Pharmacokinetic Analysis of Phenytoin and Its Derivatives in Plasma and Brain in Rats

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The derivatives of phenytoin (DPh) were synthesized by the reaction at 3 position of hydantoin ring with valproic acid and valeric acid, producing valproyl DPh (VPDPh) and valeryl DPh (VADPh), respectively. These derivatives showed much higher lipid solubilities than that of DPh. Their distribution and elimination were compared to those of DPh. Additionally, the concentration profiles of the drugs in brain and plasma were analyzed with a modified 2-compartment model. DPh and its derivatives, without hydrolysis to DPh in blood, were found rapidly distributed into brain, although the distribution of derivatives was much less, probably due to the high protein binding capacities. The distribution of DPh and its derivatives into brain regions was similar to that into the cortex cerebri. VPDPh and VADPh were more rapidly eliminated from plasma and brain than DPh, giving smaller mean residence time (MRT) values (0.92 and 0.85 h) and much smaller cortex/plasma concentration ratio than those of DPh. The VPDPh and VADPh concentrations in the cerebrospinal fluid (CSF) were also much lower than that of DPh. The time course of plasma and brain concentrations of DPh and its derivatives after i.v. administration was successfully described by the modified 2-compartment models presented.

Keywords: valproyl phenytoin; valeryl phenytoin; brain distribution; pharmacokinetic analysis; modified 2-compartment model

Phenytoin (DPh, 1), introduced by Merritt and Putnam, is used extensively in treatment of seizure disorders, in addition to compounds recently developed as antiepileptic drugs. Although DPh has strong anticonvulsant activity, improved medication is still needed because 20–30% of patients' seizures are not adequately controlled. In addition, the blood–brain barrier (BBB) is composed of restrictive endothelia with intimate cell connections in the brain capillaries. The capillaries lack fenestrae, have few pinocytic vesicles, and process tight junctions which seal the edges of the endothelial cells. It seems that an anticonvulsant drug such as DPh would be restricted from crossing the BBB since it binds strongly to plasma proteins. The penetration rate of a drug into the brain depends on its degree of ionization in the plasma and its lipid solubility. Highly lipid soluble drugs like thiopental reach the brain almost immediately after administration.

Nakamura et al. synthesized and tested 3-carbethoxy-5,5-diphenylyhdantoin. Although the compound did not improve DPh solubility, it had better anticonvulsant properties. Stella and his colleagues also synthesized water-soluble prodrugs of DPh, which were various amino acids and phosphate esters of 3-(hydroxymethyl)phenytoin, however, these prodrugs have not been evaluated for their anticonvulsant effect.

As mentioned above, the aim of this investigation was to improve distribution of DPh into brain by modification of its physicochemical properties, especially increasing its lipid solubility. For this purpose, the derivatives of DPh were synthesized by the reaction at 3 position of hydantoin ring with the drugs, valproic acid and valeric acid (Fig. 1). Based on the data obtained, the distribution and elimination of DPh and its derivatives after intravenous (i.v.) administration were then pharmacokinetically analyzed using a compartment model.

Materials and Methods

Materials: Phenytoin (JP grade) and valproic acid of reagent grade were obtained from Nacalai Tesque Inc. (Kyoto, Japan) and Tokyo Kasei Kogyo Co. (Tokyo, Japan), respectively. Valeric acid was purchased from Sigma Chemical Co. (St. Louis, MO). Acmecothen, clonazepam and medazepam, internal standards for high performance liquid chromatography (HPLC), were generous gifts of Kowa Co. (Nagoya, Japan), Sumitomo Pharmaceutical Co. (Osaka, Japan) and Shionogi Pharmaceutical Co. (Osaka, Japan), respectively. All other chemicals were of reagent grade. Male Wistar rats weighing 200–250 g were used throughout this experiment. The animals had free access to MF diet (Oriental Yeast Co.) and water during the experiment. On the day before the experiment, the rat jugular vein was cannulated with a silicone tubing.

