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Short Communication

No effect of co-administered antiepileptic drugs on in-vivo protein binding parameters of valproic acid in patients with epilepsy

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Abstract

Objectives The aim of this study was to establish the population protein binding parameters of valproic acid (VPA) in patients with epilepsy receiving VPA monotherapy and those receiving VPA combined with other antiepileptic drugs.

Methods One hundred and thirty nine data sets from 63 Japanese patients with epilepsy were analysed. These patients were separated into two groups: VPA monotherapy and VPA combined with other binding-sensitive antiepileptic drugs, including phenytoin, clonazepam, clobazam, carbamazepine and phenobarbital (VPA polytherapy). The population protein-binding parameters of VPA were obtained by non-linear least-squares method in each group.

Key findings The mean (95% confidence interval) dissociation constants were 38.9 μM (33.2–44.6 μM) and 36.9 μM (26.7–47.1 μM), and the numbers of binding sites were 1.36 (1.27–1.44) and 1.33 (1.19–1.47) in the monotherapy and polytherapy groups, respectively. No significant differences in the binding parameters of VPA to serum albumin were observed between the two groups.

Conclusions The steady-state serum albumin binding of VPA in Japanese patients with epilepsy is not affected by co-administration of other antiepileptic drugs. These findings suggest that serum VPA concentration is stable at the steady state with regard to interaction by protein binding, even when other antiepileptic drugs with moderate-to-high binding properties are co-administered.

Keywords albumin; binding parameters; co-administration; protein binding; valproic acid

Introduction

Valproic acid (VPA) is a branched-chain carboxylic acid that has been used as an antiepileptic for 40 years. VPA is a broad-spectrum antiseizure medication for the treatment of generalized tonic–clonic seizures, juvenile myoclonic epilepsy and absence seizures.[1,2] The mechanism of VPA action is not fully understood, although blockade of voltage-dependent sodium channels and potentiation of GABAergic transmission are postulated.[3] Therapeutic drug monitoring (TDM) of VPA is essential to prevent toxicities such as hyperammonemia and pancytopenia from high serum concentrations of VPA.[4,5] In particular, serum concentrations of free VPA should be monitored because they are closely related to adverse reactions as well as therapeutic efficacy.[6,7] More than 90% of VPA binds to plasma protein, mainly albumin,[8] and the free fraction of VPA varies considerably in a concentration-dependent manner within the therapeutic concentration range.[9] Several studies have shown that in-vitro protein binding of VPA is affected by other strongly albumin-binding drugs such as ketoconazole, salicylate, tolmetin, ibuprofen, naproxen, mefenamic acid and fenoprofen.[10–13] However, little is known about the in-vivo situation. On the other hand, it is known that the free fraction of antiepileptic drugs *in vivo* is altered by antiepileptic comedication. For example, the free fraction of phenytoin increases significantly by co-administration of valproic acid or carbamazepine in patients.[14,15] However, there are few reports on the effect of antiepileptic comedication on in-vivo protein binding parameters of antiepileptic drugs. We therefore established the population parameters of VPA–serum albumin binding at steady state in two Japanese epilepsy patients groups: those treated with

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VPA monotherapy and those treated with VPA combined with other antiepileptic drugs (VPA polytherapy). We report for the first time that in-vivo VPA–albumin binding at steady state is not affected by co-administration of other antiepileptic drugs with moderate-to-high binding properties.

Methods

Data sets for analysis

We performed a retrospective analysis using 169 serum VPA concentration data sets from 81 Japanese epilepsy patients (one data set from 41 patients, two data sets from 20 patients, three data sets from 7 patients, four data sets from 6 patients, five data sets from 1 patient, six data sets from 4 patients and seven data sets from 2 patients) who were treated with VPA at Oita University Hospital between August 2007 and June 2009. The drug concentrations were collected as part of the routine TDM data. The following clinical data obtained on the same day as TDM were also analysed: gender, age, body weight, co-administered drug(s) and laboratory data, including aspartate transaminase, alanine transaminase, amma-glutamyl transpeptidase (γ-GTP), total bilirubin, serum albumin, serum creatinine and blood urea nitrogen. This research was a retrospective study using data from routine clinical TDM, and was therefore exempted from approval by the institutional review board. However, the data were handled in accordance with the institutional ethical guidelines, with full consideration given to protect the patients' confidentiality.

