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Research paper

Gender- or age-related binding characteristics of valproic acid to serum proteins in adult patients with epilepsy

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Abstract

The aim of the present study was to determine the gender- or age-related binding characteristics of valproic acid (VPA) to serum proteins in the adult population. Serum samples examined in the study were obtained from 70 adult patients (36 males, 34 females) with epilepsy on VPA monotherapy. Their age ranged from 16 to 68 years (mean age with (SD), 37.7 (15.7) years; <45 years, $n = 44$; ≥45 years, $n = 26$). The in vivo population binding parameters of VPA to serum proteins and theoretical minimal unbound serum VPA fraction (*Fu*) were determined using an equation derived from the Scatchard equation in: (1), all; (2), male and female subgroups; and (3), younger (<45 years) and older (≥45 years) subgroups. There was a significant difference in serum concentration of unbound VPA between male and female patients. The mean association constant (K) was 0.010 μM^{-1} in all, male, and female patients. The mean total concentration of binding sites ($n(Pt)$) was 1453 μM for all patients, and 1561 and 1394 μM for male and female patients, respectively. The *Fu* was 0.064 for all patients, and 0.060 and 0.067 for male and female patients, respectively. There were no significant differences in the binding characteristics of VPA to serum proteins between the male and female groups. On the other hand, there were significant differences in the serum albumin concentration and molar concentration ratio of free fatty acids to albumin in serum between the younger and older patients. The mean value of K was 0.016 μM^{-1} for the younger patients and 0.007 μM^{-1} for the older patients. The mean $n(Pt)$ was 1157 μM for the younger patients and 1703 μM for the older patients. The *Fu* was 0.051 for the younger patients and 0.077 for the older patients. Thus, significant differences were observed in the binding characteristics of VPA to serum proteins between the younger and older groups. Our results show that age, but not gender, has significant influences on the binding characteristics of VPA to serum proteins in our patient population.

Keywords: Valproic acid; Gender or age effect; Protein binding characteristics; Epilepsy

1. Introduction

Valproic acid (VPA) is a branched-chained fatty acid, structurally unrelated to any other antiepileptic drugs. VPA is effective in generalized (both tonic–clonic and absence) and partial seizures [1]. However, the mechanism of action of VPA has not been fully evaluated, although its action of blockade of voltage-dependent sodium channels and potentiation of GABAergic transmission has been postulated [2]. More than 90% of VPA binds to plasma proteins, mainly albumin [3] and exhibits its concentration-dependent plasma protein binding near or within the usual therapeutic concentration ranges [4]. Therefore, the determination of binding characteristics in VPA–plasma protein interaction is useful in clinical practice, because the unbound plasma VPA concentration reflects the level in cerebrospinal fluid [5].

There are few detailed studies concerning the in vivo binding characteristics of VPA to serum or plasma proteins in adult patients receiving VPA [6,7]. Furthermore, information on gender- or age-related characteristics of VPA–serum or VPA–plasma protein interaction in these patients

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is limited. Scheyer et al. reported the in vivo population binding parameters of VPA to serum proteins in epileptic patients receiving VPA mono- or polytherapy [6]. Anderson et al. also determined the in vivo population binding parameters of VPA to plasma proteins in adult patients treated with VPA for prophylaxis of post-traumatic head injuries [7]. However, the gender- or age-related binding characteristics of VPA to serum or plasma proteins were not determined in these two studies. In view of the potential effect of serum protein binding on drug disposition, it is important that gender- or age-related characteristics in serum protein binding of drugs be evaluated.

In a previous study, we reported no effects of gender or age on the binding characteristics of VPA to serum proteins after a repeated dosing of VPA in pediatric patients with epilepsy receiving monotherapy [8]. The results also showed that the differences in VPA binding to serum proteins appear to be relatively larger in binding affinity than in binding capacity between the subgroups of male and female, or younger and older patients. In the present study, we determined the in vivo population binding parameters of VPA to serum proteins in male and female adult patients with epilepsy receiving monotherapy. The binding characteristics of VPA to serum proteins were also determined in younger (<45 years of age) and older (≥45 years) groups. The results were compared to examine the effects of gender or age on the binding characteristics of VPA to serum proteins.

