Factors Influencing the Serum Concentration of Antiepileptic Drugs
—Relationship between the Serum Valproic Acid Concentration and Daily Dose in Epileptic Patients—

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(Received September 24, 1997)
(Accepted July 8, 1998)

The relation between the serum valproic acid (VPA) concentration and the daily dose was studied using the therapeutic drug monitoring data obtained from epileptic patients who were treated with the oral administration of the sustained-release preparations of VPA. The 233 data at steady-state after repetitive dosing were used.

A multiple regression analysis revealed the serum VPA concentration to be dependent on only one variable regarding the daily dose per ideal body weight.

An 1-compartment model including two assumptions that VPA binds to plasma protein and the distribution volume of VPA is proportional to the ideal body weight, was postulated for this analysis. The plasma protein binding ratios were calculated based on the values estimated by the nonlinear least squares method and they were found to be 96.0, 94.7, 92.6 (%) when the serum VPA concentrations were 40, 70, 100 (μg/mL), respectively. These values showed a good correlation with those reported by previous investigators and this model also seemed to demonstrate good confidence level.

Key words — valproic acid, serum concentration, transforming factor of daily dose, influencing factor, protein binding, 1-compartment model

Introduction

Valproic acid (VPA)1,2, which features a wide anticonvulsant spectrum and relatively slight side effects, is widely used for the treatment of epilepsy. As VPA has a short elimination half-life ranging from 8 to 15 hours3, its serum concentration is varied widely in a day. Consequently, VPA has been supposed to have a low correlation between the serum concentration and the daily oral dose4. It is suggested for VPA dose adjustment that the body weight should be employed rather than the serum concentration5. However, a nonlinear relationship between daily dose and serum concentration is found6,7. This indicates that the monitoring of VPA concentration is worthwhile to prevent
side effects and to confirm effectiveness.

In most of papers on the serum VPA concentration (C), the data were obtained from patients who were administrated the conventional preparations of VPA whose absorption rates were relatively rapid7-9 and the plural measurements in one patient were used10. Accordingly, the values of C varied widely by the influence of inter- and intra-individual variability. These papers indicated that C is affected by the factors such as age7 and sex differences10. On the contrary, for pediatric patients, it is indicated that C is well correlated with the dosage regimens based on the body surface area11. That is to say, the reports in a large number of studies are not consistent in the relationship between daily dose and C.

In epileptic therapy, it is important to know the effects of coadministered antiepileptic drugs on C. Therefore, before investigating these effects, the authors studied on the administration of VPA alone and how the value of daily dose should be transformed to relate to C without being affected by other confounding factors such as age and sex differences.

Recently, the sustained-release preparations of VPA (VPA-R) have become available in clinical use. It shows a narrow C range in the oral administration of once or twice a day12,13. Therefore, we used the data obtained from the epileptic patients administrated VPA-R and studied statistically to clarify the factors influencing C. Subsequently, an equation, which expressed satisfactorily the relationship between C and VPA daily dose, was presented.

Method

1. Patients and Data Description

We collected the data from epileptic patients, who were treated with repetitive oral administration of VPA-R alone at both Kagawa Medical University Hospital and Kurashiki Central Hospital from April 1995 to September 1996. VPA-R was Selenica-R granules, Nikken chemicals Co., LTD., Tokyo, Japan, or Depakene-R tablets, Kyowa Hakko Kogyo Co., Tokyo, Japan. VPA-R was administrated to 233 patients. There were no abnormal findings on hepatic and renal functions in all patients. Blood samples were obtained 2 to 3 hours after the last dosing in outpatients and 2 to 15 hours after in inpatients. After centrifuging, the separated serum samples were collected.

When there were several measurements for C in one patient during the investigation period, the average value was used as a representative one. The age, body weight, height, and VPA daily dose were treated similarly. C was measured in duplicate by FPIA method (TDX, FLX system, DAINABOT, Tokyo, Japan) in both hospitals.

2. Data Analysis

Data analysis was performed by utilizing the statistical program packages, NAP (ver. 4)14 and MULTI15,16.

2-1 Investigation of the Factors Influencing the Serum VPA Concentration

For multiple regression analysis, C was assigned as a criterion variable and the transformed variable of daily dose per body weight, daily dose per body surface area or daily dose per ideal body weight as a main-explanatory variable. Also, sex differences (SEX), age (AGE), body weight (W), height (H), VPA daily dose (D) and hospital (HP) were assigned as the sub-explanatory variables. Males and females were denoted by 1 and 2 for SEX, and Kagawa Medical University Hospital and Kurashiki Central Hospital were denoted by 1 and 2 for HP, respectively. The forward selec-
tion method was used to select the variables influencing $C_i$. A level of significance, which prescribed the addition and/or elimination of variable by F-test, was taken as 0.05.

