Analysis of the Transport of Valproic Acid into Saliva from Serum

Yoshinari SUZUKI,*b Toshihiko UEMATSU;b Atsuhiro MIZUNOB; Toshiaki NINCHIO;
Kiichiro FUJI,a and Mitsuyoshi NAKASHIMA

Division of Pharmacy,a Departments of Pharmacology, b and Neurosurgery, c Hamamatsu University School of
Medicine, 3600 Handa-cho, Hamamatsu 431-31, Japan. Received August 12, 1993; accepted October 8, 1993

A study was conducted to explore whether a positive correlation between serum and salivary concentrations of the well-known antiepileptic drug, valproic acid (VPA), in epileptic patients could be explained by facilitated diffusion. The total concentration in saliva (C) would be related to the apparent ratio (Rapp 100 C/C) of C to the total concentration in serum (C) as follows: C = A Rapp + B. This equation can be illustrated with microcomputer-simulated figures by assuming a process of facilitated diffusion for the transport of VPA into saliva from blood by the mechanism of monocarboxylic acid absorption through the intestinal brush-border membrane vesicles. The above equation has been proved to be valid when applied to the data reported separately by Gugler and coworkers and by Nitsche and Mascher, who evaluated the pharmacokinetics of VPA. Moreover, we can estimate the serum concentration with the salivary concentration using the above equation.

Keywords valproic acid; saliva; serum; TDM; transport

In the therapeutic drug monitoring of anti-epileptic drugs, we have measured the concentrations of valproic acid (VPA) in blood and saliva.1,2,7 Phenytoin (PHT) and phenobarbital (PB) are generally accepted as drugs that show a good correlation between the concentrations in blood and saliva2 because they are transported into the saliva from blood by the pH distribution law.2 On the other hand, VPA has been claimed to have no correlation between its concentrations in the two body fluids.5,7

A carrier-mediated transport mechanism for monocarboxylic acids in the intestinal brush-border membrane vesicles was elucidated by Tsuji et al. in 1990.6,7 VPA (pK = 4.6) would be almost completely dissociated in blood (pH 7.4), yet it is still detectable in saliva. Therefore, the mechanism of VPA transport into saliva from blood should be similar to its absorption from the small intestine.

The present study showed that the transport of VPA in saliva from blood in human could be explained by facilitated diffusion.

MATERIALS AND METHODS

Chemicals All anti-epileptic drugs used were prescription medicines for patients in the Hamamatsu University Hospital in Japan. Depaken (200 mg of sodium valproate per tablet; Kyowa Hakkou Industry Co., Ltd., Japan), as film coated tablets, was used in this study.

Subjects 21 epileptic outpatients who had been taking VPA for at least one year (sex: 11 males and 10 females; age: 10 to 65 years; body weight: 37 to 76 kg) on a total of 28 occasions in our Hospital volunteered for this study. They had been taking sodium valproate alone or with other antiepileptic drugs such as PHT, PB, carbamazepine (CBZ) and clonazepam (CZP), as described in Table I. We obtained informed consent from each person after a full explanation of the procedures.

Study Procedure Blood samples (5 ml each) were collected at various times after the last intake of anti-epileptic drugs (2.23 ± 2.61 h; mean ± S.D.) from patients. At the time of blood sampling, saliva was collected by the subjects spitting directly into a plastic tube, and salivary pH was measured immediately with a pH meter (Hitachi-Horiba, Japan) equipped with a fine glass electrode (Fuji Kagaku Keisoku Co., Ltd., Japan). Saliva flow was not stimulated. Patients were allowed to brush their teeth prior to saliva collection. Patients had a breakfast at about 6:00—7:00 am and took their VPA tablet(s) after their meal. Saliva was collected over 5 min between noon and 1:00 pm.

Separated serum and saliva were stored at −20 °C until analyzed.