2. Subjects and methods

2.1. Subjects

Serum samples examined in the study were obtained from 70 adult patients (Table 1). The patients' ages ranged from 16 to 68 years (16–44 years, 26 subjects; 45–64 years, nine subjects; ≥65 years, one subject) for male patients and from 16 to 67 years (16–44 years, 18 subjects; 45–64 years, 15 subjects; ≥65 years, one subject) for female patients. To determine the influence of aging on the serum protein binding characteristics of VPA, these patients were divided into a younger group (<45 years) between 16 and 43 years and an older group (≥45 years) between 45 and 68 years according to the age-grouping defined by Wood et al. [9] (Table 2). All were receiving only VPA as their treatment for epilepsy. Biochemical screenings showed their renal and hepatic functions as normal.

A steady-state condition for VPA had been already obtained, and all patients had taken the same dose of VPA each for at least 2 months before the study. Patients did not take any other medications, including oral contraceptives,

Table 1
Demographic data of the patients, and their serum VPA concentrations and unbound serum VPA fraction[a]

	Male	Female	Total	Mann–Whitney *U*-test (male vs. female)
Number of patients	36	34	70	
Age (years)	35.3 (15.7)	40.2 (15.6)	37.7 (15.7)	$P = 0.2495$
Range	16–68	16–67	16–68	
Median	35.0	43.0	36.5	
Serum concentration (μM)				
Albumin	588 (48)	576 (54)	582 (51)	$P = 0.3781$
Range	465–686	468–674	465–686	
Median	591	576	585	
Free fatty acids	417 (121)	412 (141)	414 (130)	$P = 0.8555$
Range	268–718	196–714	196–718	
Median	396	371	386	
Serum free fatty acids/albumin concentration ratio	0.719 (0.233)	0.721 (0.252)	0.720 (0.241)	$P = 0.8555$
Range	0.430–1.330	0.300–1.280	0.300–1.330	
Median	0.650	0.705	0.670	
Serum VPA concentration (μM)				
Total	356 (156)	412 (140)	384 (150)	$P = 0.1087$
Range	85–641	159–716	85–716	
Median	310	439	381	
Unbound	29 (17)	38 (20)	33 (19)	$P = 0.0370$
Range	3–79	14–94	3–94	
Median	24	35	29	
Unbound serum VPA fraction	0.077 (0.024)	0.090 (0.027)	0.083 (0.026)	$P = 0.0397$
Range	0.041–0.132	0.052–0.175	0.041–0.175	
Median	0.073	0.088	0.080	

[a] Data are presented as means (SD).

Table 2
Demographic data of the younger[a] and older[b] groups, and their serum VPA concentrations and unbound serum VPA fraction[c]

	Group		Mann–Whitney *U*-test
	Younger	Older	
Number of patients	44	26	
Male/female	26/18	10/16	
Age (years)	27.3 (9.0)	55.3 (5.2)	
Range	16–43	45–68	
Serum concentration (μM)			
Albumin	603 (45)	547 (39)	$P = 0.0001$
Range	465–686	468–678	
Median	607	541	
Free fatty acids	407 (137)	428 (118)	$P = 0.3782$
Range	196–718	272–678	
Median	379	410	
Serum free fatty acids/albumin concentration ratio	0.681 (0.247)	0.785 (0.219)	$P = 0.0476$
Range	0.300–1.330	0.480–1.280	
Median	0.625	0.760	
Serum VPA concentration (μM)			
Total	383 (160)	384 (134)	$P = 0.8697$
Range	85–716	197–599	
Median	390	360	
Unbound	30 (18)	39 (20)	$P = 0.0664$
Range	3–88	10–94	
Median	26	31	
Unbound serum VPA fraction	0.075 (0.018)	0.098 (0.030)	$P = 0.0008$
Range	0.041–0.123	0.044–0.175	
Median	0.073	0.097	

[a] Age, <45 years.
[b] Age, ≥45 years.
[c] Data are presented as means with (SD).

for a chronic use. The blood sample from each patient was obtained at approximately 3 h after the morning dose of VPA with their usual breakfast. These samples were allowed to clot, and then the serum was separated. A total of 70 steady-state concentrations were analyzed in the study. All samples were obtained during routine therapeutic monitoring approved by the local ethics committee.