The patients were divided into two groups: VPA monotherapy and VPA polytherapy (VPA with other binding-sensitive antiepileptic drugs, including phenytoin, clonazepam, clobazam, carbamazepine and phenobarbital).[16–20] In the monotherapy group, patients who were receiving any other drug with the exception of levocarnitine, which is administered to prevent chronic VPA toxicity,[21,22] were excluded. All samples were collected after more than 1 week from the initiation of VPA therapy and 4 weeks from the addition of other antiepileptic drugs.

Quantification of total and free valproic acid

Serum VPA concentration was determined with a fluorescence polarization immunoassay using the TDx® analyser (Abbott Japan, Tokyo, Japan). With this method, the limit of detection was 0.7 μg/ml and the coefficient of variation was less than 5% for routine clinical TDM. In view of the high sensitivity and good reliability, the data were suitable for the present analysis. Serum-free VPA concentration was determined after ultrafiltration using a Nanosep® (Pall Life Sciences, USA). The recovery rate after ultrafiltration by Nanosep® at a VPA concentration of 5.0 μg/ml was 98.8 ± 1.2% (mean ± SD, $n = 4$).

Calculations and statistics

To calculate the binding parameters, we tested two models as candidates of the VPA protein binding model: a single binding site model (equation 1) and a two binding site model (equation 2).

$$r = \frac{n \cdot C_{free}}{K_d + C_{free}} \quad (1)$$

$$r = \frac{n_1 \cdot C_{free}}{K_{d_1} + C_{free}} + \frac{n_2 \cdot C_{free}}{K_{d_2} + C_{free}} \quad (2)$$

where r is the ratio of bound VPA to albumin concentration, n is the number of binding sites, C_{free} is the free VPA concentration and K_d is the dissociation constant. We selected the more appropriate model by comparing the Akaike information criterion (AIC) for the two models, as calculated by curve-fitting for the monotherapy group. The population binding parameters of VPA for each group were obtained by the non-linear least-squares method using a pharmacokinetic analysis program (MULTI).[23]

Differences in patient characteristics between the monotherapy and polytherapy groups were analysed by Student's *t*-test. The relationship between serum albumin concentration and free fraction of VPA for each group was analysed by Spearman's rank-correlation coefficient. Differences in the VPA–serum albumin binding population parameters between the two groups were compared by Welch's *t*-test. A P value less than 0.05 was taken as the minimal degree of statistical significance. Statistical analyses were performed using the SPSS software package (version 16.0; SPSS Inc., IL, USA).

Results

Of 81 patients, 18 patients in the monotherapy group who were receiving other drugs besides levocarnitine were excluded. Eventually, 76 data sets from 39 patients in the monotherapy group and 63 data sets from 24 patients in the polytherapy group were analysed. Table 1 shows the clinical data of the subjects analysed in this study. There were no differences in demographic characteristics between two groups. Among the biochemical data, only serum albumin, creatinine and γ-GTP were significantly different between two groups. VPA dose was significantly higher in the polytherapy than in the monotherapy group. Accordingly, mean total and free VPA concentrations were apparently higher, and the free fraction of VPA was significantly higher in the polytherapy than in the monotherapy group.

Table 2 lists the other drugs co-administered with VPA. In the monotherapy group, most patients did not receive any other drug with the exception of 7 patients (11 data sets) who received levocarnitine. On the other hand, patients in the polytherapy group were treated concomitantly with various drugs other than the antiepileptic agents.

The relationships between serum albumin concentration and free fraction of VPA, and between total and free VPA concentrations in each group are shown in Figures 1 and 2. A significant negative correlation was observed between serum albumin concentration and free fraction of VPA in both groups (Figures 1a and 2a). In addition, free VPA concentration tended to increase non-linearly with increase in total VPA concentration in both groups (Figures 1b and 2b).