Analytical Method We have established and reported the assay method of anti-epileptic agents in saliva7 as follows: The concentrations of VPA in serum and saliva were measured by a fluorescence polarization immunoassay technique (TDx, Dainabot, Tokyo, Japan). Protein-bound and unbound drugs in serum were separated by ultrafiltration at 2000 × g for 20 min using

\[
\begin{align*}
\text{external solution} & \quad \text{cell membrane} & \quad \text{internal solution} \\
C^o & + & K_1 \quad & X^o & + & K_2 & X^i & \quad + & C^i \\
& & K_1 \quad & P_1 & & K_2 & P_1 & \quad & C^i \\
K_1 = \frac{C[X^o]}{[X^o]} & \quad K_2 = \frac{C[X^i]}{[X^i]} \\
[X^i] = [X^o] + [X^i] & \quad P_1([X^i] - [X^o]) = P_2([X^i] - [X^o])
\end{align*}
\]

Fig. 1. Schematic Representation of the Facilitated Diffusion Model for the Transport of VPA into Saliva from Blood

P_1: rate constant for the transport of the carrier-ligand complex ([X^o] from the outer surface, [X^i] from the inner surface), P_2: rate constant for the movement of the unbound-carrier, C: concentration of ligand (= VPA in this case) in the outer solution. C: concentration of ligand in the inner solution. K = K_1 = K_2: equilibrium constant between carrier and ligand. X: total concentration of carriers.
Table I. Patient Characteristics, Other Details and VPA Concentrations

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (year)</th>
<th>Weight (kg)</th>
<th>VPA Dose&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Others&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Time&lt;sup&gt;a&lt;/sup&gt; (h)</th>
<th>Serum Concentration (µg/ml)</th>
<th>Saliva Concentration (µg/ml)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 x 200 mg</td>
<td>A</td>
<td>5.0</td>
<td>37.56</td>
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</tr>
<tr>
<td>1</td>
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<td>77.21</td>
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<tr>
<td>3</td>
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<td>4.6</td>
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<td>57</td>
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<td>38.84</td>
<td>4.87</td>
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<td>11.54</td>
<td>5.7</td>
<td>61.00</td>
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<tr>
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<td>39</td>
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<td>30.77</td>
<td>5.6</td>
<td>24.20</td>
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<tr>
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<td>67.19</td>
<td>1.08</td>
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<tr>
<td>25</td>
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<td>6.67</td>
<td>3.5</td>
<td>45.87</td>
<td>2.21</td>
</tr>
</tbody>
</table>

<sup>a</sup> Time after last dose.  <sup>b</sup> Dose of sodium valproate (mg/kg/d).  <sup>c</sup> Other epileptic drugs used concurrently (A = PB, E = CZP, AD = PB + CBZ, ACDE = PB + PHT + CBZ + CZP). VPA = number and size of sodium valproate tablets taken.

MPS, a device for the separation of free drugs (Amicon Co., U.S.A.) at room temperature. This ultrafiltration device has been proved not to bind VPA.<sup>8</sup> Each saliva sample was mixed well and centrifuged at 1500 × g for 10 min. The supernatant obtained was diluted with an equal volume of saline to reduce the viscosity. These diluted samples could be assayed with the TDX system in the same way as the serum.

**Facilitated Transport Model for VPA**  A general model to delineate facilitated diffusion can be constructed as shown in Fig. 1 according to Schultz.<sup>9</sup> and Hanano.<sup>10</sup> Where, $J_{in} =$ influx; $J_{out} =$ efflux; $J_0 =$ transport maximum and $K_0 =$ the apparent Michaelis constant, the following equations from Schultz<sup>9</sup> (see Fig. 1) apply.

$$J_{in} = J_0 \cdot \frac{C}{K_0 + C}$$

(1)

where

$$K_0 = \frac{K[2K + (n+1)C]}{K(n+1) + 2nC}$$

(2)

and

$$J_0 = nP[X]_0 \frac{K + C}{K(n+1) + 2nC}$$

(3)

As shown later, we empirically found that the apparent ratio ($R_{app}$) of the concentration of VPA in serum ($C_i$) to that in saliva ($C_s$) was well described by the following Eq. 4.

$$C_i = A \cdot R_{app} + B$$

(4)

where $A$ and $B$ are the constants obtained by the least-squares regression for $C_i$ against $R_{app}$. The apparent ratio ($R_{app}$) value was only approximate, but was nevertheless the same order of magnitude as the true ratio ($100 \cdot C_i/C_s$) in the facilitated diffusion model as shown below in the discussion section.