2.2. Sample analysis

Serum levels of total and unbound VPA were measured by a fluorescence polarization immunoassay (TDx; Abbott Laboratories, Chicago, IL). The day-to-day coefficient of variation (CV) of the total VPA assay was 3.7% at 260 μM, 3.2% at 520 μM, and 2.6% at 867 μM. The CV of the unbound VPA assay was 3.8% at 28 μM, 2.5% at 83 μM, and 2.4% at 139 μM.

2.3. Protein binding study

Protein binding of VPA was evaluated by ultrafiltration with a commercially available MPS-3 device (Amicon, Tokyo, Japan) [10] under current laboratory routine conditions (25 ± 3°C). All serum samples were ultrafiltered as soon as possible after separation from blood, because pH change affects the serum protein binding of drugs [11]. The time interval was about 1 h between blood sampling and ultrafiltration. The degree of protein binding was calculated as the ratio of drug in the ultrafiltrate to that in serum and was expressed as the unbound fraction.

2.4. Estimation of binding parameters

The in vivo binding parameters of VPA were determined in all patients, and subgroups of male and female, and younger and older patients. Data analyses were performed using the SYSTAT statistical package [12], as was the case in the studies of Scheyer et al. [6] or Anderson et al. [7] for the determination of VPA binding parameters to plasma proteins. The association constant (K) and total concentration of binding sites ($n(Pt)$) were estimated by iteratively reweighed least squares regression analysis of the following binding Eq. (1) [13,14] derived from the Scatchard equation for a one-site binding model.

$$Cf = \frac{1}{2}\left\{Ct - n(Pt) - \frac{1}{K} + \left[\left((n(Pt) - Ct + \frac{1}{K}\right)^2 + \frac{4Ct}{K}\right]^{\frac{1}{2}}\right\} \quad (1)$$

where Ct is the concentration of total VPA in serum, and Cf

is the concentration of VPA not bound to proteins. The number of binding sites per albumin molecule (n) was calculated by dividing the mean value of $n(Pt)$ by mean serum albumin concentration.

2.5. Calculation of theoretical minimal unbound serum fraction

The unbound serum fraction (fu) of drugs with a single population of binding sites on serum proteins depends on the association constant for the drug–protein interaction, and the concentration of free protein (P) according to Eq. (2) [15]:

$$fu = \frac{1}{1 + K \cdot P} \tag{2}$$

and Eqs. (3) and (4)

$$P = n(Pt) - Cb \tag{3}$$

$$Cb = Ct - Cf \tag{4}$$

where Cb is the concentration of VPA bound to serum proteins. Thus, by substitution of Eq. (3) into Eq. (2) (see Eq. (5)):

$$fu = \frac{1}{1 + K(n(Pt) - Cb)} \tag{5}$$

The theoretical minimal unbound serum fraction (Fu) of VPA can be defined as a value of fu at 0 μM of bound serum VPA concentration and therefore, is calculated by the following equation, Eq. (6).

$$Fu = \frac{1}{1 + K \cdot n(Pt)} \tag{6}$$

2.6. Statistical analysis

The Mann–Whitney U-test was used to determine statistically significant differences in age, total and unbound serum VPA concentrations, serum concentrations of albumin and free fatty acids, and serum concentration ratio of free fatty acids to albumin between two groups of patients. Simple regression analysis was performed for the unbound serum VPA fraction and serum albumin concentration, or the serum concentration ratio of free fatty acids to albumin in all patients. Comparison of binding characteristics of VPA to serum proteins was made by the F-test, which was an approximation to the likelihood ratio test, to determine differences between two groups of patients, as reported previously [8]. The predetermined level for significance was $P < 0.05$.

3. Results

The demographic data and serum VPA concentration of the patients are shown in Table 1. No significant difference was observed in age between male and female subgroups. The mass (μg/ml)-to-molar (μM) conversion factor for serum VPA concentrations was 6.934 [16].