Synthesis of Derivatives of DPh and Its Derivatives
1) 3(2-Propylpentanoyl)-5,5-diphenylyhdantoin (2, VPDPh): A mixture of valproic acid (1.0 ml, 6.3 mmol) and acetic anhydride (1.8 ml, 18.9 mmol) was heated under reflux for 10 h and evaporated under reduced pressure to give 300 mg of a brown oil. To the residue was added 1 (800 mg, 3.2 mmol), and the mixture was heated under reflux for 48 h. The excess valproic anhydride and valproic acid formed were distilled off in vacuo. The residual brown oil was triturated with n-hexane, and precipitated solid was taken up in ether. The insoluble starting material was filtered, and removal of the solvent gave 2 (182 mg, 15%) as a colorless crystalline, mp 138–140 °C (needles from acetonitrile). IR (Nujo): 3400, 2950, 1790, 1755 cm⁻¹. 1H-NMR (CDCl₃): 8 0.77 (6H, t, J=7.0 Hz), 1.05 (4H, m), 1.20 (2H, m), 1.53 (2H, m), 3.78 (1H, dt, J=7.8, 5.6 Hz), 7.27 (10H, m), 8.80 (1H, br). MS m/z: 379 (M⁺). Anal. Calcd for C₂₅H₂₅N₂O₄•H₂O: C, 72.99; H, 6.92; N, 7.40; Found: C, 72.94; H, 6.90; N, 7.33.
2) 3-Pentanoyl-5,5-diphenylyhdantoin (3, VADPh): A solution of 1 (10 g, 39.6 mmol) in n-valeric anhydride (20 ml, 100 mmol) was heated under reflux for 96 h. The excess anhydride and valeric acid formed were

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removed under reduced pressure. The residual brown oil was triturated with n-hexane, and precipitated solid was taken up in CHCl₃. The insoluble starting material was filtered, and removal of the solvent gave 3 (11 g, 33%) as a colorless crystalline, mp 154–156 °C (needles from carbon tetrachloride). IR (Nujol): 3400, 2920, 1785, 1755 cm⁻¹, 1H-NMR (CDCl₃) δ: 0.84 (3H, t, J = 7.2 Hz), 1.24 (2H, sextet, J = 7.2 Hz), 1.52 (2H, quint, J = 7.2 Hz), 2.95 (2H, t, J = 7.2 Hz), 7.36 (10H, o), 8.8 (1H, t). MS m/z: 336 (M⁺). Anal. Caled for C₂₃H₂₄O₂N₂: C, 71.41; H, 5.99; N, 8.33. Found: C, 71.10; H, 6.04; N, 8.26.

Measurement of Partition Coefficient in n-Octanol/Water System DPH and its derivatives were dissolved in the n-octanol at a concentration of 1 x 10⁻⁴ M. The drug solutions were vigorously shaken for 1h with 1N HCl in glass-stoppered vessels at 20 °C. After centrifugation, drug concentrations in both phases were determined by the HPLC method. The partition coefficients (PC) were calculated according to the following formula:

\[ \log PC = \log(C_o/C_i) \]

where \( C_o \) and \( C_i \) are drug concentration in n-octanol and aqueous phases, respectively.

Intravenous (i.v.) Administration Each drug (20 mg/kg, DPH equivalent) was administered intravenously as a solution of saline-propylene glycol (20:80, v/v). Blood samples were collected at designated time intervals from the canulated jugular vein into heparinized syringes. The plasma was separated immediately by centrifugation and stored frozen until assay.

Analytical Methods for DPH, VPDPH and VADPH in Plasma, Brain and Cerebrospinal Fluid (CSF) Drug concentrations in plasma were determined by the methods of Sato et al.¹³ and Dyken and Ecobichon¹⁴ with slight modifications [acemethacin (10 µg/ml in acetone) as internal standard and methanol-acetone-water-0.025 M phosphate buffer (pH 7.0) (2:3:5:5, v/v) with 0.5 M tetra-n-butylammonium phosphate as the mobile phase were used]. The samples were injected onto a reversed-phase Inertsil ODS C₁₈ column (4.6 x 150 mm, particle size 5 µm, GL Sciences Inc., Tokyo) using a Shimadzu liquid chromatograph (model LC-6A, Kyoto) equipped with an ultraviolet (UV) detector (model SPD-6AD). The minimum detectable concentrations were approximately 1.0 µg/ml for DPH and 1.2 µg/ml for VADPH and VPDPH.

