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Comparison of Two Binding Equations for Prediction of the Concentration of Unbound Valproic Acid in the Serum of Adult Epileptic Polytherapy Patients

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Abstract

Because binding of valproic acid to plasma proteins affects the efficacy of the drug in the treatment of epilepsy (only the unbound fraction of the drug is effective) we have compared two methods which use different binding parameters to predict the in-vivo concentration of unbound valproic acid in serum.

The study was performed on 46 serum samples from 29 polytherapy adult patients with epilepsy. Mean prediction error, mean absolute prediction error and root mean squared error were calculated for each method; these values served as a measure of prediction bias and precision. The mean absolute prediction errors and root mean squared errors for the two methods were similar in magnitude (Method 1, mean absolute prediction error = 10·0 μM, root mean squared error = 15·0 μM; Method 2, mean absolute prediction error = 10·3 μM, root mean squared error = 13·5 μM). Method 2 had a general tendency to over-predict unbound valproic acid; both methods had a tendency to over-prediction for total concentrations above 500 μM. Method 1 had a tendency to under-prediction at total concentrations below 250 μM. Within the total concentration range of valproic acid investigated, Method 1 was superior to Method 2 for prediction of unbound serum valproic acid.

Our approach using Method 1 may be useful for prediction of unbound serum valproic acid concentration in patients with total valproic acid concentrations ranging from 250 to 500 μM; Method 2 may be useful for patients with total valproic acid below 500 μM. Our results suggest that there is wide and unpredictable variability in valproic acid binding to serum proteins among study populations.

Valproic acid is an alternative antiepileptic drug for absence seizures and is becoming the drug of choice for other types of generalized seizures (Pugh & Garnett 1991).

Valproic acid has characteristic pharmacokinetic properties. It is over 90% bound to plasma proteins, mainly albumin (Zaccara et al 1988) and exhibits concentration-dependent plasma protein binding near or within the usual therapeutic concentration ranges (Ludden 1991). The most important clinical implication of concentration-dependent binding is that it makes the use of total serum concentration misleading for therapeutic drug monitoring, particularly if there is significant inter-subject variability in the apparent binding constants. Diurnal variation of valproic acid binding has also been reported (Riva et al 1983; Bauer et al 1985). It would seem appropriate that the concentration of unbound serum valproic acid, i.e. the fraction that is pharmacologically active, is used to monitor valproic acid efficacy. In some cases, however, the concentration of unbound valproic acid may not be readily obtainable, because of time constraints, cost or assay availability, or a combination of these. Predicting unbound serum valproic acid is, therefore, useful for the clinician requiring accurate assessment of valproic acid efficacy and has the potential to provide cost and time savings.

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In-vivo studies show that valproic acid binds with high affinity to one type of site on the albumin molecule (Yu 1984; Scheyer et al 1990). For a single population of binding sites, the relationship between unbound and total serum valproic acid concentrations can be expressed in terms of the binding parameters for drug–serum protein interaction. Valproic acid is not displaced from albumin binding-sites by phenobarbital, phenytoin or carbamazepine (Patel & Levy 1979) and plasma-protein binding interaction is not observed between valproic acid and other alternative antiepileptic drugs such as ethosuximide, sulthiame and benzodiazepines (Mackichan 1989). Unbound serum valproic acid concentration in adult polytherapy patients might, therefore, be estimated by using in-vivo binding parameters of subjects receiving valproic acid monotherapy.

In a previous study, we determined the in-vivo population binding parameters for binding of valproic acid to serum proteins for a single dose of valproic acid in nine healthy young adults, and defined a binding equation that was derived from the Scatchard equation (Kodama et al 1993). Scheyer et al (1990) also determined in-vivo population binding parameters in patients with seizure receiving valproic acid mono- or polytherapy. In this study, we have compared the performance of binding equations employing the in-vivo population binding parameters of Kodama et al (1993) or Scheyer et al (1990) for prediction of unbound serum valproic acid con-

centration in adult patients with epilepsy receiving valproic acid in combination with other alternative antiepileptic drugs.

