Empirical Approach for Improved Estimation of Unbound Serum Concentrations of Valproic Acid in Epileptic Infants by Considering Their Physical Development

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The unbound serum concentration of valproic acid (VPA) is closely related to its therapeutic efficacy. In epileptic infants, the unbound VPA concentration varies largely from patient to patient, being difficult to predict using the reported equations for older children. To establish an equation to estimate the unbound concentration in infants, we empirically characterized the relationship between total and unbound VPA concentrations, taking their growth and development into consideration. Data were retrospectively collected from archived clinical records of 30 epileptic infants aged 0—11 months old. The relationship between total and unbound VPA concentrations was analyzed according to the Langmuir equation, in which the patient’s body weight, height, and body surface area were considered as physical development indices. Inter- and intra-individual variabilities in the VPA concentrations were also considered. It was shown that the unbound VPA concentration in infants is properly estimated when their body weights are taken into account, in which the parameter for the maximum binding site concentration (Bm) increases as the body weight increases, while that for the dissociation constant (Kd) is unaltered. Additionally, the relationship was shown to slightly change when the infants are concomitantly treated with VPA and the other antiepileptics. These findings provide useful information to adjust the VPA dosage to achieve optimal therapeutic efficacy in epileptic infants.

Key words valproic acid; infant; body weight; development; protein binding; therapeutic drug monitoring

Valproic acid (VPA) is known to have a broad antiepileptic spectrum for partial and generalized seizures, and it has also been shown to be effective for intractable epilepsy when used at a higher dose than that generally recommended.1,2) With these properties, it is one of the widely prescribed compounds for epilepsy, but it should be noted that an excess increase in the serum VPA concentration inevitably elevates the risk of adverse reactions, such as hepatic dysfunction,3) thrombocytopenia,4) and hyperammonemic encephalopathy.5) Since it has been considered that the unbound serum VPA concentration is closely related to the antiepileptic action of VPA and its adverse effects,6) determination of the unbound VPA concentration via therapeutic drug monitoring (TDM) is essential to optimize the therapeutic efficacy of VPA.7,8) However, the total serum VPA concentration is commonly analyzed instead of its unbound concentration in TDM activities, due to the cost-ineffective and time-consuming procedures necessary for measuring the unbound concentration.

One approach to evaluate the unbound serum VPA concentration without performing such procedures is to estimate it based on its total serum concentration. In fact, there have been several studies on the estimation and/or a regression equation to predict the unbound from the total concentration.9—11) However, as revealed in our previous study, the equation for epileptic children or adult patients is not applicable for epileptic infants aged less than 1 year old. The unbound serum VPA concentrations in infants vary patient by patient more markedly than those in children, and, thereby, their total and unbound VPA concentrations are poorly correlated (Fig. 1).12) This means that the unbound VPA concentration in an epileptic infant needs to be directly measured through TDM activities, but he/she is usually so small that it is difficult to draw a volume of blood large enough for measurement. In conjunction with the fact that the adverse reactions of VPA frequently occur in infants,13,14) it is therefore important to develop an equation to properly estimate the unbound VPA concentration for epileptic infants.

To devise such an equation, we chose an empirical approach involving compensating for the equation in children by identifying a factor affecting the unbound VPA concentration. In addition, the parameter to be used for the compensation should be noninvasive, or some measurable value that can be determined without blood drawing. In this study, we
retrospectively characterized the non-linear relationship between total and unbound VPA concentrations in epileptic infants, taking their physical development into consideration, and a regression equation for infants to estimate the unbound VPA concentration from its total concentration was developed.

MATERIALS AND METHODS

Data Source for Analysis This study was conducted in accordance with the Declaration of Helsinki with the approval of the advisory boards of the Clinical Ethics Committee of Okayama University Hospital for the protection of the dignity and human rights of patients.