Table 1 Description of patient data used in the study

Variable	Monotherapy	Polytherapy
No. of samples	76 (from 39 patients)	63 (from 24 patients)
Males/females	26/13	14/10
Age (year)	11.4 ± 8.2 [0–29]	14.0 ± 10.9 [0–53]
Body weight (kg)	39.5 ± 27.6 (54)[a] [6.9–149]	30.4 ± 19.0 [7.09–77.0]
AST (IU/l)	23.3 ± 7.7 [7.7–56.1]	24.9 ± 12.5 [8.0–64.3]
ALT (IU/l)	16.4 ± 14.9 [3.7–81.7]	17.5 ± 18.2 [4.3–120.2]
γ-GTP (IU/l)	21.2 ± 15.6 (64)[a] [8.7–81.7]	70.9 ± 86.7 (52)[a] [10.8–383.1]†
Total bilirubin (mg/dl)	0.55 ± 0.38 (56)[a] [0.21–2.21]	0.39 ± 0.39 (53)[a] [0.06–2.27]
Serum albumin (g/dl)	4.38 ± 0.43 [3.20–5.48]	3.91 ± 0.62 [2.80–4.95]†
Serum creatinine (mg/dl)	0.44 ± 0.19 [0.14–0.96]	0.33 ± 0.15 (62)[a] [0.12–0.95]†
BUN (mg/dl)	12.9 ± 3.8 [4–22.5]	12.4 ± 5.1 (62)[a] [2.1–26.7]
Dose (mg/day)	504 ± 295 [100–1300]	711 ± 304 [200–1500]†
Total VPA concentration (μg/ml)	71.4 ± 41.7 [3.3–164.1]	81.8 ± 28.4 [15.8–142.8]
Free VPA concentration (μg/ml)	8.02 ± 8.38 [0.1–41.1]	9.97 ± 6.74 [0.9–29]
Free fraction of VPA	0.089 ± 0.049 [0.022–0.259]	0.112 ± 0.043 [0.038–0.207]†

γ-GTP, gamma-glutamyl transpeptidiase; ALT, alanine aminotransaminase; AST, aspartate aminotransaminase; BUN, blood urea nitrogen; VPA, valproic acid.
[a]Number of samples with laboratory test data available. †$P < 0.01$ compared with VPA monotherapy group. Data are mean ± SD; [range].

Table 2 Description of co-administered drugs

Monotherapy ($n = 76$)		Polytherapy ($n = 63$)					
Other comedications	Number of samples	Other binding-sensitive antiepileptic drugs	Number of samples	Other comedications	Number of samples	Other comedications	Number of samples
Levocarnitine chloride	11	Carbamazepine	11	Alprazolam	1	Lansoprazole	1
		Clobazam	14	Ambroxol hydrochloride	1	L-Carbocisteine	6
		Clonazepam	26	Amoxicillin hydrate	1	Levocarnitine chloride	19
		Phenobarbital	12	Ampicillin hydrate	1	Levomepromazine hydrochloride	1
		Phenytoin	3	Azulene	1	Lysozyme hydrochloride	5
				Biperiden hydrochloride	1	Magnesium oxide	10
				Camostat mesilate	1	Mecobalamin	3
				Cefozopran hydrochloride	1	Minocycline hydrochloride	1
				Chlorpromazine hydrochloride	1	Polaprezinc	2
				Clarithromycin	7	Potassium bromide	1
				Daikenchuto	1	Pranlukast hydrate	3
				Dantrolene sodium hydrate	8	Pyridoxal calcium phosphate	2
				Erythromycin stearate	2	Quetiapine fumarate	1
				Ethosuximide	2	Risperidone	1
				Famotidine	10	Sulfamethoxazole, Trimethoprim	2
				Ferric pyrophosphate, Soluble	5	Tizanidine hydrochloride	10
				Fluconazole	1	Tocopherol nicotinate	3
				Haloperidol	1	Triclofos sodium	2
				Hydrocortisone	7	Tulobuterol	6
				Lactic acid bacteriae	1	Ursodeoxycholic acid	1
				Lactitol hydrate	1	Zonisamide	4