Eq.(1) for body surface area ($A$)\(^{17}\) and eqs.(2) and (3) for ideal body Weight ($W_i$)\(^{18}\) have been reported. These equations are supposed to offer the adequate values of $A$ and $W_i$ for the Japanese.

\begin{align}
A &= 0.007246 \times W (kg)^{0.725} \times H (cm)^{0.725} \\
W_i &= 50 + 2.3 \times (H(cm) - 152.4)/2.54 \quad (152.4 < H : male) \\
W_i &= 45 + 2.3 \times (H(cm) - 152.4)/2.54 \quad (152.4 < H : female)
\end{align}

There are no proper equation proposed from which $W_i$ can be derived for $H \leq 152.4$ cm. In our study, there were only a few patients of both $H \leq 152.4$ cm and $AGE \geq 16$. Thus, we defined a modified body weight ($W_m$) as a substitution for $W_i$. As the value of $W_m$, eqs.(2) and (3) were used for the patients of $AGE \geq 16$ for males and females, respectively, and the actual body weight was used for the patients of $AGE < 16$. Then $D/W_m$ instead of $D/W_i$ was employed for analysis.

2-2. A Model Representing the Serum VPA Concentration at Steady-state

We supposed an 1-compartment model (Fig. 1), where the VPA binding to plasma protein was considered. In this model, the free-VPA concentration is assumed to be constant in the body.

Under the repetitive administration, few errors would be caused by assuming that the daily amount of VPA administrated is equal to that eliminated, and that the daily variation of $C_i$ is negligible.

At steady-state, the mass balance equations for VPA in plasma can be expressed as eqs.(4) and (5).

\begin{align}
D \cdot F &= k_{el} C_i V_d \\
k_i C_i (C_b - C_{bs}) &= k_d C_i
\end{align}

Where, $D$ is the VPA daily dose [mg/day], $F$ is the bioavailability [-], $k_{el}$ is the elimination rate constant [1/day], $C_i$ is the free-VPA concentration in plasma [$\mu g/mL$], $V_d$ is the distribution volume of VPA [L], $k_i$ is the binding rate constant [mL/(µg·sec)], $k_d$ is the dissociation rate constant [1/sec], $C_b$ is the bound-VPA concentration in plasma [$\mu g/mL$] and $C_{bs}$ is $C_b$ in saturation [$\mu g/mL$].

On the other hand, $C_i$ can be assumed to be equal to the plasma VPA concentration and can be
expressed as the sum of \( C_f \) and \( C_b \),

\[
C_v = C_f + C_b
\]  \hspace{1cm} (6)

Eqs. (4), (5) and (6) are the basic equations for VPA disposition.

**Results**

1. **Characteristics of the Patients**

Table 1 shows the characteristics of the patients administrated VPA-R alone in each hospital. As most of the patients in Kagawa Medical University Hospital were under 15 years old, significant differences in \( AGE \), \( W \), and \( H \) were detected between two hospitals. By assembling the data of two hospitals together, we could collect a lot of data from a wide age range.

2. **Factors Influencing the Serum VPA Concentration**

Table 2 shows the results obtained by the stepwise multiple regression analysis when the main-explanatory variable was \( D/W \), \( D/A \) or \( D/W_m \). The standard partial regression coefficients of the selected explanatory variables, namely the factors influencing \( C_v \), the simple correlation coefficient \( (R_s) \) between the main-explanatory variable and \( C_v \), and the multiple correlation coefficient \( (R_m) \) between all selected explanatory variables and \( C_v \) are shown in Table 2.

(1) **In the Case where \( D/W \) is the Main-explanatory Variable**

\( D/W \) was selected as the first factor. Subsequently, \( SEX \) and \( W \) were selected. The values of \( R_m \) and \( R \), were 0.665 and 0.632, respectively. The differences between \( R_m^2 \) and \( R^2 \) indicates the contributions of \( SEX \) and \( W \) to \( C_v \).

As described above, males and females were denoted by 1 and 2, respectively. Consequently, if \( C_v \) was expressed by only one variable of \( D/W \), the values of \( C_v \) would have been higher in females than in males, because the standard partial regression coefficient for \( SEX \) was positive.