To obtain brain tissue, rats receiving drugs were killed at scheduled time intervals after administration. The brain was rapidly removed and dissected into the following regions: hippocampus, thalamus, cerebellum and cerebral cortex. The weight of the isolated brain tissue was measured and samples were kept frozen until assay. The concentration of drugs in the brain was determined by the method of Chou and Levy¹⁵ with a minor modification. Briefly, the brain (0.6 g) was homogenized with 2 ml of a solution consisting of 0.5 M potassium dihydrogen phosphate, 1 M sodium chloride and 0.71 M sodium fluoride (adjusted to pH 5.0) and 600 µl of internal standard (acemethacin, 1 µg/ml in acetone). The drugs in the homogenate were extracted with 10 ml of chloroform, followed by centrifugation. The organic phase was evaporated to dryness under reduced pressure and the residue, dissolved in 200 µl of mobile phase, was injected onto a reversed-phase Inertsil ODS C₁₈ column. The flow rate was 1 ml/min and detection was at 254 nm. The mobile phases for drug determination were: methanol-acetone-water-0.025 M phosphate buffer (pH 7.0) (2:3:5, v/v) containing 0.5 M tetra-n-butylammonium phosphate for DPH and VADPH, and methanol-acetone-water-0.025 M phosphate buffer (pH 7.0) (2:3:5, v/v) for VPDPH. The limit of detection was about 0.2 µg/g for DPH and VADPH and 0.5 µg/g for VPDPH.

CSF was taken by cisternal puncture with the sharp end of a 25-gauge needle connected to about 1.1 cm length of silicone tubing.¹⁶ The determination for DPH was performed by the method described above. For VADPH and VPDPH, a 10 µl of internal standard (clonazepam and medazepam, respectively, 5 µg/ml in acetone) was added to 35 µl of CSF and the mixture was injected onto Inertsil ODS C₁₈ column. The mobile phases were: methanol-acetone-water-0.025 M phosphate buffer (pH 7.0) (1:4:5, v/v) for VADPH and acetone-water-0.025 M phosphate buffer (pH 7.0) (3:2:5, v/v) for VPDPH. The limit of detection was about 0.3 µg/ml for the parent drug, 0.09 µg/ml for VADPH and 0.03 µg/ml for VPDPH.

Analysis of Data Pharmacokinetic parameters were computed using the nonlinear least squares regression program, MULTI.¹⁶ The plasma concentration-time data after i.v. administration were fitted to the equation:

\[ C_t = A \cdot e^{-\lambda t} + B \cdot e^{-\beta t} \]

where \( C_t \) is the drug concentration at time \( t \), and \( A, \lambda, B \) and \( \beta \) are the biequational parameter constants. The pharmacokinetic parameters were calculated from the usual equations.¹⁷

The area under the plasma concentration–time curve (AUC) up to the last sampling point was calculated by the trapezoidal method, and the AUC beyond the last observed plasma concentration (C) was extrapolated according to \( t \cdot C \). The area under the first moment curve (AUMC) and the mean residence time (MRT) were calculated by means of the moment analysis¹⁸:

\[ AUMC = \int_0^\infty t \cdot C \, dt \]

\[ MRT = \frac{\text{AUMC}}{\text{AUC}} \]

The means of all data are presented with their standard deviation (mean ± S.D.). Statistical analysis was performed using the non-paired Student’s t-test, and the significance level adopted was \( p < 0.05 \).

Results

Partition Coefficient The PC of derivatives synthesized was determined in comparison with that of DPH. The log PC for VADPH and VPDPH was 2.75 and 3.28, respectively, these values being significantly higher than that (2.35) of DPH. Therefore, it seems that the synthesized derivatives had higher lipopolarity than DPH.

Plasma Concentration of DPH and Its Derivatives after Single i.v. Administration The plasma concentration–time curves for DPH, VADPH, and VPDPH after a single i.v. administration are shown in Fig. 2. The plasma decay curves for DPH and VPDPH showed clear biequponential kinetics, however, the pharmacokinetic behavior of VADPH was not clear on the distribution between the 1- or 2-compartment. The pharmacokinetics of VADPH were evaluated based on Akaike’s information of criterion (AIC). The AIC values calculated were 34.6 and 24.1 for the 1- and 2-compartment models, respectively. Accordingly, the 2-compartment model was applied to the kinetics of VADPH.