Materials and Methods

Subjects

The serum samples used in the study were obtained from 29 adult epilepsy patients (15 males, 14 females; Table 1). The patients' ages ranged from 16 to 67 years (mean ± s.d. = 38·2 ± 16·4). All patients were receiving valproic acid combined with one or more other antiepileptic drugs as their treatment (Table 2) and had normal renal and hepatic functions. Twenty-three patients were also receiving phenobarbital, six received phenytoin, two received ethosuximide, and each received sulthiame, carbamazepine, clonazepam or diazepam. Steady-state had been attained for each drug, and all patients had taken the same dose of drugs for at least 3 months before the study. No patients received other chronic medication. Blood samples were drawn before the morning dose of drugs or at approximately 2 to 4 h after administration of a dose. A total of 46 concentrations was analysed in the study. All serum samples were obtained during routine therapeutic monitoring.

Sample analysis

Serum levels of total and unbound valproic acid were measured by fluorescence polarization immunoassay (TDx; Abbott Laboratories, Chicago, IL, USA). The day-to-day coefficient of variation of total valproic acid assay was 3·7% at 260 μM, 3·2% at 520 μM, and 2·6% at 867 μM. For unbound valproic acid assay, the coefficient of variation was 3·8% at 28 μM, 2·5% at 83 μM, and 2·4% at 139 μM.

Protein binding of valproic acid was evaluated by ultrafiltration with a commercially available MPS-3 device (Amicon, Tokyo, Japan; March & Blanke 1985). Because the changes in pH were determined in frozen and thawed serum (Brørs & Jacobsen 1985), all serum samples were ultrafiltered as soon as possible after collection. Serum (1 mL) was placed in the reservoir and the device was centrifuged at 1000 *g* until 100 μL ultrafiltrate had been collected. Ultrafiltration was performed under the current laboratory routine conditions (25 ± 3°C).

Calculations

Predicted values for unbound serum valproic acid were calculated according to the binding equation (Kodama et al 1993):

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

where Cf is the unbound serum concentration, Ct is the total serum concentration, K is the population mean association constant, and n(Pt) is the population mean total concentration of binding sites. The mean values of in-vivo binding parameters of Kodama et al (1993) (Method 1) or Scheyer et al (1990) (Method 2) were used as population parameters for K and n(Pt) (Table 3). Binding parameters of patients receiving valproic acid monotherapy were used to evaluate the predictive performance of Method 2. In these two studies, the binding of valproic acid to serum proteins was determined by ultrafiltration with an MPS-3 device at 37°C (Kodama et al 1993) or 25°C (Scheyer et al 1990). The predicted and observed unbound serum concentrations were compared for all serum samples.

Statistical analysis

Simple or polynomial regression analysis was performed to determine the relationship between unbound serum valproic acid fraction and total serum valproic acid concentration. Simple regression analysis was performed for the predicted and observed unbound serum valproic acid concentrations. Comparison of predicted and observed unbound serum concentrations was performed by the Wilcoxon signed-rank test for paired data. In each analysis, *P* values less than 0·05 were considered to be statistically significant.

The predictive performance was evaluated by the Sheiner & Beal (1981) method of calculating the mean prediction error, mean absolute prediction error and root mean squared error:

$$\text{Mean prediction error} = 1/n\sum_{i=1}^{n}(\text{predicted Cf} - \text{observed Cf})$$

$$\text{Mean absolute prediction error} = 1/n\sum_{i=1}^{n}|\text{predicted Cf} - \text{observed Cf}|$$

Table 1. Demographic data of the patients.