\( W \) was considered to be in inverse proportion to \( G \) in the main-explanatory variable as \( D/W \). Nevertheless, \( W \) was selected as the sub-explanatory variable, and its standard partial regression coefficient was positive. This means that the influence of \( W \) to \( C_v \) should be greater than the minus first power. On the other hand, none of \( AGE \), \( H \) and \( HP \) were selected. That is, they are not the influencing factors to \( C_v \). Also, \( D \) was not selected. However, \( D \) was already considered in the main-explanatory variable as \( D/W \). That is, \( C_v \) is proportional to \( D \).

(2) **In the Case where \( D/A \) is the Main-explanatory Variable**

\( D/A \) was selected as the first factor. Subsequently, \( SEX \) and \( H \) were selected. \( A \) is related with \( W \) and \( H \), as expressed in eq. (1). The contributions of \( SEX \) and \( H \) to \( C_v \) can be explained similar to the case where \( D/W \) is the main-explanatory variable.

(3) **In the Case where \( D/W_m \) is the Main-explanatory Variable**

Only the main-explanatory variable, \( D/W_m \), was selected, and no sub-explanatory variables were selected. That is, \( C_v \) can be correlated to only one factor of \( D/W_m \).

From these findings, \( D/W_m \) was reliable to relate with \( C_v \).

3. **A Model Representing the Serum VPA Concentration at Steady-state**

Fig. 2 shows the relationship between \( D/W_m \) and \( C_v \). Though the plots are considerably scattered, it seems that the increment of \( C_v \) decreases gradually with \( D/W_m \). This tendency suggests that the bound VPA to plasma protein increased gradually, because of its high binding property\(^{[9]} \). Then VPA disposition was analyzed with the model shown in Fig. 1.
Table 1. Comparison of Patients Administrated VPA-R Alone in Each Hospital

<table>
<thead>
<tr>
<th></th>
<th>Sum or Mean ± S.D.</th>
<th>Kagawa Medical University Hospital</th>
<th>Kurashiki Central Hospital</th>
<th>t or χ² test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>233</td>
<td>57</td>
<td>176</td>
<td></td>
</tr>
<tr>
<td>SEX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>119</td>
<td>28</td>
<td>91</td>
<td>n.s.</td>
</tr>
<tr>
<td>Female</td>
<td>114</td>
<td>29</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>AGE [years]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 ~ 15</td>
<td>95</td>
<td>45</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>16 ~ 30</td>
<td>47</td>
<td>10</td>
<td>37</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>31 ~ 45</td>
<td>26</td>
<td>1</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>46 ~ 60</td>
<td>24</td>
<td>1</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>61 ~</td>
<td>41</td>
<td>0</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>29.7 ± 23.2</td>
<td>11.8 ± 8.7</td>
<td>35.5 ± 23.5</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Body Weight : W [kg]</td>
<td>46.5 ± 17.6</td>
<td>35.2 ± 17.9</td>
<td>50.1 ± 15.8</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Height : H [cm]</td>
<td>148 ± 22</td>
<td>133 ± 28</td>
<td>153 ± 17</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>VPA Daily Dose : D [mg/day]</td>
<td>615 ± 267</td>
<td>577 ± 329</td>
<td>627 ± 243</td>
<td>n.s.</td>
</tr>
<tr>
<td>Serum VPA Concentration : C₀ [μg/mL]</td>
<td>54.3 ± 22.0</td>
<td>61.3 ± 21.1</td>
<td>52.1 ± 21.9</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D..

Table 2. Standard Partial Regression Coefficients of Selected Explanatory Variables and Correlation Coefficients Calculated by Multiple Regression Analysis

<table>
<thead>
<tr>
<th>Main-Explanatory Variable</th>
<th>D/W : 0.731</th>
<th>D/A : 0.616</th>
<th>D/W₀ : 0.660</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-Explanatory Variable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEX : SEX</td>
<td>0.176</td>
<td>0.135</td>
<td>n.s.</td>
</tr>
<tr>
<td>AGE : AGE [years]</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Body Weight : W [kg]</td>
<td>0.170</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Height : H [cm]</td>
<td>n.s.</td>
<td>-0.172</td>
<td>n.s.</td>
</tr>
<tr>
<td>Hospital : HP</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>VPA Daily Dose : D [mg/day]</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Simple Correlation Coefficient between the Main-Explanatory Variable and C₀ : Rs  

| Multiple Correlation Coefficient between All Selected Explanatory Variables and C₀ : Rm |
|------------------------------------------|------------------------------------------|
|                                         |                                         |

n.s. : not significant

We indicated that C₀ could be correlated to only D/W₀ in the section of “Results 2(3)”. Then, an equation which expressed the relationship between C₀ and D, was derived from eqs.(4), (5) and (6)
Fig. 2. Correlation between VPA Daily Dose per Modified Body Weight ($D/W_M$) and Serum VPA Concentration ($C_i$)

The serum VPA concentration is plotted against the VPA daily dose per modified body weight. The solid curve represents the fits using eq.(9).

in the following procedure.