The serum concentration of albumin was within the normal range of 30–49 g/l (435–710 μM) in all patients. No significant relationships were observed between the serum unbound VPA fraction and the serum albumin concentration ($r = -0.092$, $P = 0.4502$), or the serum concentration ratio of free fatty acids to albumin ($r = -0.046$, $P = 0.7039$) in all patients. On the other hand, the serum free fatty acids in 64 patients were within the normal range of 150–640 μEq/l (150–640 μM), except for two male (641 and 718 μEq/l) and four female patients (658, 678, 682, and 714 μEq/l). There were no significant differences in the serum concentrations of albumin and free fatty acids between the two subgroups (Table 1). The percentage of samples with a total serum concentration of more than 560 μM, above which level a saturable binding of VPA to serum proteins might occur [17], was similar between male (17%, six samples) and female (15%, five samples) patients. Significant differences were observed in the serum concentration of unbound VPA and the unbound serum VPA fraction between the two subgroups (Table 1).

There were significant differences in the serum albumin concentration and serum concentration ratio of free fatty acids to albumin between younger and older patients (Table 2). However, no significant difference was observed in the serum free fatty acid concentration between the two subgroups. The percentage of samples with a total serum concentration of more than 560 μM was higher in the younger (18%, eight samples) than in the older (12%, three samples) patients. The serum concentration of unbound VPA in the older patients was greater than that in the younger patients, but the difference was not statistically significant. A significant difference was observed in the unbound serum VPA fraction between the two subgroups (Table 2).

The mean binding parameters characterizing VPA binding to serum proteins are shown in Table 3. The affinity of VPA to serum proteins is identical between male and female patients, and the total concentration of binding sites is approximately 1.1 times greater in male than in female patients. Fig. 1 shows the relationships between total and unbound serum VPA concentrations obtained by applying mean binding parameters for all patients, and male and female subgroups (Table 3) to Eq. (1). No significant differences were observed in the binding characteristics of VPA to serum proteins between the two subgroups ($P = 0.2629$). Consequently, the similar theoretical minimal unbound serum fraction of VPA was calculated in each subgroup (Table 3).

The mean binding parameters of VPA in the younger and older subgroups are shown in Table 3. The affinity of VPA to serum proteins in the younger patients is approximately 2.3 times higher than that in the older patients, while the total concentration of binding sites is approximately 1.5 times greater in the older than in the younger patients.

Table 3
Mean binding parameters and theoretical minimal unbound serum fraction of VPA in all patients, male and female subgroups, and younger[a] and older[b] subgroups[c]

Group	Number	K (μM^{-1})[d]	$n(Pt)$ (μM)[d]	n	Fu
All	70	0.010 (0.003–0.017)	1453 (771–2136)	2.50	0.064
Male	36	0.010 (0.001–0.018)	1561 (600–2522)	2.65	0.060
Female	34	0.010 (−0.001–0.022)	1394 (380–2407)	2.42	0.067
Younger	44	0.016 (0.010–0.023)	1157 (897–1417)	1.92	0.051
Older	26	0.007 (−0.007–0.021)	1703 (−805–4211)	3.11	0.077

[a] Age, <45 years.
[b] Age, ≥45 years.
[c] K, association constant; $n(Pt)$, total concentration of binding sites; n, number of binding sites per albumin molecule; Fu, theoretical minimal unbound serum fraction.
[d] Figures in parentheses represent a symptotic 95% confidence intervals (CI) of the mean.

Fig. 2 shows the relationships between total and unbound serum VPA concentrations obtained by applying mean binding parameters for younger and older patients (Table 3) to Eq. (1). There were significant differences in the binding characteristics of VPA to serum proteins between the two subgroups ($P = 0.00045$). Consequently, the theoretical minimal unbound serum fraction of VPA in the older patients is approximately 1.5 times higher than that in the younger patients (Table 3).

4. Discussion

The in vivo binding characteristics of VPA to serum proteins was determined in adult patients with epilepsy receiving monotherapy. In vivo binding parameters of VPA to serum or plasma proteins have been evaluated previously by Scatchard analysis in populations of otherwise healthy patients with epilepsy or head trauma [6–8]. Scheyer et al. found mean values of $K = 0.013$ μM^{-1}, $n(Pt) = 1035$ μM, and $n = 1.64$ in 37 patients with epilepsy receiving VPA mono- or polytherapy [6]. Anderson et al. reported mean values of $K = 0.008$ μM^{-1} and $n = 2.0$ in 50 patients (age, >18 years) treated with VPA for prophylaxis of post-traumatic head injuries [7]. On the other hand, we showed mean values of $K = 0.014$ μM^{-1} and $n(Pt) = 1160$ μM in 61 pediatric patients with epilepsy receiving VPA monotherapy [8]. The association constant and total concentration of binding sites or number of binding sites per albumin molecule in these studies were comparable with those found in our group of all patients with epilepsy ($K = 0.010$ μM^{-1}, $n(Pt) = 1453$ μM, and $n = 2.50$; Table 3).