Variable	Mean ± s.d.	Range
Number of patients	29	
Sex (M/F)	15/14	
Age (years)	38·2 ± 16·4	16–67
Number of serum samples	46	
Serum concentration (μM)*		
Total valproic acid	357 ± 136	123–644
Observed unbound valproic acid	30 ± 14	8–72
Predicted unbound valproic acid		
Method 1†	32 ± 22	7–94
Method 2†	37 ± 19	10–83

*The mass (μg mL^{-1})-to-molar (μM) conversion factor for serum concentration is 6·934. †Data from Kodama et al (1993). ‡Data from Scheyer et al (1990).

Table 2. Antiepileptic medications co-administered with valproic acid during polytherapy.

Medication	Number of patients	Number of serum samples
Phenobarbital	23	40
Phenytoin	6	7
Ethosuximide	2	2
Sulthiame	1	1
Carbamazepine	1	1
Clonazepam	1	1
Diazepam	1	1

Table 3. Population mean binding parameters used in the study.

	Association constant (μM^{-1})	Total concentration of binding sites (μM)
Method 1*	0·0281	757
Method 2†	0·0110	1176

*Data from Kodama et al (1993). †Data from Scheyer et al (1990).

Table 4. Comparison of Scatchard binding equation method.

Method*	n†	Correlation coefficient‡ (r)	Mean prediction error (95% CI) (μM)**	Mean absolute prediction error (95% CI) (μM)**	Root mean squared error (95% CI) (μM)**
1	46	0·758	2·4 (− 2·1 to 6·8)	10·0 (6·7 to 13·3)	15·0 (9·0 to 19·2)
2	46	0·786	6·7 (3·2 to 10·2)	10·3 (7·7 to 12·9)	13·5 (9·7 to 16·4)

*Method 1, data from Kodama et al (1993). Method 2, data from Scheyer et al (1990). †Number of serum samples. ‡Correlation coefficient between observed and predicted unbound valproic acid. 95% confidence intervals (CI) of the mean. **The mass (μg mL^{-1})-to-molar (μM) conversion factor for serum concentration is 6·934.

Root mean squared error

$$= \left(1/n \sum_{i=1}^{n} (\text{predicted Cf} - \text{observed Cf})^2 \right)^{\frac{1}{2}}$$

where n is the number of serum samples. The relative performance was evaluated by comparing 95% confidence intervals. All data analysis was performed using the StatView statistical package (Abacus Concepts, Berkeley, CA, USA).

Results

The patients' demographic data are presented in Table 1. The mass (μg mL^{-1})-to-molar (μM) conversion factor for serum valproic acid concentrations is 6·934 (Mcleod 1988). The mean total concentration in all serum samples was 357 μM (range 123–644 μM). The observed unbound concentration ranged from 8 to 72 μM, mean 30 μM. The population mean binding parameters used in the study are presented in Table 3. The predicted unbound concentrations calculated by use of Method 1 ranged from 7 to 94 μM, with a mean of 32 μM, whereas those for Method 2 ranged from 10 to 83 μM, with a mean of 37 μM.

Comparison of predicted and observed unbound serum concentrations showed no significant difference for Method 1 ($P = 0.6532$). For Method 2, however, there was a significant difference ($P = 0.0004$).

The precision and bias of different prediction methods for all serum samples are summarized in Table 4. Method 2 was significantly biased, showing a tendency to over-predict unbound concentration (i.e., the 95% confidence interval of mean prediction error did not include zero). The mean absolute prediction errors and root mean squared errors for each method were similar in magnitude and overlapped with each other. The correlation between observed and predicted values was significant and slightly higher for Method 2 ($r = 0.786$, $P = 0.0001$) than for Method 1 ($r = 0.758$, $P = 0.0001$).

Table 5 summarizes the number and percentage of predictions for each method that had an absolute prediction error > 7 μM (i.e., 1 μg mL^{-1}) or 14 μM (i.e., 2 μg mL^{-1}). Method 1 had the slightly lower percentage of total errors > 7 μM than Method 2. On the other hand, each method had the same percentage of total errors > 14 μM. The scatter diagrams of unbound concentration prediction error against total serum concentration used in making the prediction for each method are shown in Fig. 1. For each method there was a similar tendency to over-prediction for total concentrations above 500 μM. For Method 1, a tendency to under-prediction was also observed for total concentrations below 250 μM.

