groups in the serum free fatty acid concentration and the ratio of the serum concentration of free fatty acids to the serum level of albumin.

The percentage of samples with a total serum valproic acid concentration of $>560 \mu\text{M}$ ($>80 \mu\text{g/mL}$)—a level at which a saturable binding of valproic acid to serum proteins might occur¹⁸—was higher in group A (40%, 19 of 48 samples) than in group B (13%, 6 of 48 samples). Although there were significant differences between groups A and B in the total and unbound serum valproic acid concentrations, there was no significant difference in the unbound fraction.

Table 2 shows the demographics of the patients in groups A, B—*younger*, and B—*older* and their total and unbound serum valproic acid concentrations and unbound serum fractions. The serum albumin concentration in group A was significantly lower than in group B—*younger* ($p = 0.0007$) and significantly higher than in group B—*older* ($p = 0.0008$). Group A’s serum free fatty acid concentration was significantly lower than in group B—*younger* ($p = 0.0161$) and group B—*older* ($p = 0.0057$). Consequently, the serum concentration ratio of free fatty acids to albumin in group A was significantly lower than in group B—*older* ($p = 0.0014$) and lower than in group B—*younger* ($p = 0.0544$).

The total serum concentration of valproic acid was significantly higher in group A than in group B—*younger* ($p = 0.0002$) and group B—*older* ($p = 0.0008$). Similarly, the unbound valproic acid concentration was significantly higher in group A than in group B—*younger* ($p = 0.0001$) and group B—*older* ($p = 0.0411$). Consequently, group A’s unbound serum fraction was significantly higher than in group B—*younger* ($p = 0.0084$); no significant difference was observed between groups A and B—*older*.

The serum albumin concentration was significantly higher in group B—*younger* than in group B—*older* ($p =$

0.0001). However, no significant differences were observed between these two groups in the serum free fatty acid concentration or the serum concentration ratio of free fatty acids to albumin. Although there were no significant differences in the total serum valproic acid concentration between these groups, the unbound serum valproic acid concentration was significantly higher in group B—*older* than in group B—*younger* ($p = 0.0433$). Consequently, the unbound serum fraction was significantly higher in group B—*older* ($p = 0.0065$).

Data for the variables characterizing valproic acid binding to serum proteins in each group are shown in Table 3. The affinity of valproic acid for serum proteins was higher in group A than in group B, while the total concentration of binding sites was higher in group B. Figure 1 shows the relationship between the total and unbound serum valproic acid concentrations determined by applying the mean binding data for groups A and B in Table 3 to equation 1. No significant differences were observed between these two groups in the binding of valproic acid to serum proteins, and similar theoretical minimum unbound serum fractions were calculated.

The affinity of valproic acid for serum proteins was higher in group A than in group B—*younger* and group B—*older*, while group A’s total concentration of binding sites was lower than in either of the B subgroups. Figure 2 shows the relationship between the total and unbound serum valproic acid concentrations obtained by applying the mean binding data for all three groups in Table 3 to equation 1. No significant differences in the binding of valproic acid to serum proteins were observed between group A and groups B—*younger* and B—*older*, but there was a significant difference between groups B—*younger* and B—*older* ($p = 0.0258$).

Table 1. Demographic and Valproic Acid Protein Binding Data for Groups A and B^a

Variable	Group		<i>p</i> ^b
	A (1–15 yr)	B (18–63 yr)	
No. patients (male/female)	48 (22/26)	48 (18/30)	
Age (yr)	7.9 ± 4.3	42.0 ± 14.0	
Albumin conc. (μM)	575 ± 28	576 ± 47	0.9912
Free fatty acid conc. (μM)	346 ± 133	429 ± 136	0.0015
Serum free fatty acid-to-albumin conc. ratio	0.604 ± 0.236	0.749 ± 0.245	0.0017
Serum valproic acid conc. (μM) ^c			
Total	519 ± 144	386 ± 130	0.0001
Unbound	53 ± 27	35 ± 18	0.0002
Unbound serum valproic acid fraction	0.098 ± 0.032	0.089 ± 0.027	0.1649

^aData (except number of patients) are mean ± S.D.

^bMann–Whitney *U* test.

^cDividing values by 6.934 will yield values in micrograms per milliliter.¹⁷

Table 2. Demographic and Valproic Acid Protein Binding Data for Groups A, B—Younger, and B—Older^a

Variable	Group			p ^b
	A (1–15 yr)	B—Younger (18–44 yr)	B—Older (45–63 yr)	
No. patients (male/female)	48 (22/26)	24 (9/15)	24 (9/15)	
Age (yr)	7.9 ± 4.3	29.7 ± 8.2	54.3 ± 3.9	
Albumin conc. (μM)	575 ± 28	603 ± 41	550 ± 38	<0.0001
Free fatty acid conc. (μM)	346 ± 133	430 ± 150	428 ± 123	0.0065
Serum free fatty acid-to-albumin conc. ratio	0.604 ± 0.236	0.718 ± 0.264	0.780 ± 0.227	0.0045
Serum valproic acid conc. (μM) ^c				
Total	519 ± 144	374 ± 130	397 ± 131	<0.0001
Unbound	53 ± 27	30 ± 14	40 ± 20	0.0002
Unbound serum valproic acid fraction	0.098 ± 0.032	0.078 ± 0.017	0.100 ± 0.031	0.0107

^aData (except number of patients) are mean ± S.D.