**RESULTS**

Patient characteristics, together with VPA doses, concomitant medications, VPA concentrations in serum and saliva, etc., are summarized in Table I, including repeated studies in the same patients. The mean ± S.D. percentage of protein-unbound VPA in Table I was 9.39 ± 2.30 µg/ml. The concentrations of protein-unbound VPA in serum were neither equal to nor had any linear relation to those in saliva. When the apparent ratio ($R_{app}$) of the concentration of VPA in saliva to that of the total VPA in serum was calculated, the regression line for this ratio against the concentration of VPA in saliva was linear, with a coefficient of correlation ($r = 0.843$) as shown in Fig. 2 and Table II. Similar $r$-values were obtained using the data of Gugler et al. and of Nitsche and Mascher<sup>5</sup> (Table II).
Fig. 2. Relationships between Salivary ($C_s$) and Serum ($C_i$) Concentration of VPA (I) or the Ratio (II) of $C_s/C_i$

The regression line (straight line) and the 95% confidence intervals (dotted lines) are shown. II, $Y = 0.320X + 0.383, r = 0.843, n = 28$.

TABLE II. Linear Regression between Salivary Concentrations and Ratios ($R_{app}$) of Salivary Concentrations against Serum Concentrations

<table>
<thead>
<tr>
<th>Linear regression ($C_s$ vs. $R_{app}$)</th>
<th>Concentration ($\mu g/ml$)</th>
<th>Range of $R_{app}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>0.320</td>
<td>0.383</td>
</tr>
<tr>
<td>Gugler et al.</td>
<td>0.595</td>
<td>0.153</td>
</tr>
<tr>
<td>Nitsche et al.</td>
<td>0.954</td>
<td>0.009</td>
</tr>
</tbody>
</table>

$C_s$: salivary concentration. $C_i$: serum concentration. $R_{app}$: $100 \cdot C_s/C_i$. $r$: coefficient of correlation.

Fig. 3. Relationships between $Y$ ($C_s/K$) and the Ratio ($T\% = 100 \cdot C_s/C_i$)

Simulations were obtained using Eq. 5 in the Text assuming three cases of a facilitated diffusion mechanism transport–stimulation ($n=5$) unstimulated ($n=1$) and transport inhibition ($n=0.2$) at various influx values ($Z$: $a = 0.04$, $b = 0.06$, $c = 0.08$, $d = 0.10$, $e = 0.12$ and $f = 0.14$) described in Discussion.

DISCUSSION

The percentage of protein-unbound VPA in the serum of anti-epileptic patients has been reported to be about 10%—20%\textsuperscript{11,12} and this percentage appeared to vary because of saturable VPA binding. Values for the ratio of serum ($C_i$) to salivary ($C_s$) concentrations were as high as 12.5%. This amount of VPA transport into saliva from blood in patients could not be explained from results in human erythrocytes in vitro, where VPA is transported by the pH distribution law. These results could be explained if VPA was assumed to penetrate the cell membrane using the monocarboxylic acid transport system, a facilitated diffusion mechanism (Fig. 1) similar to its absorption through the intestinal brush-border membrane vesicles, as reported by Tsuji et al.\textsuperscript{7} who described a bicarbonate exchange system for the transport of monocarboxylic acids like VPA in intestinal brush-border membrane vesicles. We hypothesize that the same system is located in acinar cells, which are the primary source of saliva secretion.

An almost equivalent, but not better, correlation ($r = 0.657$) would be obtained using the unbound concentrations of VPA as the outer concentrations ($C_i^*$) instead of the total concentration. So, we assumed that the rate-determining transport step would be the formation of a carrier ligand complex rather than unbound concentrations of VPA in serum in our model (Fig. 1). When we substitute $X$ for $C_s/K$, $Y$ for $C_i/K$, and $Z$ for influx/$nP_2[X]$ in Eq. 1, $X$ can be expressed as follows (see Appendix):
However this model may not fit perfectly with a complex system consisting of vessel and salivary glands. The hypothetical concentrations of both C₀ and C₁ do not coincide well with the actual serum (Cᵢ) and salivary (Cₛ) concentrations, respectively. But, our consideration is about the same as that of a simple diffusion—the salivary concentrations are fairly easily estimated with the observed serum concentrations according to the pH distribution law, and besides, a model of simple diffusion consists of a single membrane, and saliva is not concentrated further nor diluted in the salivary glands.