Table 5. Number of predictions with absolute prediction errors > 7 μM or > 14 μM (> 1 or 2 μg mL^{-1}) and its percentage ratio to all serum samples.

	Number/ratio (%)			
	Method 1*		Method 2†	
	> 7 μM	> 14 μM	> 7 μM	> 14 μM
Over-prediction	12/26·1	7/15·2	19/41·3	9/19·6
Under-prediction	9/9·6	4/8·7	4/8·7	2/4·3
Total	21/45·7	11/23·9	23/50·0	11/23·9

*Kodama et al (1993). †Scheyer et al (1990).

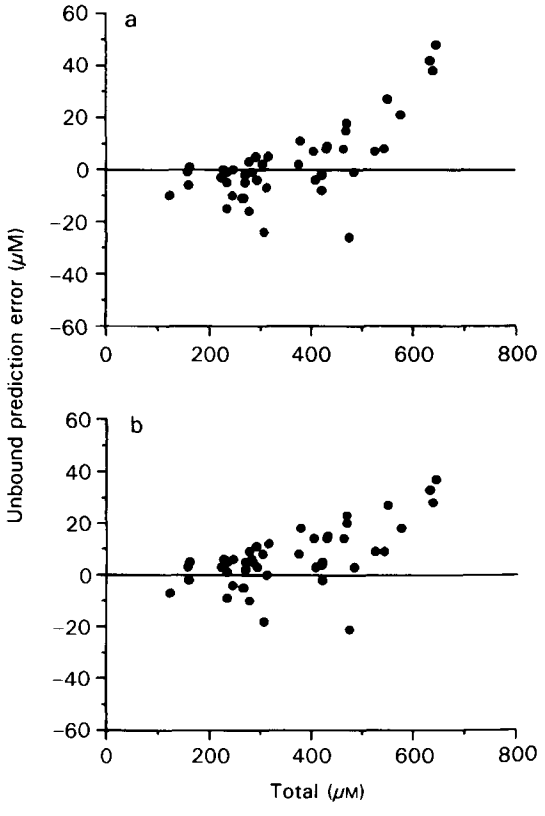

FIG. 1. Scatter diagram of unbound valproic acid prediction error against total valproic acid concentration used in making the prediction for each method. a, Method 1; b, Method 2.

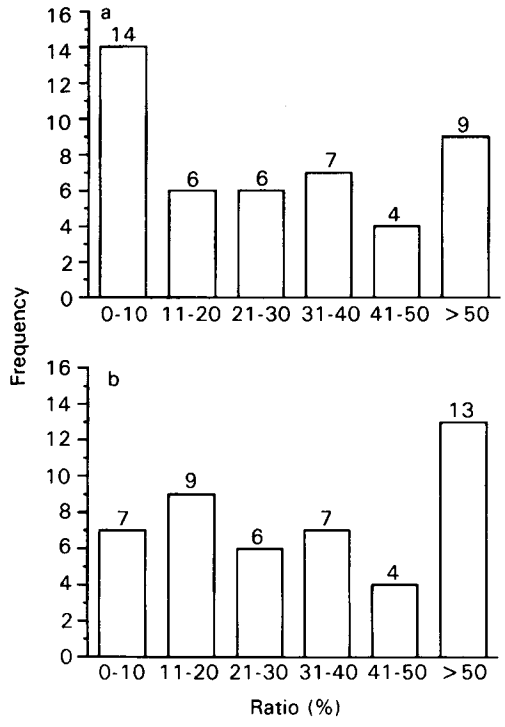

FIG. 2. Frequency of percentage ratio of absolute prediction error/observed unbound valproic acid concentration for each method. a, Method 1; b, Method 2.