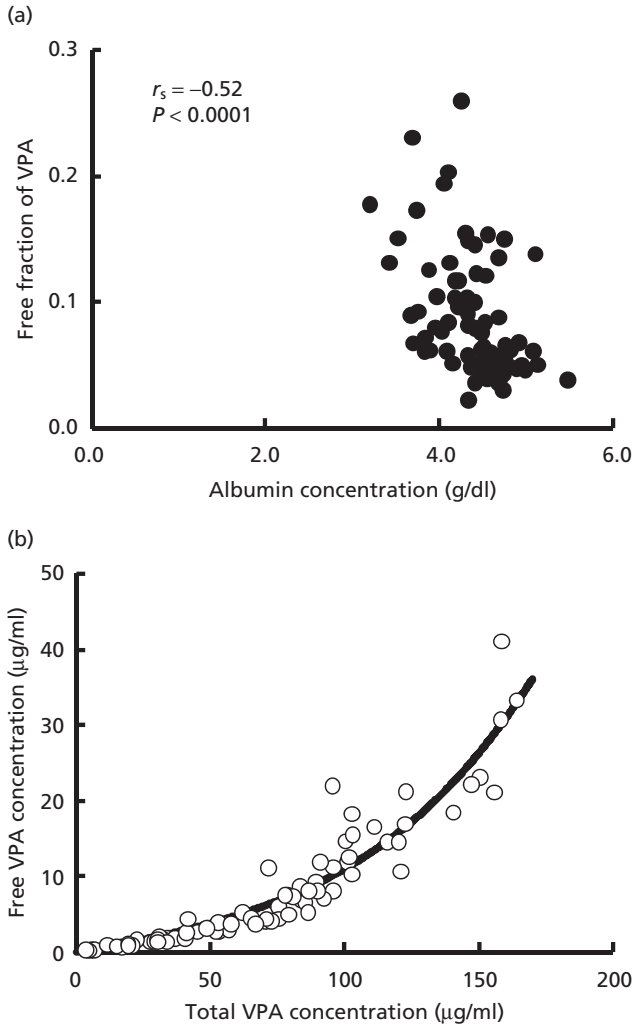

Figure 1 Relationship between serum albumin concentration and free fraction of VPA (a), and between total and free VPA concentration (b) in monotherapy group.

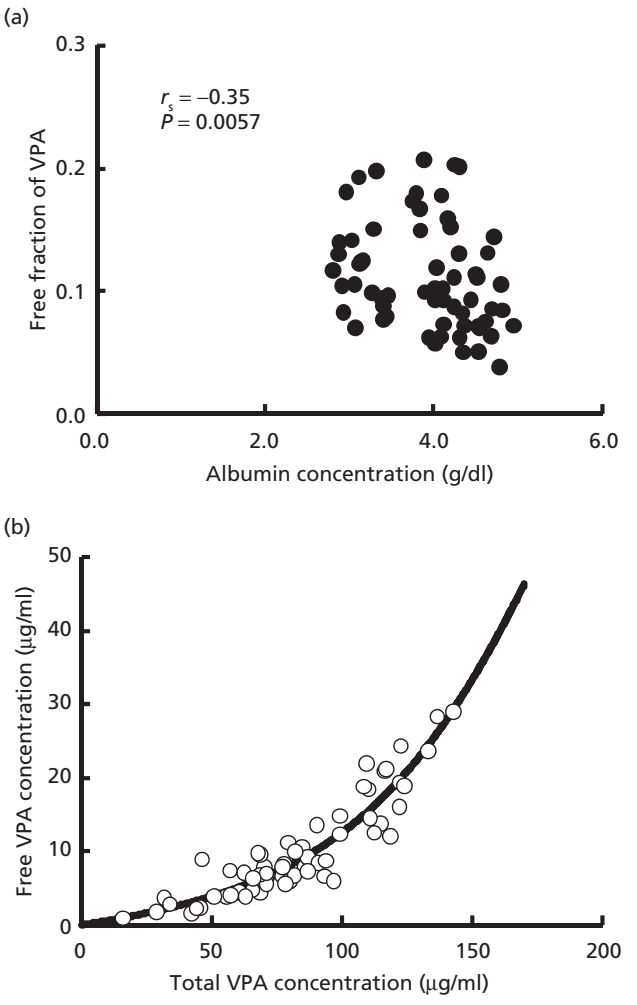

Figure 2 Relationship between serum albumin concentration and free fraction of VPA (a), and between total and free VPA concentration (b) in polytherapy group.

To select the more appropriate model for protein binding of VPA, we compared the AIC of two protein-binding models. The single-binding-site model had a lower AIC (−159.3) than the two-binding-site model (−156.3), therefore the single-binding-site model was more suitable for analysing the protein-binding characteristics of VPA.

Table 3 shows the VPA–serum albumin binding population parameters in the monotherapy and polytherapy groups. The mean (95% confidence interval) dissociation constants were 38.9 μM (33.2–44.6 μM) and 36.9 μM (26.7–47.1 μM), and the numbers of binding sites were 1.36 (1.27–1.44) and 1.33 (1.19–1.47) in the monotherapy and polytherapy groups, respectively. No significant differences in the binding parameters were observed between the two groups. These results indicate that the serum albumin binding of VPA at steady state is not affected by co-administration of the other tested antiepileptic drugs with moderate-to-high binding properties.

Table 3 Binding parameters for valproic acid monotherapy and polytherapy

Parameter	Monotherapy	Polytherapy	*P* value
K_d (μM)	38.9 (33.2–44.6)	36.9 (26.7–47.1)	0.746
n	1.36 (1.27–1.44)	1.33 (1.19–1.47)	0.748

K_d, dissociation constant; *n*, number of binding sites. Data are means (95% confidence interval).