Two groups that have slow (\( \beta = 0.42 ± 0.06 \) h⁻¹) and fast (\( \beta = 0.85 ± 0.09 \) h⁻¹) elimination of DPH after i.v. administration were observed in the rats used in this experiment (in Table I, \( \beta \) is expressed as the mean value), this being in approximate agreement with the data of Colburn and Gibaldi.¹⁸ The derivatives, VADPH and VPDPH, were more rapidly eliminated from plasma than DPH, giving small MRT values (0.85 and 0.92 h) compared to that.

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Fig. 2. Time Course of Concentrations of DPH and Its Derivatives in Plasma after Intravenous Administration

○ DPH, □ VADPH, □ VPDPH. Each value represents the mean ± S.D. of 4–6 rats. Solid lines are fitting curves. Dose: 20 mg/kg (DPH equivalent).
I. Since the pharmacokinetic parameters of DPH were the mean values for all individuals showing slow and fast elimination, relatively large S.D. values were observed for some parameters. VADPH and VPDPH gave high plasma concentrations at the initial time stage after i.v. administration, resulting in relatively large AUC values.

**Brain and CSF Concentrations of DPH and Its Derivatives after Single i.v. Administration** The time curves of DPH, VADPH and VPDPH in the cortex cerebri and CSF after i.v. administration are depicted in Fig. 3. DPH concentration in the cortex was the highest 45 min after dosing. Consequently, the brain concentration of DPH was in disequilibrium with the plasma concentration during the distribution phase, although the equilibrium was observed at the late time stage. The cortex/plasma concentration ratio (\(K_p\)) of DPH was about 1 and the value was significantly higher than that (0.056 and 0.009) of VADPH and VPDPH. These results also suggest a low uptake of the derivatives into brain, in contrast to our expectations.

The CSF concentration of DPH was much lower than the plasma and cortex concentrations and was not detected 3 h after dosing. On the other hand, the CSF concentrations of VADPH and VPDPH were significantly lower than that of DPH, suggesting a poor distribution of the derivatives into CSF. The CSF concentrations of all drugs were generally in equilibrium with the plasma concentrations during the time period measured. VADPH and VPDPH in the cortex and CSF as shown in the insert in Fig. 3b were cleared relatively rapidly, in accordance with the plasma concentrations.

### Table 1. Pharmacokinetic Parameters of DPH and Its Derivatives after Single Intravenous Administration

<table>
<thead>
<tr>
<th></th>
<th>DPH</th>
<th>VADPH</th>
<th>VPDPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (pg/ml)</td>
<td>38.7 ± 24.4</td>
<td>69.9 ± 5.1</td>
<td>162.3 ± 64.7</td>
</tr>
<tr>
<td>(\kappa) (h⁻¹)</td>
<td>14.9 ± 7.9</td>
<td>12.2 ± 1.8</td>
<td>28.5 ± 7.9</td>
</tr>
<tr>
<td>B (pg/ml)</td>
<td>17.8 ± 2.5</td>
<td>115.7 ± 3.5</td>
<td>66.2 ± 9.9</td>
</tr>
<tr>
<td>(k_{12}(\text{h}^{-1}))</td>
<td>0.60 ± 0.26</td>
<td>1.08 ± 0.08</td>
<td>1.32 ± 0.21</td>
</tr>
<tr>
<td>(t_{1/2,b}(\text{h}))</td>
<td>1.3 ± 0.45</td>
<td>0.64 ± 0.05</td>
<td>0.54 ± 0.08</td>
</tr>
<tr>
<td>(k_{12}(\text{h}^{-1}))</td>
<td>8.9 ± 6.0</td>
<td>4.0 ± 1.0</td>
<td>16.2 ± 5.4</td>
</tr>
<tr>
<td>(k_{21}(\text{h}^{-1}))</td>
<td>4.9 ± 1.3</td>
<td>8.0 ± 1.2</td>
<td>9.5 ± 2.7</td>
</tr>
<tr>
<td>(V_f(\text{l/kg}))</td>
<td>1.8 ± 1.3</td>
<td>1.7 ± 0.2</td>
<td>4.0 ± 1.1</td>
</tr>
<tr>
<td>(V_f(\text{l/kg}))</td>
<td>0.43 ± 0.18</td>
<td>0.14 ± 0.01</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>(V_f(\text{l/kg}))</td>
<td>0.55 ± 0.23</td>
<td>0.07 ± 0.03</td>
<td>0.23 ± 0.04</td>
</tr>
<tr>
<td>(AUC(\text{pg.h/ml}))</td>
<td>34.4 ± 9.9</td>
<td>111.2 ± 10.0</td>
<td>56.0 ± 4.7</td>
</tr>
<tr>
<td>(MRT(\text{h}))</td>
<td>1.8 ± 0.6</td>
<td>0.88 ± 0.06</td>
<td>0.92 ± 0.03</td>
</tr>
<tr>
<td>(C_{\text{mav}}(\text{h}))</td>
<td>0.64 ± 0.20</td>
<td>0.24 ± 0.02</td>
<td>0.54 ± 0.05</td>
</tr>
<tr>
<td>(V_{\text{mav}}(\text{l/kg}))</td>
<td>1.07 ± 0.18</td>
<td>0.21 ± 0.03</td>
<td>0.49 ± 0.04</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 4–6 rats. The drug plasma concentration data were fitted to a 2-compartment model. a) Calculated by \(k_{12}, V_f/k_{12}\). b) Calculated by \(C_{\text{mav}}=\text{dose}/\text{AUC}\). c) Calculated by \(V_{\text{mav}}=\text{dose}/\text{AUC} \cdot \text{C}\). d) \(p<0.05\), e) \(p<0.01\), f) \(p<0.001\), compared to DPH.