As $V_e$ would be proportional to $W_M$,

$$V_e = \beta W_M \tag{7}$$

where, $\beta$ is the arbitrary constant [L/kg]. Replacing $F/(\beta \cdot k_0)$ by $\alpha$ and $D/W_M$ by $X$, eqs.(4) and (7) give $C_i$ as

$$C_i = \alpha X \tag{8}$$

where $\alpha$ is the arbitrary constant [day] and $X$ is the VPA daily dose per modified body weight [mg/(kg\cdot day)]. Replacing $k_e/k_0$ by $K_e$ and substituting eqs.(4) and (5) into eq.(6), $C_i$ can be expressed as a function of $X$ as

$$C_i = \alpha X (1 + K_eC_{in} + \alpha K_eX)/(1 + \alpha K_eX) \tag{9}$$

Using 233 data shown in Fig. 2, three parameters, $\alpha$, $K_e$, and $C_{in}$ in eq.(9) were estimated by a nonlinear least squares method. A modified Marquardt method written by Yamaoka\textsuperscript{[5]} was used. The estimated parameter values and their standard deviations were as follows.

$$\alpha = 0.177 \pm 0.859 \quad \text{[day]}$$

$$K_e = 0.212 \pm 0.950 \quad \text{[mL/\mu g]}$$

$$C_{in} = 151 \pm 69.8 \quad \text{[\mu g/mL]} \tag{10}$$

The parameters $\alpha$ and $K_e$ in eq.(9) are inferred to be correlative and the data are considerably scattered, so their standard deviations of the estimated parameter values became very large.

The solid curve in Fig. 2 represents the regression curve, in fair agreement with the plotted points.

To obtain $dC_i/dX$, eq.(9) was differentiated with respect to $X$. Then the tangent line to the curve at $X=0$ was obtained using the estimated parameter values, which is shown in Fig. 2 as line 1; $C_i$.
= 5.84 X.

On the other hand, VPA binding to plasma protein at \( X \to \infty \) would have been saturated. Under this condition, the following first order equation can be derived as

\[
C_a = \alpha X + (1/K_a + C_a)
\]

(11)

where the subscript \( s \) shows saturated situation. Employing the estimated parameter values of eq (10), we obtain \( C_a = 0.177 X + 156 \), which is also shown in Fig. 2 as line 2.

It can be said that \( C \) increases along line 1 in a lower region of \( D/W_M \) and increases along line 2 in a higher region of \( D/W_M \).

**Discussion**

When \( C \) is related with \( D \), \( D \) is often transformed by dividing by a factor (transforming factor) such as \( W \), \( W_I \), or \( A \). However, it is not clarified which transformed variable is reliable to relate with \( C_a \), or whether \( C \) can be explained by only one transformed variable\(^7,10,11\). So, it is suggested that the data should be classified according to \( AGE \) or \( SEX \).

The purpose of this study is to find the transforming factor which correlates \( C \) with \( D \) without being affected by other confounding factors such as \( AGE \) and \( SEX \). By the stepwise multiple regression analysis, \( SEX \) was selected as a factor influencing \( C \) in the relation between \( C \) and \( D/W \), and also between \( C \) and \( D/A \). As \( AGE \) was not selected in both cases, the transforming factor which could exclude \( SEX \) in their relations was examined.

The sex differences in VPA distribution seemed to be caused by \( V_a \). Because of a water-soluble property, the distribution of VPA in the body is restricted to the circulating blood and the rapidly exchangeable extracellular water\(^20\). The ratio of the total body water volume to the actual body weight is higher than in adult males than females at the same age\(^21\). As the sum of the volumes of both tissues would have been proportional to the total body water volume, the differences in the fraction of body water between adult males and females might have been reflected by \( V_a \). This coincides with our result that \( C \) is higher in females than in males when \( C \) is related with \( D/W \) (Table 2).

Since \( W_I \) is indicated to be proportional to the total body water volume\(^{21} \), \( W_I \) would be reliable as the transforming factor to exclude the influence of \( SEX \). As \( AGE \) was widely distributed in our patients, the value of \( W_I \) could not be estimated in some patients. Then, \( W_M \) corresponding to \( W \) was defined.