The clinical examination records of epileptic infants who were treated with VPA at the Department of Child Neurology, Okayama University Hospital and who were aged 0—11 months were analyzed in this study. These records had been routinely archived via TDM activities in the hospital from April 1, 2003 to July 31, 2009. Following data anonymization, each infant was numbered at random to ensure the privacy of information. According to the physician’s comments on the infant’s condition and the abnormality of biochemical markers (alanine aminotransferase; >100 IU/l, aspartate aminotransferase; >100 IU/l, and serum creatinine concentration; >1.0 mg/dl), records for infants suffering from severe hepatic or renal dysfunction were excluded. As a result, the screened data encompassed 179 records from 30 patients. Their total and unbound serum VPA concentrations were both determined. All infants were administered VPA syrup, or powder preparations (Depakene®, Kyowa Hakko Co., Ltd., Tokyo, Japan) two to three times a day for at least 5 d. Based on the examination records, all blood samples were drawn approximately 3 h after the morning dose. These samples were processed for VPA determination within 24 h. As for the physical development indices of those epileptic infants, their age in months (AGE), height in centimeters (HT), and body weight in kilograms (WT) were also collected from the examination records. Their body surface area in square meters (BSA) and total body water in liters (TBW) were calculated according to the following equations

\[
\text{BSA} = 0.009568 \times \text{WT}^{0.445} \times \text{HT}^{0.453}
\]

(1)

\[
\text{TBW} = \begin{cases} 0.887 \times \text{WT}^{0.93} & \text{(AGE < 3 months old)} \\ 0.0846 \times 0.95^{\text{AGE} - 0.30} & \text{(AGE ≥ 3 months old)} \end{cases}
\]

(2)

where the Boolean parameter F is equal to 1 if the infant is female, otherwise it is set to 0.

The screened data were divided into two datasets (Table 1). The first dataset consisted of data from infants who were solely treated with VPA (monotherapy group). The second dataset comprised data from infants who were treated with VPA in combination with other antiepileptic drugs, such as phenytoin, phenobarbital, primidone, carbamazepine, zonisamide, clonazepam, or clobazam (combination therapy group). In the combination therapy group, the antiepileptic drug used with VPA was often changed one after another in a short period, according to the patient’s condition. It was therefore difficult to properly characterize the effect of each of those concomitantly used antiepileptics on the VPA concentration profile, and such a characterization could not be performed in this study. Additionally, it should be mentioned that there were 8 infants who were treated with monotherapy at first, but then their treatment was switched to combination therapy. In such a case, a part of their data belongs to the first dataset, and the remainder to the second dataset.

VPA Assay According to the examination records, the determination of serum VPA concentrations was performed with blood samples from patients during TDM activities. The blood sample was centrifuged at 3000 rpm for 10 min at an ambient temperature to collect the proper volume of serum specimen for the determination (300 µl). The total serum concentration of VPA was then determined with a fluorescence polarization immunoassay using an AxSYM® analyzer (Abbott Diagnostics, Santa Clara, CA, U.S.A.). To determine the unbound VPA fraction, the serum specimen was separated using an Amicon® YM-30 ultrafiltration membrane (Millipore, Billerica, MA, U.S.A.). After the centrifugation at 3500 rpm for 20 min, the volume of the separated faction was adjusted to be the proper volume for the determination, and the VPA concentration in the fraction was then determined as described above.

Population-Based Analysis of the VPA Concentrations in Infants Treated with VPA Monotherapy With the first dataset described above, the relationship between the total and unbound serum VPA concentrations was analyzed using the non-linear mixed effects modeling program (NONMEM® version V; GloboMax, Ellicott City, MD, U.S.A.).

In this analysis, the Langmuir equation was used to characterize the relationship. That is, the relationship between the total (\(C_T\)) and unbound (\(C_f\)) serum VPA concentrations in the \(i\)th record of the \(i\)th infant is described as follows, taking into account intra-individual variability, \(\varepsilon\):

\[
C_{T_i} = B_m \frac{C_f_i}{K_{di} + C_{f_i}} \exp(\varepsilon_i)
\]

(3)

where \(B_m\) designates the maximum concentration of the VPA binding site on the serum protein in the \(i\)th infant, and \(K_{di}\) designates the dissociation constant between the serum protein and VPA in the \(i\)th infant. Then, these parameters are described with population mean parameters (\(\theta\)) as follows:

\[
B_m = \theta_1 \exp(\eta_{1g}) \quad K_{di} = \theta_2 \exp(\eta_{2g})
\]