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**Fig. 3.** Time Course of Concentrations of DPH (a) and Its Derivatives (b) in Brain and CSF after Single Intravenous Administration

Circular, triangular and square symbols represent DPH, VADPH and VPDPH, respectively. Open and closed symbols represent brain and CSF concentrations, respectively. Each point represents the mean ± S.D. of 4–6 rats. When the S.D. was small, it was included in the symbol. Solid lines are fitting curves.

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**Fig. 4.** Uptake of DPH (a), VADPH (b) and VPDPH (c) by Various Brain Regions after Intravenous Administration

clearances.

The distributions of DPH and its derivatives into other brain regions were also measured after i.v. administrations. The results are shown in Fig. 4. The concentrations of DPH and its derivatives in the cerebellum, hippocampus and thalamus were similar to the relative cortex concentrations, indicating that there were no significant changes in the transport of the drugs in the brain regions.

**Pharmacokinetic Analysis of DPH and Its Derivatives in Brain and CSF Distributions Using the Model**

The pharmacokinetics of DPH is nonlinear and analyzed by the 2-compartment open model with Michaelis-Menten kinetics in man. In the dose (20 mg/kg) used in our study, since the elimination of the drugs followed first-order kinetics, linear kinetics was used to describe the concentration profiles. The brain and tissue distributions of DPH were pharmacokinetically analyzed with a modified 2-compartment model including the brain and CSF compartments as shown in Fig. 5a; this model being the same as that reported by Yata and his colleagues for study of caffeine pharmacokinetics.

The mutual transfer of DPH between the brain and CSF was neglected in the model, since the concentration of DPH in the CSF was fairly small compared to the concentration in the brain and it was thought that the transfer of DPH to CSF would mainly depend on the high plasma concentrations.

The differential equations employed for the calculation were as follows:

\[
\begin{align*}
\frac{dX_1}{dt} &= k_{21} \cdot X_2 + k_{31} \cdot X_3 + k_{41} \cdot X_4 - (k_{10} + k_{12} + k_{13} + k_{14}) \cdot X_1 \\
\frac{dX_2}{dt} &= k_{12} \cdot X_1 - k_{21} \cdot X_2 \\
\frac{dX_3}{dt} &= k_{13} \cdot X_1 - k_{31} \cdot X_3 \\
\frac{dX_4}{dt} &= k_{14} \cdot X_1 - k_{41} \cdot X_4
\end{align*}
\]

where \(X_1, X_2, X_3\) and \(X_4\) denote the amount of the drugs in the central, peripheral, brain and CSF compartments, respectively. When \(C_1\) and \(V_1\) were defined as drug concentration and the distribution volume in the central compartment, respectively, \(X_1\) at time 0 is the dose (\(X_0 = C_1 \times V_1\)). The transfer of DPH (i.e., \(k_{13}, k_{31}, k_{14}\) and \(k_{41}\)) from plasma to the brain and CSF and vice versa were assumed not to affect the plasma concentration of DPH, since the amount of DPH uptake by the central nervous system was fairly small compared to the amount in plasma. When the initial conditions are \(X_1 = X_0\) and \(X_2 = X_3 = X_4 = 0\), these equations may be solved to give:

\[
\begin{align*}
X_1 &= \frac{X_0 \cdot (k_{31} - \alpha)}{V_1 \cdot (\alpha - \beta)} e^{-\alpha t} + \frac{X_0 \cdot (k_{21} - \beta)}{V_1 \cdot (\alpha - \beta)} e^{-\beta t} \\
X_2 &= k_{12} \cdot X_1 \cdot (k_{31} - \alpha) e^{-\alpha t} + \frac{X_0 \cdot k_{13} \cdot (k_{21} - \beta)}{(\alpha - \beta)(k_{31} - \alpha)} e^{-\beta t} \\
X_3 &= \frac{X_0 \cdot k_{13} \cdot (k_{21} - \beta) \cdot (\alpha - \beta)(k_{31} - \alpha)}{(\alpha - k_{31})(\beta - k_{13})} e^{-\beta t} \\
X_4 &= \frac{X_0 \cdot k_{14} \cdot (k_{21} - \beta) \cdot (\alpha - \beta)(k_{31} - \alpha)}{(\alpha - k_{41})(\beta - k_{14})} e^{-\beta t}
\end{align*}
\]

where \(\alpha\) and \(\beta\) are hybrid first-order rate constants, \(k_{12}\) and \(k_{21}\) are transfer rate constants between the central and tissue compartments, \(k_{13}\) and \(k_{31}\) are transfer rate constants between the central and brain compartments, \(k_{14}\) and \(k_{41}\) are transfer rate constants between the central and CSF compartments, \(V_3\) is the mean weight of brain and \(V_4\) is the mean volume of CSF.

For the analysis of derivatives, the CSF compartment was disregarded in the model as shown in Fig. 5a, since the CSF concentrations of the derivatives were significantly lower than that of DPH and were not detected over 1 h after dosing. Figure 5b shows this compartment model used for the analysis. Similarly, the mutual transfer of DPH between plasma and the brain was assumed not to affect the plasma concentration of the derivatives. The differential equations for the calculation were Eqs. 8, 2 and 3, and Eqs. 5 and 6.

\[
\frac{dX_i}{dt} = k_{21} \cdot X_2 + k_{31} \cdot X_3 - (k_{10} + k_{12} + k_{13} + k_{14}) \cdot X_1
\]

In these analyses, the main disposition parameters (i.e., \(\alpha, \beta, k_{21}, k_{12}\) and \(V_1\)) of DPH were fixed at the mean values obtained after i.v. administration. Also, the transfer rate constants between plasma and brain (\(k_{13}\) and \(k_{31}\)) and plasma and CSF (\(k_{14}\) and \(k_{41}\)) were estimated by curve fitting.

The fitting curves, analyzed using a computer, are shown in Fig. 3 with the observed drug concentrations in brain and CSF. Curves calculated were in good agreement with the measured concentrations for DPH and its derivatives. These findings indicate the validity of this pharmacokinetic model and the analysis technique. The pharmacokinetic parameters obtained by simultaneous fitting are shown in Table II. The larger values of \(k_{13}\) for VADPH and VPDPH suggest the apparently low uptake by brain and the rapid elimination from brain of the derivatives.
TABLE II. Pharmacokinetic Parameters of DPH and Its Derivatives Calculated Following Single Intravenous Administration

<table>
<thead>
<tr>
<th>Estimate</th>
<th>DPH</th>
<th>VADPH</th>
<th>VPDPH</th>
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<tbody>
<tr>
<td>$k_{12}$ (h⁻¹)</td>
<td>$12.9 \pm 1.9$</td>
<td>$9.4 \pm 3.6$</td>
<td>$14.3 \pm 3.2$</td>
</tr>
<tr>
<td>$k_{21}$ (h⁻¹)</td>
<td>$12.4 \pm 0.2$</td>
<td>$17.4 \pm 7.5$</td>
<td>$23.4 \pm 3.5$</td>
</tr>
<tr>
<td>$V_r$ (ml)</td>
<td>$0.50$</td>
<td>$0.50$</td>
<td>$0.50$</td>
</tr>
<tr>
<td>$k_{14}$ (h⁻¹)</td>
<td>$0.80 \pm 2.5$</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$AUC_{brain}$ (µg·h/g)</td>
<td>$43.4$</td>
<td>$53$</td>
<td>$6.6$</td>
</tr>
</tbody>
</table>

Parameters were obtained by the simulation of mean data of each time course. 