The multiple regression analysis cleared that \( C \) could be related with \( D/W_M \) without being affected by \( SEX \). In addition, when \( W_M \) was used as the transforming factor, \( W \) and \( H \) did not affect on \( C \) (Table 2). As the fraction of the total body water volume is different with the degree of obesity, which is related with \( W \) and \( H^{10,21} \), the effect of obesity seemed to be excluded by using \( W_I \) as the transforming factor.

As \( C \) could be related with only one variable of \( D/W_M \), the VPA pharmacokinetic behavior was analyzed with this relation, subsequently. The behavior has been analyzed according to an 1-compartment model\(^{22-24} \). In our studies, however, the regression line for \( C \) against \( D/W_M \) seemed not to intersect the origin, that is, the simple 1-compartment model seemed to lead to poor fitting with the data (Fig. 2). The nonlinearity, caused by the saturation kinetics of VPA to plasma albumin, is noted in the relation of \( D \) and \( C \)\(^7,20\). However, no models considering this property have
been proposed. Thus, we analyzed the data using such a model in which the protein binding and the proportion of $V_d$ to $W_m$ were considered (Fig. 1), resulting in a good agreement with data (Fig. 2).

The plasma protein binding ratio of VPA, $C_b/C_t$, was calculated using the estimated parameter values, and compared with the value reported previously. This ratio can be expressed as

$$ (C_b/C_t) \times 100\% = \frac{K_r C_{bs}}{(1 + K_r C_{bs} + \alpha K_r X)} \times 100 $$

(12)

By giving an arbitrary value into $C_t$ in eq.(9), we could obtain the value of $X (X > 0)$. Subsequently, giving this $X$ into eq.(12), we obtained the value of $C_b/C_t$. Employing the estimated values shown in eq.(10), the values of the ratio were calculated as 96.0, 94.7, 92.6($\%$) at $C_t = 40, 70, 100$ ($\mu g/mL$), respectively. These values have a good agreement with those obtained from in vitro studies\(^{26,27}\). Thus, the model shown in Fig. 1 and the estimated parameter values seem to have a good confidence.

These results indicate that the ideal body weight (or the modified body weight) was a useful transforming factor for VPA dosage regimens.

### Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td>years</td>
</tr>
<tr>
<td>$A$</td>
<td>$[m^2]$</td>
</tr>
<tr>
<td>$C_b$</td>
<td>$[\mu g/mL]$</td>
</tr>
<tr>
<td>$C_{bs}$</td>
<td>$[\mu g/mL]$</td>
</tr>
<tr>
<td>$C_t$</td>
<td>$[\mu g/mL]$</td>
</tr>
<tr>
<td>$C_t$</td>
<td>$[\mu g/mL]$</td>
</tr>
<tr>
<td>$D$</td>
<td>$[mg/day]$</td>
</tr>
<tr>
<td>$F$</td>
<td>$[-]$</td>
</tr>
<tr>
<td>$H$</td>
<td>$[cm]$</td>
</tr>
<tr>
<td>$HP$</td>
<td>hospital (Kagawa medical university hospital: 1, Kurashiki central hospital: 2)</td>
</tr>
<tr>
<td>$K_r$</td>
<td>$[mL/\mu g]$</td>
</tr>
<tr>
<td>$k_s$</td>
<td>$[mL/(\mu g \cdot sec)]$</td>
</tr>
<tr>
<td>$k_d$</td>
<td>$[1/sec]$</td>
</tr>
<tr>
<td>$k_{el}$</td>
<td>$[1/day]$</td>
</tr>
<tr>
<td>$R_m$</td>
<td>multiple correlation coefficient between all selected explanatory variables and $C_t$</td>
</tr>
<tr>
<td>$R_s$</td>
<td>simple correlation coefficient between the main-explanatory variable and $C_t$</td>
</tr>
<tr>
<td>$SEX$</td>
<td>sex (male: 1, female: 2)</td>
</tr>
<tr>
<td>$V_d$</td>
<td>$[L]$</td>
</tr>
<tr>
<td>$W$</td>
<td>$[kg]$</td>
</tr>
<tr>
<td>$W_i$</td>
<td>$[kg]$</td>
</tr>
<tr>
<td>$W_m$</td>
<td>$[kg]$</td>
</tr>
<tr>
<td>$X$</td>
<td>$[mg/(kg \cdot day)]$</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>$[day]$</td>
</tr>
</tbody>
</table>
\[ \beta \text{ [L/kg]} \] constant

References

14) S. Aoki, "Tokei program package NAP (ver. 4.0) manual", Igaku Shoin, Tokyo, 1995.