(4)

where \(\theta_1\) and \(\theta_2\) are the parameters to be determined in this study. Inter-individual variabilities for \(B_m\) and \(K_{di}\) are described with population mean parameters (\(\vartheta\)) as follows:

\[
\eta_{1g} = \vartheta_1 + \varepsilon_{1g} \quad \eta_{2g} = \vartheta_2 + \varepsilon_{2g}
\]

(5)

As for the physical development indices of those epileptic infants, their age in months (AGE), height in centimeters (HT), and body weight in kilograms (WT) were also collected from the examination records. Their body surface area in square meters (BSA) and total body water in liters (TBW) were calculated according to the following equations:
noted as $\eta_1$ and $\eta_2$, respectively. Hereafter, the combination of Eq. 3 with Eq. 4 is referred to as a ‘basic model.’ If the basic model is found not to be suitable to characterize the relationship between the total and unbound VPA concentrations, we can modify Eq. 4 to prepare another equation set for $Bm_i$ and $Kd_i$ to be used with Eq. 3 instead of Eq. 4. Through repeating this modification process, we tried to identify a suitable equation set to describe the relationship between the total and unbound serum VPA concentrations in epileptic infants. That is, in addition to Eq. 4, we prepared several equation sets for $Bm_i$ and $Kd_i$ based on Eq. 4, in which the physical development indices of the epileptic infants were empirically incorporated. The following are examples:

$$Bm_i = \theta_1 \cdot (1 + AG^e_i) \cdot \exp(\eta_{1i})$$  
$$Kd_i = \theta_2 \cdot \exp(\eta_{2i})$$  

(5)

$$Bm_i = \theta_1 \cdot (1 + \theta_1 \cdot AG^e_i) \cdot \exp(\eta_{1i})$$  
$$Kd_i = \theta_2 \cdot \exp(\eta_{2i})$$  

(6)

where $AG^e_i$ denotes the age of the $i$th infant. Hereafter, the parameters for the $i$th infant are denoted with the subscript $i$. Other equation sets used in this study are listed in Table 2. These equation sets were used with Eq. 3 to characterize the relationship between the total and unbound VPA concentrations. To determine the equation set which, in combination with Eq. 3, most suitably describes the relationship between the total and unbound VPA concentrations, a comparison based on the objective function (Obj) was performed. The Obj is the value calculated using the NONMEM program with the population means of $Bm$ and $Kd$ when the dataset is analyzed with it. Briefly, if the Obj value calculated with Eq. 5 in combination with Eq. 3 is significantly smaller than that calculated with the basic model previously mentioned, we judge that Eq. 5 in combination with Eq. 3 is more suitable to characterize the relationship than the basic model.

### Population-Based Analysis of the Effects of Combination Therapy on the Relationship between the VPA Concentrations

The effects of combination therapy on the relationship between the VPA concentrations were evaluated using a similar method to that described above. Based on the equation set which most suitably described the relationship, several modified equation sets were further prepared, in which the effects of additional antiepileptic drugs are taken into account. The following are examples prepared by modifying Eqs. 5 and 6, respectively:

$$Bm_i = \theta_1 \cdot (1 + \theta_3 \cdot AG^e_i) \cdot \exp(\eta_{1i})$$  
$$Bm_i = \theta_1 \cdot (1 + \theta_3 \cdot \theta_4 \cdot AG^e_i) \cdot \exp(\eta_{1i})$$  

(7)  

(8)

where the Boolean parameter $A$ is equal to 1 if patients are treated with combination therapy, otherwise it is set to 0. These equation sets were used in combination with Eq. 3 to characterize the second dataset. To determine the equation set which most suitably describes the relationship between the VPA concentrations, a comparison based on the Obj value was performed in the same manner as described above.

**Statistics** Data are expressed as the median values with minimum and maximum values, unless otherwise indicated. For multiple comparisons against a single control group, significance was evaluated employing the Kruskal–Wallis test. Significant differences between two Obj values were evaluated using the chi-square test. Significance was set at $p<0.05$.