Our study shows that there is a significant difference in serum unbound VPA concentration between male and female patients (Table 1). Since the total serum concentration of VPA was greater in female than in male patients (Table 1), whereas the total concentration of binding sites was smaller in female than in male patients (Table 3), saturable binding of VPA to serum proteins may be apparent in female than in male patients. No significant differences in the binding characteristics of VPA to serum proteins were observed between the two groups. The capacity of serum

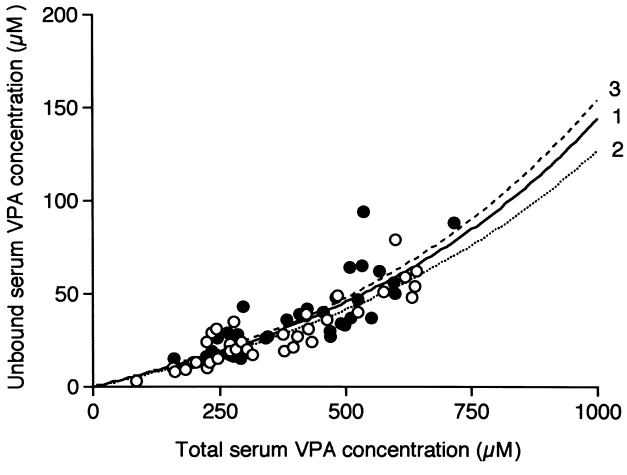

Fig. 1. Relationship between total and unbound serum VPA concentrations simulated by using the mean binding parameters determined in all patients, and male and female subgroups: (1), all patients ($n = 70$); (2), male patients (open circles; $n = 36$); (3), female patients (filled circles; $n = 34$).

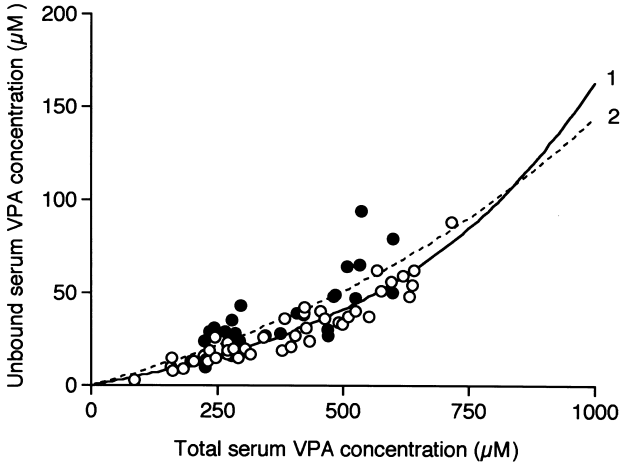

Fig. 2. Relationship between total and unbound serum VPA concentrations simulated by using the mean binding parameters determined in younger and older subgroups: (1), younger group of patients (open circles; $n = 44$); (2), older group of patients (filled circles; $n = 26$).

proteins for drug–binding site interaction seems to be apparently similar between the two groups, because the theoretical minimal unbound serum fraction of VPA in male patients is approximate to that in female patients. The binding of VPA to serum proteins is affected by some factors, such as total serum VPA concentration, and serum concentrations of albumin and free fatty acids [3]. As no significant differences were observed in these variables between male and female patients, differences in the binding characteristics of VPA to serum proteins may not be apparent between the two groups. It appears, therefore, that there may not be gender-related differences in the VPA–binding site interaction.