Discussion

The brain is the target organ of anticonvulsants such as DPH, so that its clinical efficacy cannot be attained without penetration of the BBB. Factors affecting the BBB penetration of drugs are lipid–water partition coefficient, protein binding and $pK_a$, with lipophilicity thought to be the most important factor. In this study, the lipophilic derivatives of DPH were synthesized and their BBB penetration was compared with that of DPH. Additionally, compartmental model analysis using of simultaneous curve fitting was carried out to ascertain the pharmacokinetic relationship between DPH and its derivatives in plasma, brain and CSF after i.v. administration.

The derivatives were found to have much higher lipophilicity than DPH. This is due to the N-acetylation at 3 position of the hydantoin ring. Rapid distribution of the derivatives and DPH into the brain regions after their i.v. administration was observed. However, the brain concentrations of the derivatives, VADPH and VPDPH, were much less than that of DPH in spite of the high lipophilicity, suggesting that their distribution into the brain is inferior to that of DPH, probably due to the high plasma protein binding capacity (98.7 and 99.2% for VADPH and VPDPH, respectively). Lin and Lin have reported that plasma protein binding of benzodiazepines in rats significantly decreased the uptake by the brain but to a lesser extent than that predicted from the unbound drug fraction in vitro. Since the concentrations of VADPH and VPDPH in the four examined brain regions showed no significant difference, it is suggested that there is no difference in drug transport in these regions, and that the even distribution can be attributed to their similar rates of distribution into and elimination from the brain. The uptake of lipophilic drugs by the brain is generally assumed to be by simple diffusion so that the drugs used in this study would be transferred to the brain by this means.

The CSF is virtually protein free and, in the case of DPH, is equivalent to an ultrafiltrate of plasma under steady-state conditions. Thus, the CSF appears to reflect much better than plasma the concentrations of DPH and its derivatives at the site of action. The CSF concentrations of VPDPH and VADPH, however, were about one-tenth and one-fifty of the brain concentrations, respectively. This findings also indicated the poor distribution of the derivatives into the site of action, brain, and their high protein binding capacities.

The relatively rapid elimination of the derivatives from plasma and brain, in spite of the high plasma protein binding capacity, cannot be fully explained at this time. Nonetheless, this result is interesting in view of the report that rats showing relatively high plasma protein binding of DPH eliminated the drug much more rapidly than those with relatively low plasma protein binding of DPH, in which the presence of an endogenous inhibitor is assumed responsible for the decreased plasma protein binding and reduced metabolic clearance.

There has been no report of the pharmacokinetic analysis of DPH concentrations in brain using the linear compartment model, although the pharmacokinetics of plasma DPH in patients and of amitriptyline and nortriptyline in serum and brain have been investigated. To further characterize the process of distribution and elimination of DPH and its derivatives, VADPH and VPDPH, we have described here pharmacokinetic models including brain and CSF compartments or brain compartment and applied the models to the experimental data. The time course of plasma and brain concentrations of DPH and its derivatives after i.v. administration were successfully described by these models (Fig. 5), so that the validity of these modified 2-compartment models was fully proven by the agreement with the observed data. Pharmacokinetic analyses of the drugs after oral administration are currently being evaluated and will be presented in the next paper. Further study on their physicochemical properties and pharmacological effects is under way.

In conclusion, DPH, VADPH and VPDPH were rapidly distributed into the brain regions after their i.v. administration. However, VADPH and VPDPH, eliminated rapidly from plasma, distributed into brain and CSF much less than DPH, resulting in a small cortex/plasma concentration ratio. The distributions of the derivatives without hydrolysis to DPH into brain regions were similar to that into the cortex cerebri. The proposed compartment models were adequately able to describe the time course of plasma, brain and CSF levels of DPH and its derivatives following i.v. administration.

References and Notes


25) The result will be reported in a subsequent paper.