### RESULTS

**Characterization of the Serum Unbound VPA Concentration Profile in Infants Treated with VPA Monotherapy**

To establish the equation for infants, through which their unbound serum VPA concentration can be suitably estimated from their total serum VPA concentration, we first prepared several equations to evaluate the relationship between the total and unbound VPA concentrations (Table 2). The first dataset was used for this analysis. As shown in Table 2, the Obj values calculated with the models, in which the infant’s physical development is taken into account, are generally smaller than that calculated with the basic model. This means that the physical development must be considered when the relationship between the total and unbound VPA concentrations in infants is characterized. Among the equations involving a single development index, the equation involving the infant’s body weight (Model 80) yields the smallest Obj value. This equation is therefore considered to be the most suitable to characterize the relationships (Table 2). When two development indices are involved in the equation (Models 81—84), the Obj value seems to become even smaller than that calculated with Model 80, but the difference does not reach significance (Table 2). We also tested several equations, in which the development indices are assumed to also influence the $Kd$ value, in addition to the $Bm$ value, but none of these equations resulted in a significant decrease in the Obj value compared to that calculated with Model 80 (data not shown).

**Effects of Combination Therapy on the Relationship between the VPA Concentrations** The effects of combination therapy on the relationship between the VPA concentrations were then examined. This analysis was performed with the consolidated dataset comprising the first and second datasets. The equations used in this analysis were prepared by modifying Model 80, as it yielded the most favorable result in the aforementioned analysis (Tables 2, 3). As shown in Table 3, the Obj value calculated with Model 85 is significantly smaller than that calculated with Model 80, indicating that the relationship between the VPA concentrations is al-

<table>
<thead>
<tr>
<th>Model No.</th>
<th>$Bm$</th>
<th>$Kd$</th>
<th>$\Delta$Obj$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic</td>
<td>$\theta_1 \cdot (1 + AG^e)$</td>
<td>$\theta_2$</td>
<td>0$^b$</td>
</tr>
<tr>
<td>10</td>
<td>$\theta_1 \cdot (1 + HT)^e$</td>
<td>$\theta_2$</td>
<td>-9.35$^c$</td>
</tr>
<tr>
<td>20</td>
<td>$\theta_1 \cdot (1 + WT)^e$</td>
<td>$\theta_2$</td>
<td>-6.32$^c$</td>
</tr>
<tr>
<td>30</td>
<td>$\theta_1 \cdot (1 + TH)^e$</td>
<td>$\theta_2$</td>
<td>-2.64</td>
</tr>
<tr>
<td>40</td>
<td>$\theta_1 \cdot (1 + BSA)^e$</td>
<td>$\theta_2$</td>
<td>-9.40$^c$</td>
</tr>
<tr>
<td>50</td>
<td>$\theta_1 \cdot (1 + TBW)^e$</td>
<td>$\theta_2$</td>
<td>-6.89$^c$</td>
</tr>
<tr>
<td>60</td>
<td>$\theta_1 \cdot (1 + TBE)^e$</td>
<td>$\theta_2$</td>
<td>-4.51</td>
</tr>
<tr>
<td>70</td>
<td>$\theta_1 \cdot (1 + HT)^e$</td>
<td>$\theta_2$</td>
<td>-7.97$^c$</td>
</tr>
<tr>
<td>80</td>
<td>$\theta_1 \cdot (1 + WT)^e$</td>
<td>$\theta_2$</td>
<td>-9.62$^c$</td>
</tr>
<tr>
<td>81</td>
<td>$\theta_1 \cdot (1 + WT) \cdot (1 + TBE)^e$</td>
<td>$\theta_2$</td>
<td>-9.69$^c$</td>
</tr>
<tr>
<td>82</td>
<td>$\theta_1 \cdot (1 + WT) \cdot (1 + TH)^e$</td>
<td>$\theta_2$</td>
<td>-9.62$^c$</td>
</tr>
<tr>
<td>83</td>
<td>$\theta_1 \cdot (1 + WT) \cdot (1 + AG^e)^e$</td>
<td>$\theta_2$</td>
<td>-9.62$^c$</td>
</tr>
<tr>
<td>84</td>
<td>$\theta_1 \cdot (1 + WT) \cdot (1 + HT)^e$</td>
<td>$\theta_2$</td>
<td>-9.62$^c$</td>
</tr>
</tbody>
</table>