^bKruskal–Wallis test.

^cDividing values by 6.934 will yield values in micrograms per milliliter.

Table 3. Binding of Valproic Acid and Theoretical Minimum Unbound Serum Fraction^a

Group	n	K (95% CI), L/μmol	n(P) (95% CI), μM	K × n(P)	f _u
A (1–15 yr)	48	0.016 (0.007 to 0.024)	1077 (805 to 1350)	17.23	0.055
B (18–63 yr)	48	0.011 (–0.001 to 0.022)	1365 (399 to 2331)	15.02	0.062
B—younger (18–44 yr)	24	0.013 (0.001 to 0.025)	1291 (517 to 2065)	16.78	0.056
B—older (45–63 yr)	24	0.006 (–0.008 to 0.021)	1827 (–1421 to 5074)	10.96	0.084

^aValues are means. K = association constant, CI = confidence interval (asymptotic), n(P) = total concentration of binding sites, K × n(P) = binding potential, f_u = theoretical minimum unbound serum fraction.

Discussion

We studied the in vivo binding of valproic acid to serum proteins in pediatric and adult patients with epilepsy receiving valproic acid monotherapy. We found no significant differences in protein binding between pediatric (group A) and adult (group B) patients. The capacity of serum proteins for drug-binding site interaction appeared to be similar between the two groups, because the theoretical minimum unbound serum fraction of valproic acid was similar.

No significant differences in the binding of valproic acid were observed between group A and the two group B subgroups, B—younger (18–44 years) and B—older (45–63 years). However, the capacity of serum proteins for valproic acid bind-

ing seemed different between group B—older and the other two groups, because the theoretical minimum unbound serum fraction was higher in group B—older. On the other hand, there was a significant difference in binding between groups B—younger and B—older. It appears, therefore, that there may be age-related differences in valproic acid-protein binding-site interaction.

Age has been found to be positively correlated with the unbound plasma fraction of valproic acid.⁴ The binding of valproic acid to serum proteins is affected by certain factors, such as the total serum valproic acid concentration and the serum levels of albumin and free fatty acids.⁴ Furthermore, there is a significant negative correlation between the association constant for valproic

acid-albumin binding-site interaction and the serum concentration ratio of free fatty acids to albumin.¹⁴ We found a significantly higher serum concentration ratio of free fatty acids to albumin in group B than in group A and consequently a higher association constant for valproic acid-serum protein binding in group A. However, no significant difference in binding to serum proteins was observed between the two groups, suggesting a similar capacity of serum proteins for drug-binding-site interaction.

No significant differences in the protein binding of valproic acid were observed between group A and groups B—younger and B—older, although there was a significant difference between B—younger and B—older. The serum concentration ratio of free fatty acids to albumin in group A was significantly lower than in group B—older and was lower than in group B—younger. Consequently, the association constant for the binding of valproic acid to serum proteins in group A was higher than in group B—older and similar to that in group B—younger. Furthermore, binding potential in group B—older seemed different from that in groups A and B—younger. However, there was no significant difference in binding between groups A and B—older, perhaps because binding was determined for relatively narrow serum concentration ranges.

Figure 1. Relationship between total serum valproic acid concentration (C_t) and unbound serum valproic acid concentration (C_f) determined by using the mean binding data for pediatric (group A) and adult (group B) patients with epilepsy. The binding equation for group A (curve 1, open circles) ($n = 48$): $C_f = 1/2\{C_t - 1077 - 1/0.016 + [(1077 - C_t + 1/0.016)^2 + 4C_t/0.016]^{1/2}\}$. The binding equation for group B (curve 2, filled circles) ($n = 48$): $C_f = 1/2\{C_t - 1365 - 1/0.011 + [(1365 - C_t + 1/0.011)^2 + 4C_t/0.011]^{1/2}\}$.