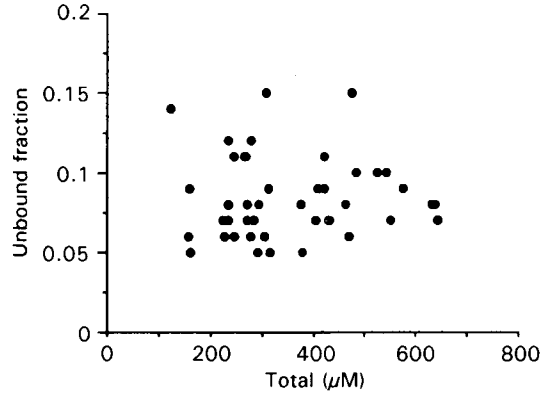

FIG. 3. Relationship between total serum concentration of valproic acid and unbound fraction. Simple regression analysis ($r = 0.004$, $P = 0.9805$); Second-degree polynomial regression analysis ($r = 0.030$, $P = 0.9803$).

The frequency of percentage ratio calculated by dividing absolute prediction errors by the corresponding observed unbound valproic acid for each method is shown in Fig. 2. This demonstrates that for Method 1, the absolute prediction errors were within 10% of the observed unbound concentration for 14 serum samples, within 30% for 26 samples and within 50% for 37 samples; for Method 2, they were within 10% for 7 serum samples, within 30% for 22 samples and within 50% for 33 samples.

The observed unbound serum valproic acid fraction ranged from 0·05 to 0·15 (mean ± s.d. = 0·083 ± 0·025). The scatter diagram of unbound valproic acid fraction against total valproic acid concentration is shown in Fig. 3. The plots of the unbound fraction were evenly distributed among total concentration and consequently, there was no significant relationship between them by simple ($r = 0.004$, $P = 0.9805$) or second-degree ($r = 0.030$, $P = 0.9803$) polynomial regression analysis.

Discussion

Our study indicates that each method with different population mean binding parameters has a characteristic ability to predict levels of unbound serum valproic acid. Method 2 shows a poor predictive performance because of its having a bias when compared with Method 1. The precision of method 1 (root mean squared error = 15·0 μM) is, however, slightly inferior to that of Method 2 (root mean squared error = 13·5 μM) (Table 4). The absolute prediction errors for Method 1 deviated > 50% from the observed unbound valproic acid in 9 of 46 serum samples (19·6%; Fig. 2a), whereas for Method 2, such deviation was observed for 13 samples (28·3%; Fig. 2b). The results also show that on the basis of percentage of over- or under-prediction of unbound valproic acid, Method 1 is more accurate (> 7 μM or < − 7 μM) than Method 2 (Table 5). The mean absolute prediction error is slightly smaller for Method 1 than for Method 2 (Table 4). Within the total concentration range of valproic acid investigated, Method 1 is superior to Method 2 in predictive performance of unbound serum valproic acid.

Binding potential, a parameter reflecting the capacity of the serum proteins for drug-binding-site interaction (Mintun et al 1984), defined as the product of association constant and total concentration of binding sites, is 21·27 for Method 1 and 12·94 for Method 2. Simulations with binding parameters used in each method demonstrate that the relationship between unbound and total serum valproic acid concentrations found using Method 1 is more curvilinear than that obtained by use of Method 2. The differences in binding potential of each method may, therefore, cause large differences in predictive performance of unbound serum valproic acid.

The extent of valproic acid binding to serum proteins is perturbed by a number of endogenous and exogenous factors. Binding of valproic acid to plasma proteins is temperature-dependent (Gugler & Mueller 1978). In this study, the temperature conditions used for ultrafiltration were similar to those of Scheyer et al (1990). The characteristics of the binding isotherm for valproic acid to serum proteins in our patients receiving polytherapy might, therefore, be similar to those of the patient population of Scheyer et al (1990). The results obtained by Method 2 show poor performance at predicting unbound serum valproic acid, however. This suggests that other factors affect valproic acid binding to serum proteins in our patients receiving polytherapy.