$^a$ The difference from the value calculated with the basic model is expressed to two decimal places.  
$^b$ The Obj value calculated with the basic model was 494.13.  
$^c$ $p<0.05$: significantly different from the value calculated with the basic model.
tered when epileptic infants are treated with the combination therapy. Based on this result, Model 85 was judged to be the most suitable to describe the relationship between the VPA concentrations in epileptic infants, taking into account the alteration occurring in the combination therapy. Then, the relationship between the total and unbound VPA concentrations was characterized with Model 85, and the population means of $B_m$ and $K_d$ in epileptic infants were estimated (Table 4, Fig. 2). It was indicated that the parameter $B_m$ increases when the body weight increases, while it slightly decreases when infants are treated with the combination therapy (Table 4). As a result, the relationship profile shifts downward when the infant’s body weight increases, while it shifts upward when infants are treated with the combination therapy (Fig. 2). Lastly, the predicted value calculated with Model 85 was compared with the value calculated using the previously reported equation, indicating that the prediction improves and the variability of the prediction is smaller when the calculation is performed with Model 85 (Fig. 3).

### DISCUSSION

In this study, we retrospectively and empirically characterized the non-linear relationship between the total and unbound VPA concentrations in epileptic infants by considering their physical development. It is known that the serum albumin concentration in infants gradually increases to the mature level according to their physical growth and develop-

### Table 3. Comparison of the Obj Values Calculated with Various Models for the Combination Therapy

<table>
<thead>
<tr>
<th>Model No.</th>
<th>$B_m$</th>
<th>$K_d$</th>
<th>$\Delta$Obj$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>$\theta_1 (1 + \theta_1 \cdot WT)$</td>
<td>$\theta_2$</td>
<td>0$^b$</td>
</tr>
<tr>
<td>85</td>
<td>$\theta_1 (1 + \theta_1 \cdot \theta_1^{eq} \cdot WT)$</td>
<td>$\theta_2$</td>
<td>-6.24$^c$</td>
</tr>
<tr>
<td>86</td>
<td>$\theta_1 (1 + \theta_1 \cdot WT)$</td>
<td>$\theta_2 \cdot \theta_3^{eq}$</td>
<td>-2.54</td>
</tr>
<tr>
<td>87</td>
<td>$\theta_1 (1 + \theta_1 \cdot \theta_1^{eq} \cdot WT)$</td>
<td>$\theta_2 \cdot \theta_3^{eq}$</td>
<td>-5$^d$</td>
</tr>
</tbody>
</table>

$^a$ The difference from the value calculated with Model 80 is expressed to two decimal places. $^b$ The Obj value calculated with Model 80 was 1095.72. $^c$ p<0.05: significantly different from the value calculated with Model 80. $^d$ Calculation of the Obj value was not performed.

### Table 4. Estimates of Population Means for the Parameters in the Equation to Describe the Relationship between the Total and Unbound Serum Concentrations of VPA in Infants