The theoretical relationships between Y and T% are shown in Fig. 3, with values for Z from 0.04 to 0.14 (using Eq. 5) simulated with a microcomputer when the following three cases were assumed: n = 5 (transport stimulation), n = 1 (steady state) and n = 0.2 (transport inhibition). A sufficiently linear relation was obtained in all cases of transport (n = 5, 1, 0.2) within 12.5% of the true ratio. A completely linear relationship was obtained in the case of unstimulated transport (n = 1) in Eq. 5. Therefore, X is expressed by a constant value of Z as follows in Eq. 6.

\[
X = \frac{Z}{0.5 - Z}
\]  

In conclusion, the apparent ratio (Rₘₐₓ) of the concentration of VPA in saliva against that in serum was shown to correlate linearly with the VPA concentration in saliva in patients. This phenomenon can be explained by assuming the facilitated transport of VPA by the monocarboxylic transport system previously demonstrated in intestinal brush-border membrane vesicles. Moreover, we can estimate the serum concentration with the salivary concentration by transforming Eq. 4 into Eq. 7, as follows:

\[
Cᵢ = \frac{100 \cdot A \cdot Cₛ}{Cᵢ - B}
\]  

APPENDIX

\[
Jₘ = \frac{Jₘ \cdot C₀}{Kₐ \cdot Cᵢ}
\]

In Eq. 1, the numerator and denominator are divided by K, and C₀/K is substituted for X; giving:

\[
Jₘ = \frac{Jₘ \cdot C₀}{Kₐ + Cᵢ}
\]

\[
Jₘ = \frac{Jₘ \cdot X}{Kₐ + X}
\]

where the constant Kₐ/K in the denominator is transformed as follows using Y (= Cᵢ/K):

\[
Kₐ = \frac{K[2K + (n+1)Cᵢ]}{K(n+1) + 2nCᵢ}
\]

\[
= \frac{2 + \frac{(n+1)Cᵢ}{K}}{(n+1) + \frac{2nCᵢ}{K}}
\]

\[
= \frac{2 + \frac{(n+1)Cᵢ}{K}}{(n+1) + \frac{2nY}{K}}
\]

\[
Jₘ = \frac{nPₗ[Xₗ](K + nCᵢ)}{K(n+1) + 2nCᵢ}
\]

\[
= \frac{nPₗ[Xₗ](1 + nY)}{(n+1) + 2nY}
\]

This may be rearranged to

\[
Jₘ = \frac{1 + nY}{nPₗ[Xₗ]} = \frac{1 + nY}{(n+1) + 2nY}
\]

Eq. 1a is expressed with X, Y, Z (= Jₘ/Xₗ[Xₗ])

\[
Jₘ = \frac{Jₘ \cdot X}{Kₐ + X}
\]

\[
Jₘ = \frac{Jₘ \cdot Z}{nPₗ[Xₗ]} = \frac{Jₘ \cdot Z}{nPₗ[Xₗ]}
\]

The solution of Eq. 1a is found to be

\[
X = \frac{Z \cdot nPₗ[Xₗ]}{Kₐ} = \frac{Jₘ \cdot Z}{nPₗ[Xₗ]}
\]

\[
X = \frac{Z \cdot Kₐ}{nPₗ[Xₗ]}
\]

\[
X = \frac{Z \cdot Kₐ}{nPₗ[Xₗ]}
\]

X can be expressed by using Eqs. 2a and 3a as follows:

\[
X = \frac{Z \cdot 2 + (n+1)Y}{(n+1) + 2nY}
\]

\[
X = \frac{1 + nY}{(n+1) + 2nY}
\]

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REFERENCES