There was a significant difference in the serum concentration of albumin and the serum concentration ratio of free fatty acids to albumin between the younger and older patients. The theoretical minimal unbound serum fraction of VPA in the older patients is approximately 1.5 times higher than that in the younger patients and, therefore, the capacity of serum proteins for drug–binding site interaction seems to be different between the two groups. Anderson et al. showed that the unbound plasma fraction of VPA varied from six- to seven-fold as the plasma albumin concentration ranged from 15 (218 μM) to 48 g/l (696 μM) [7]. A significant positive correlation is also shown between the plasma concentration ratio of free fatty acids to albumin and the unbound plasma fraction of VPA [18]. In the previous study, we also showed a negative correlation between the association constant for VPA–albumin binding site interaction and the molar concentration ratio of free fatty acids to albumin in serum [13]. This indicates that the binding characteristics of VPA to serum proteins were significantly affected by hypoalbuminemic states or changes in the molar concentration ratio of free fatty acids to albumin in serum. Furthermore, Bauer et al. indicated that the unbound serum fraction of VPA was significantly larger in the elderly than in the younger subjects [19]. Our finding of significant differences in the binding characteristics of VPA to serum proteins between younger and older patients is consistent with these observations. Thus, the present study indicates that age has a significant effect on the binding characteristics of VPA to serum proteins.

VPA is almost entirely eliminated by metabolism [20], and its major metabolic pathways are direct glucuronide conjugation of the intact molecule and β-oxidation with a wide variation in the patterns of urinary metabolite excretion [21]. Unfortunately, we could not examine the concentration of metabolites of VPA because of a small volume of serum samples. Some metabolites of VPA, as well as parent drug, are thought to bind primarily to albumin, because both saturated and unsaturated long- and medium-chain fatty acids have a selective affinity for plasma albumin [22–24]. Nau et al. showed that the metabolites of VPA have displacing effects on VPA binding to human sera [25]. Although the metabolic pattern of VPA may affect the binding characteristics of VPA to serum proteins, gender or age effects on the metabolite pattern of VPA are not well established in the adult population.

Alterations in serum albumin concentration occur as a result of altered synthesis, loss, or a shift of albumin from the intravascular to extravascular spaces [26,27]. The most common alteration, hypoalbuminemia, is associated with a wide variety of pathological and physiological conditions. For albumin-bound drugs such as VPA, this decrease in binding capacity results in significant decreases in the binding potential that is defined as the product of the association constant and total concentration of binding sites. Therefore, it is needed to determine which binding parameters of the association constant or total concentration of binding sites have significant effects on alteration in the binding characteristics of VPA to serum proteins in patients with disease states affecting the serum albumin concentration.

Generally, plasma protein binding of drugs remains unchanged or decreases with age [28]. The results of this study show that age, but not gender, has a significant effect on the binding characteristics of VPA to serum proteins. It appears that differences in the binding characteristics of VPA to serum proteins between age groups are caused by the age-related changes of albumin and free fatty acid concentrations in the serum. Changes of greater than 50% in the unbound serum fraction of VPA were observed in elderly subjects [19]. Our results also show the approximately 50% greater values of theoretical minimal unbound serum VPA fraction in the older patients. Care should be taken in choosing the VPA dosage in the older or elderly patients and in interpreting VPA concentrations in serum, especially when only total concentrations, rather than unbound concentrations, are measured.

References

[1] M. Feely, Drug treatment of epilepsy, Br. Med. J. 318 (1999) 106–109.
[2] E. Perucca, The new generation of antiepileptic drugs: advantages and disadvantages, Br. J. Clin. Pharmacol. 42 (1996) 531–543.
[3] G. Zaccara, A. Messori, F. Moroni, Clinical pharmacokinetics of valproic acid – 1988, Clin. Pharmacokinet. 15 (1988) 367–389.
[4] T.M. Ludden, Non-linear pharmacokinetics: clinical implications, Clin. Pharmacokinet. 20 (1991) 429–446.
[5] W.G. Rapeport, A.D. Mendelow, G. French, P. Macpherson, E. Teasdale, E. Agnew, G.G. Thompson, M.J. Brodie, Plasma protein-binding and CSF concentrations of valproic acid in man following acute oral dosing, Br. J. Clin. Pharmacol. 16 (1983) 365–369.
[6] R.D. Scheyer, J.A. Cramer, B.R. Toftness, J.M. Hochholzer, R.H. Mattson, In vivo determination of valproate binding constants during sole and multi-drug therapy, Ther. Drug Monit. 12 (1990) 117–123.
[7] G.D. Anderson, B.E. Gidal, R.J. Hendryx, A.B. Awan, N.R. Temkin, A.J. Wilensky, H.R. Winn, Decreased plasma protein binding of valproate in patients with acute head trauma, Br. J. Clin. Pharmacol. 37 (1994) 559–562.
[8] Y. Kodama, H. Kodama, M. Kuranari, K. Tsutsumi, S. Ono, A. Fujimura, No effect of gender or age on binding characteristics of valproic acid to serum proteins in pediatric patients with epilepsy, J. Clin. Pharmacol. 39 (1999) 1070–1076.
[9] A.J.J. Wood, R.E. Vestal, G.R. Wilkinson, R.A. Branch, D.G. Shand,