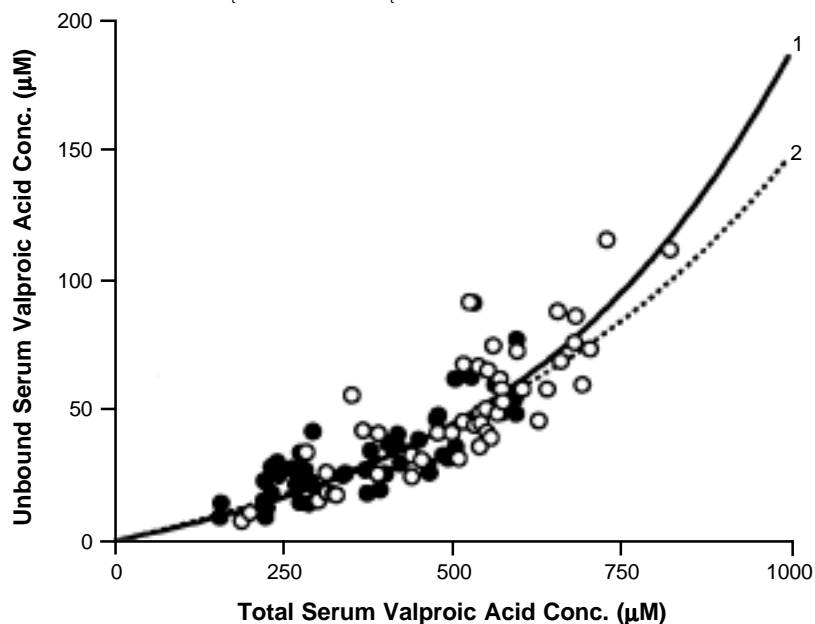
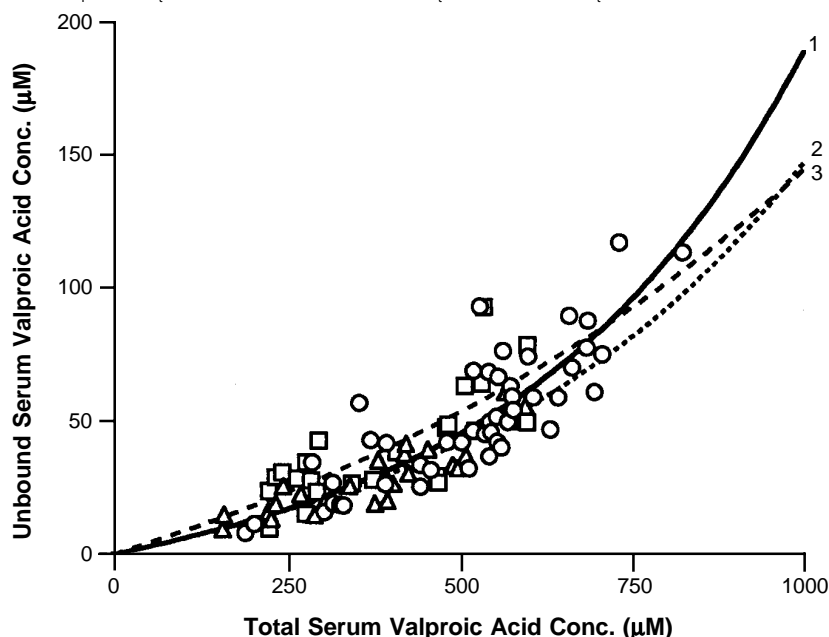


Figure 2. Relationship between total serum valproic acid concentration (C_t) and unbound serum valproic acid concentration (C_f) determined by using the mean binding data for pediatric (group A) and younger adult (group B—younger) and older adult (group B—older) patients with epilepsy. The binding equation for group A (curve 1, open circles) ($n = 48$): $C_f = 1/2\{C_t - 1077 - 1/0.016 + [(1077 - C_t + 1/0.016)^2 + 4C_t/0.016]^{1/2}\}$. The binding equation for group B—younger (curve 2, open triangles) ($n = 24$): $C_f = 1/2\{C_t - 1291 - 1/0.013 + [(1291 - C_t + 1/0.013)^2 + 4C_t/0.013]^{1/2}\}$. The binding equation for group B—older (curve 3, open squares) ($n = 24$): $C_f = 1/2\{C_t - 1827 - 1/0.006 + [(1827 - C_t + 1/0.006)^2 + 4C_t/0.006]^{1/2}\}$.



On the other hand, the serum albumin concentration in group B—younger was significantly higher than in group B—older, although there were no significant differences between these groups in the serum free fatty acid concentration and the serum concentration ratio of free fatty acids to albumin. The association constant was higher in group B—younger than in group B—older, and the binding potential was higher in group B—younger than in group B—older. Bauer et al.¹⁹ reported that the average unbound serum fraction of valproic acid was higher in healthy elderly adults (10.7%) than in healthy younger adults (6.4%). Similarly, we found that the drug's unbound serum fraction was significantly higher in group B—older than in group B—younger. There may be age-related differences in valproic acid-protein binding-site interactions.