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B_m = \theta_1 (1 + \theta_1 \cdot \theta_1^{eq} \cdot WT)$</td>
<td>57.7</td>
<td>34.6—80.8</td>
</tr>
<tr>
<td>$\theta_2$ (µg/ml)</td>
<td>0.13</td>
<td>0.03—0.23</td>
</tr>
<tr>
<td>$\theta_3$</td>
<td>0.80</td>
<td>0.62—0.98</td>
</tr>
<tr>
<td>$K_d = \theta_1$</td>
<td>6.97</td>
<td>5.04—8.96</td>
</tr>
<tr>
<td>$\eta_1$ (%)</td>
<td>19.7</td>
<td></td>
</tr>
<tr>
<td>$\eta_2$ (%)</td>
<td>16.9</td>
<td></td>
</tr>
<tr>
<td>$\epsilon$ (%)</td>
<td>11.1</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Model 85 was chosen as the best model to characterize the unbound-to-total relationship of VPA (see text). $^b$ $\eta_1$ and $\eta_2$ denote the inter-individual variabilities for $B_m$ and $K_d$, respectively. $\epsilon$ denotes the intra-individual variability.
In addition, albumin concentrations largely vary among infants since the rates of physical development markedly differ among them. These factors may be mainly responsible for the fact that the unbound VPA concentrations in infants are wide-ranging, and that, generally, the measured value is much higher than the predicted value using the total-to-unbound relationship profile of VPA provided for older children (Fig. 1). Using their body weight in the characterization of the total-to-unbound relationship of VPA probably compensates for such individuality in physical development. In fact, as shown in Table 2, with the assumption that the parameter Bm varies according to the infant’s body weight (Model 80), the prediction of the unbound VPA concentrations is markedly improved. This seems to be consistent with the recommendation that the VPA dosage for infant is adjusted according to the body weight to avoid the adverse reactions.\textsuperscript{11}

In addition, the analysis also indicated that the total-to-unbound relationship of VPA is altered when epileptic infants are treated with combination therapy, in which the relationship profile shifts upward as compared to the profile of the infants treated with VPA monotherapy (Table 3, Fig. 2). Therefore, the unbound VPA concentration in epileptic infants is properly estimated from their total VPA concentration on consideration of the altered profile with the combination therapy in conjunction with the aforementioned compensatory measure of the body weight (Table 4, Fig. 3). The primary mechanism underlying the alteration with the combination therapy may be a competition for the same binding site on the serum protein between VPA and antiepileptics. It is plausible that the concomitantly used antiepileptics, such as phenytoin, phenobarbital, and carbamazepine, displace the protein-bound VPA from the binding site. In addition, these antiepileptics are reported to expedite VPA metabolism by inducing drug-metabolizing enzymes. As one of the VPA metabolites, E-\(\Delta^2\)-valproic acid, exhibits a higher binding affinity to the serum protein than VPA, an increased concentration of the metabolite may be involved in altered VPA protein-binding properties by displacing the protein-bound VPA from the binding site.\textsuperscript{23,24}

As shown in Table 4, the parameter \(K_d\) was determined not to be related to the physical development of the infants. Such a property of the parameter \(K_d\) was also observed in various studies previously reported\textsuperscript{10,25} and these results can be interpreted to reflect the fact that the serum albumin concentration in infants increases according to their physical development, while the protein-binding affinity of VPA is little affected by the physical development.\textsuperscript{20,21} However, an additional investigation may be necessary to clearly explain that the protein-binding affinity in infants is not affected by the physical development, since it has been reported for various compounds that the protein-binding affinity to albumin decreases in infants, due to increased plasma concentrations of endogenous substances, including bilirubin and fatty acids.\textsuperscript{21,26,27} It was also indicated that the binding ability of the serum albumin with various drugs is reduced in infants, due to the persistence of a functionally immature serum albumin.\textsuperscript{7,28} As a limitation of this study, the inconsistency on the property of parameter \(K_d\) remains unexplained, and this will be clarified in the future study by confirming the usefulness of the devised equation using another population composed of newly corrected TDM data from epileptic infants.

It should be noted that the drawing of a large volume of blood sufficient to directly determine the unbound serum VPA concentration is rarely allowed if the epileptic patient is an infant. Even if blood drawing from such a patient is allowed, the blood specimen is used not for the determination of the unbound VPA concentration, but for various other clinical examinations. Since the body weight of an epileptic infant can easily be determined in a noninvasive manner, it is clinically applicable to estimate the patient’s unbound VPA concentration based on the routinely determined total VPA concentration, employing the body weight as an index of the physical development as a compensatory factor.

In summary, we examined the relationship between total and unbound serum VPA concentrations in epileptic infants, and developed a regression equation to suitably estimate their unbound VPA concentrations from the total VPA concentration while taking the physical development of the infants into consideration. This may provide useful information to facilitate appropriate VPA treatments for epileptic infants.

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