Effect of aging and cigarette smoking on antipyrine and indocyanine green elimination, Clin. Pharmacol. Ther. 26 (1979) 16–20.
[10] C. March, R.V. Blanke, Determination of free valproic acid concentrations using the amicon micropartition MPS-1 ultrafiltration system, Ther. Drug Monit. 7 (1985) 115–120.
[11] O. Brϕrs, S. Jacobsen, pH lability in serum during equilibrium dialysis, Br. J. Clin. Pharmacol. 20 (1985) 85–88.
[12] L. Wilkinson, SYSTAT: The System for Statistics, SYSTAT Inc, Evanston, IL, 1988.
[13] Y. Kodama, Y. Koike, H. Kimoto, F. Yasunaga, M. Takeyama, I. Teraoka, I. Fujii, Binding parameters of valproic acid to serum protein in healthy adults at steady state, Ther. Drug Monit. 14 (1992) 55–60.
[14] Y. Kodama, K. Tsutsumi, I. Teraoka, I. Fujii, M. Takeyama, Effect of unbound clearance on binding parameters of valproic acid to serum proteins, J. Clin. Pharmacol. 33 (1993) 130–135.
[15] J. Barre, F. Didey, F. Delion, J.P. Tillement, Problems in therapeutic drug monitoring: free drug level monitoring, Ther. Drug Monit. 10 (1988) 133–143.
[16] D.C. McLeod, SI units in drug therapeutics, Drug Intell. Clin. Pharm. 22 (1988) 990–993.
[17] J.A. Cramer, R.H. Mattson, Valproic acid: in vitro plasma protein binding and interaction with phenytoin, Ther. Drug Monit. 1 (1979) 105–116.
[18] I.H. Patel, R. Venkataramanan, R.H. Levy, C.T. Viswanathan, L.M. Ojemann, Diurnal oscillations in plasma protein binding of valproic acid, Epilepsia 23 (1982) 283–290.
[19] L.A. Bauer, R. Davis, A. Wilensky, V. Raisys, R.H. Levy, Valproic acid clearance: unbound fraction and diurnal variation in young and elderly adults, Clin. Pharmacol. Ther. 37 (1985) 697–700.
[20] M.J. Eadie, Formation of active metabolites of anticonvulsant drugs: a review of their pharmacokinetic and therapeutic significance, Clin. Pharmacokinet. 21 (1991) 27–41.
[21] R.G. Dickinson, W.D. Hooper, P.R. Dunstan, M.J. Eadie, Urinary excretion of valproate and some metabolites in chronically treated patients, Ther. Drug Monit. 11 (1989) 127–133.
[22] T. Peters Jr., Serum albumin: recent progress in the understanding of its structure and biosynthesis, Clin. Chem. 23 (1977) 5–12.
[23] S.W.M. Koh, G.E. Means, Characterization of a small apolar anion binding site of human serum albumin, Arch. Biochem. Biophys. 192 (1979) 73–79.
[24] I.Y. Lee, R.H. McMenamy, Location of the medium chain fatty acid site on human serum albumin, J. Biol. Chem. 255 (1980) 6121–6127.
[25] H. Nau, H. Helge, W. Luck, 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. 104 (1984) 627–634.
[26] W.J. Jusko, M. Gretch, Plasma and tissue protein binding of drugs in pharmacokinetics, Drug Metab. Rev. 5 (1976) 43–140.
[27] J.P. Tillement, F. Lhoste, J.F. Giudicelli, Diseases and drug protein binding, Clin. Pharmacokinet. 3 (1978) 144–154.
[28] M.K. Grandison, F.D. Boudinot, Age-related changes in protein binding of drugs: implications for therapy, Clin. Pharmacokinet. 38 (2000) 271–290.