We could not examine the concentrations of metabolites of valproic acid because of the small volume of the serum samples. Some metabolites of valproic acid displace the binding of valproic acid to human serum proteins.²⁰ Because higher serum clearance and lower serum half-life of valproic acid were observed in children ages 2–10 years with epilepsy receiving valproic acid monotherapy or concurrent antiepileptic drugs compared with adult patients,²¹ the metabolism of valproic acid may differ between pediatric and adult patients. However, no significant difference in the binding of valproic acid to serum proteins was observed between groups A and B. Thus, the effects of age on the drug's metabolism may have been small in our patient sample.

Assessments of alterations in the plasma or serum protein binding characteristics of drugs have not always been made in studies of the effects of age on pharmacokinetics. Our study identified a significant difference in the protein binding of valproic acid between younger and

older adult patients, although no significant difference was seen between the children and either adult subgroup. Furthermore, the binding potential was lower in the older adults than in the pediatric patients or the younger adults. Dosage adjustment of valproic acid on the basis of age-related serum protein-binding characteristics seems to be necessary in older adult patients with epilepsy.

Conclusion

The serum protein binding of valproic acid was similar in pediatric and adult patients with epilepsy, but binding characteristics differed between younger and older adults.

References

1. Brodie MJ. Established anticonvulsants and treatment of refractory epilepsy. *Lancet*. 1990; 336:350-4.
2. Rylance GW. Treatment of epilepsy and febrile convulsions in children. *Lancet*. 1990; 336:488-91.
3. Perucca E. The new generation of antiepileptic drugs: advantages and disadvantages. *Br J Clin Pharmacol*. 1996; 42:531-43.
4. Zaccara G, Messori A, Moroni F. Clinical pharmacokinetics of valproic acid—1988. *Clin Pharmacokinet*. 1988; 15:367-89.
5. Ludden TM. Nonlinear pharmacokinetics: clinical implications. *Clin Pharmacokinet*. 1991; 20:429-46.
6. Rapeport WG, Mendelow AD, French G et al. Plasma protein-binding and CSF concentrations of valproic acid in man following acute oral dosing. *Br J Clin Pharmacol*. 1983; 16:365-9.
7. Yu HY. Clinical implications of serum protein binding in epileptic children during sodium valproate maintenance therapy. *Ther Drug Monit*. 1984; 6:414-23.
8. Scheyer RD, Cramer JA, Toftness BR et al. In vivo determination of valproate binding constants during sole and multi-drug therapy. *Ther Drug Monit*. 1990; 12:117-23.
9. Anderson GD, Gidal BE, Hendryx RJ et al. Decreased plasma protein binding of valproate in patients with acute head trauma. *Br J Clin Pharmacol*. 1994; 37:559-62.
10. Wood AJ, Vestal RE, Wilkinson GR et al. Effect of aging and cigarette smoking on antipyrine and indocyanine green elimination. *Clin Pharmacol Ther*. 1979; 26:16-20.
11. March C, Blanke RV. Determination of free valproic acid concentrations using the amicon micropartition MPS-1 ultrafiltration system. *Ther Drug Monit*. 1985; 7:115-20.
12. Brørs O, Jacobsen S. pH lability in serum during equilibrium dialysis. *Br J Clin Pharmacol*. 1985; 20:85-8.
13. SAS/STAT user's guide. Cary, NC: SAS Institute; 1988.
14. Kodama Y, Koike Y, Kimoto H et al. Binding parameters of valproic acid to serum protein in healthy adults at steady state. *Ther Drug Monit*. 1992; 14:55-60.
15. Kodama Y, Tsutsumi K, Teraoka I et al. Effect of unbound clearance on binding parameters of valproic acid to serum proteins. *J Clin Pharmacol*. 1993; 33:130-5.
16. Barre J, Didey F, Delion F et al. Problems in therapeutic drug monitoring: free drug level monitoring. *Ther Drug Monit*. 1988; 10:133-43.
17. McLeod DC. SI units in drug therapeutics. *Drug Intell Clin Pharm*. 1988; 22:990-3.
18. Cramer JA, Mattson RH. Valproic acid: in vitro plasma protein binding and interaction with phenytoin. *Ther Drug Monit*. 1979; 1:105-16.
19. Bauer LA, Davis R, Wilensky A et al. Valproic acid clearance: unbound fraction and diurnal variation in young and elderly adults. *Clin Pharmacol Ther*. 1985; 37:697-700.
20. Nau H, Helge H, Luck W. Valproic acid in the perinatal period: decreased maternal serum protein binding results in fetal accumulation and neonatal displacement of the drug and some metabolites. *J Pediatr*. 1984; 104:627-34.
21. Hall K, Otten N, Johnson B et al. A multivariable analysis of factors governing the steady-state pharmacokinetics of valproic acid in 52 young epileptics. *J Clin Pharmacol*. 1985; 25:261-8.