Discussion

In this study, we examined the effect of co-administration of antiepileptic drugs on the steady-state binding parameters of VPA to serum albumin in epilepsy patients. As shown in Table 2, patients in the polytherapy group were treated with a diversity of drugs, including binding-sensitive antiepileptic drugs other than VPA. In contrast, most monotherapy group patients received no concomitant drugs with the exception of 7 patients (11 data sets) who had levocarnitine. Levocarnitine

Table 4 Binding parameters for valproic acid monotherapy and polytherapy with the highly and moderately bound antiepileptic drugs

Parameter	Monotherapy	Polytherapy	
		Highly bound	Moderately bound
K_d (μM)	38.9 (33.2–44.6)	32.7 (20.5–44.8) $P = 0.381$	38.4 (23.4–53.4) $P = 0.954$
n	1.36 (1.27–1.44)	1.22 (1.04–1.39) $P = 0.163$	1.39 (1.18–1.59) $P = 0.802$

K_d, dissociation constant; n, number of binding sites. Data are means (95% confidence interval).
P values are calculated by comparing with VPA monotherapy group.

prevents hyperammonemia and chronic VPA toxicity, and does not bind serum albumin.[24] Since it is unlikely that levocarnitine interferes with the binding of VPA to serum albumin, we included this drug as the only permissive drug in the monotherapy group.

Free VPA fraction decreased significantly as serum albumin concentration increased in both groups (Figures 1a and 2a), and free VPA concentration increased non-linearly depending on the total VPA concentration in both groups (Figures 1b and 2b). This is consistent with previous reports.[25–27] These results suggest that both serum albumin and total VPA concentration have to be considered to predict the protein-binding characteristics of VPA.

We used the Langmuir equation to obtain the binding parameters, the dissociation constant and number of binding sites, which takes into consideration the effects of serum albumin and total VPA concentration. By calculating the population binding parameters of VPA using the non-linear least-squares method, the mean (95% confidence interval) dissociation constants were 38.9 μM (33.2–44.6 μM) and 36.9 μM (26.7–47.1 μM), and the numbers of binding sites were 1.36 (1.27–1.44) and 1.33 (1.19–1.47) for the monotherapy and polytherapy groups, respectively. No significant differences in the VPA–serum albumin binding parameters were observed between the two groups. To examine the effect of the binding property of co-administered drugs on VPA–serum albumin binding parameters, we divided the polytherapy group into a group ($n = 28$) co-administered highly bound drugs, including phenytoin and clonazepam (free fractions of 0.11 and 0.13, respectively,[16,17]), and a group ($n = 35$) co-administered moderately bound drugs, including clobazam, carbamazepine and phenobarbital (free fractions of 0.15, 0.26 and 0.49[18–20]). We observed no significant differences in either group compared to the monotherapy group (Table 4). A recent study has reported that the apparent maximum number of binding sites and the dissociation constant decrease by 18.6% and 46.2%, respectively, when VPA is used with two or three additional antiepileptic agents.[25] However, that study did not consider serum albumin concentration in individual patients. When the effect of serum albumin concentration is taken into consideration, our study reveals that the steady-state VPA–serum albumin binding parameters in epilepsy patients are not affected by co-administration of other antiepileptic drugs. The present results suggest that serum VPA concentration is stable at steady state with regard to interaction by protein binding, even when other antiepileptic drugs with moderate-to-high binding properties are co-administered. However, it should be remembered that co-administration of extensively bound drugs such as ketoconazole, salicylate, tolmetin, ibuprofen, naproxen, mefenamic acid and fenoprofen[10–13] may still affect VPA–serum albumin binding parameters, and that high serum VPA concentration and low serum albumin concentration tend to increase the free fraction of VPA as shown in Figures 1 and 2.

It is important to evaluate the pharmacokinetics of antiepileptic drugs at steady state because epilepsy treatment lasts for a long time. From this aspect, the present results are relevant to the treatment of epilepsy with VPA.

Conclusions

This study demonstrates that the binding parameters of VPA to serum albumin at steady state in Japanese epilepsy patients are not affected by co-administration of other antiepileptic drugs. This finding suggests that serum VPA concentration is stable at steady state with regard to interaction by protein binding even when other antiepileptic drugs with moderate-to-high binding properties are administered concomitantly.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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