For each method, a similar tendency to over-prediction was observed for total concentrations above 500 μM, as is shown in Fig. 1. For Method 1, a tendency to under-prediction was also observed for total concentrations below 250 μM. These findings suggest that the binding characteristics of valproic acid to serum proteins in patients receiving polytherapy are different from those of subjects receiving valproic acid only, who were studied previously (Scheyer et al 1990; Kodama et al 1993). As a consequence, binding equations with a curvilinear relationship between unbound and total serum valproic acid con-

centrations cannot be used for accurate prediction of unbound valproic acid over a wide range of concentrations of total valproic acid. Because our patients were otherwise healthy and not experiencing medical conditions affecting albumin, it appears that the effects of albumin on predictive performance of each method are negligible in our patient population. The characteristic predictive performance of each method may be explained largely by a relationship between unbound serum fraction and total serum concentration of valproic acid in our patient population, as shown in Fig. 3.

Binding of valproic acid to serum proteins is saturable at total concentrations above 560 μM (i.e., 80 μg mL^{-1}; Levy & Lai 1982) and the unbound fraction of the drug increases at higher values of total concentration. Only seven serum samples with total concentrations greater than 500 μM were encountered with this study population, because the majority of patients receiving polytherapy were being administered a relatively low dose of valproic acid. From each of the population mean binding parameters used in Methods 1 and 2, it is predicted that 50% saturation of valproic acid binding to serum proteins would occur at an unbound serum concentration of 36 μM for Method 1 and 91 μM for Method 2. The number of serum samples with unbound serum concentrations higher than 36 μM was relatively small (14) and there were no serum samples with unbound serum concentrations higher than 91 μM. This suggests that in this study, the prediction of unbound serum valproic acid was performed over a relatively low concentration range of binding isotherm when serum bound valproic acid concentration was plotted against unbound serum concentration of valproic acid with different population mean binding parameters used in each prediction method. Fig. 3 illustrates the relatively small variability in unbound valproic acid fraction with given total valproic acid concentration. Simple and second-degree polynomial regression analysis showed no significant relationships between unbound serum fraction and total serum concentration of valproic acid. This suggests that within the total concentration range of valproic acid investigated, the unbound serum fraction of valproic acid can be assumed to be relatively constant. This might be the reason why Methods 1 and 2 both tend to over-predict unbound serum valproic acid concentration for total concentrations above 500 μM and Method 1 has a tendency to under-predict unbound serum valproic acid for total concentrations below 250 μM.

In this study, we have evaluated the ability of binding equations to predict unbound serum valproic acid in adult polytherapy patients whose albumin concentrations were assumed to be normal. Because albumin levels directly influence the binding of valproic acid to plasma proteins (Zaccara et al 1988), our methods may not accurately predict unbound serum valproic acid in patients with hypoalbuminaemia. In some cases, unbound serum valproic acid concentrations may not be readily obtainable because of cost or time restraints or assay availability. Binding equations with a curvilinear relationship between unbound and total serum valproic acid concentrations appear to be available for predicting unbound serum valproic acid within a limited range of total valproic acid. Our approach using Method 1 may be useful for predicting unbound serum valproic acid concentration in patients with total valproic acid concentrations ranging from 250 to 500 μM; Method 2 may be useful for patients with total valproic acid lower than 500 μM.

Unfortunately, it appears that for our patients receiving polytherapy, the two sets of mean binding parameters used in each prediction method are not suitable for predicting unbound serum valproic acid over a wide range of total concentration. This suggests that there is wide and unpredictable variability in valproic acid binding to serum proteins among the study populations. From the results obtained by Method 2, it is predicted that in our patient population, the association constant of valproic acid to serum proteins may be similar to that reported by Scheyer et al (1990) whereas the total concentration of binding sites may be higher than that of Scheyer et al (1990). We consider that the performance at predicting unbound serum valproic acid would be better with the use of binding equations, if suitable binding parameters for the patient population were employed.

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