PERCUTANEOUS ABSORPTION OF VALPROIC ACID AND ITS PLASMA CONCENTRATION AFTER APPLICATION OF OINTMENT

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In order to develop a percutaneous (p.c.) dosage form of valproic acid (VPA), ointments containing VPA and both VPA and its calcium salt (VPA-Ca) were prepared. The p.c. absorption was studied by determining the plasma concentrations of VPA in rabbits following application of either ointment. In addition, the in vitro penetration study with excised full-thickness rabbit skin was done to evaluate the pharmaceutical availability. VPA was readily and rapidly absorbed through the skin from the gel ointment. The plasma concentrations of VPA higher than 30 µg/ml were maintained for 6 h after application of 5% VPA - 5% VPA-Ca gel ointment, and for 4 h after application of 5% VPA ointment. The bioavailability of VPA following dosing of both ointments was above 97%. The in vitro penetration rate through the skin and skin/solvent partition coefficient were significantly lower with 5% VPA - 5% VPA-Ca solution than with 5% VPA, suggesting the slower penetration of VPA from the 5% VPA - 5% VPA-Ca. A small difference was observed in the release rate of drugs from these ointments.

The present results demonstrated the pharmaceutical effectiveness of the VPA - VPA-Ca ointment for seizure disorders.

Keywords — valproic acid; calcium valproate; percutaneous absorption; ointment; rabbit; release experiment; skin penetration; plasma concentration

INTRODUCTION

Valproic acid (VPA) is a broad spectrum anticonvulsant, which has been proved useful, especially in the treatment of primary generalized epilepsies, and is frequently administered orally against various types of seizures. VPA is rapidly absorbed when given orally, peak serum concentration being attained 1–2 h after administration of a conventional tablet and 3–4 h after an enteric coated tablet. Because of the relatively short half-life of VPA, the currently available formulations such as oral tablets have to be administered 2–3 times daily. In clinical therapy for seizure disorders, especially child epilepsy, VPA is used extensively. Thus, it is of increasing interest to develop a convenient and effective formulation which is applied to patients easily and to improve compliance. A transdermal system seems to be advantageous in carrying out an effective dosage regimen for epileptic patients. Percutaneous dosage form, as a rule, is convenient for application and is expected to exert a prolonged effect compared with conventional dosage forms.

We therefore attempted to develope percutaneous ointments containing VPA and both VPA and its calcium salt (VPA-Ca) aimed at the systemic delivery of an antiepileptic drug to maintain the drug blood levels in a therapeutic range. The pharmaceutical availability of the ointments in comparison with ointments of VPA alone was evaluated, using rabbits. In addition, some in vitro parameters, release rate of VPA and VPA-Ca from the vehicle and their penetration parameters through the skin were determined.

MATERIALS AND METHOD

Materials — 1) Reagent: VPA and cyclohexanecarboxylic acid, an internal standard for gas-liquid chromatography, were purchased from Tokyo Chemical Industry Co., Ltd. Sodium salt of VPA (VPA-Na) was a generous gift of Kyowa Hakko Industry Co., Ltd. Hiviswako 104 (gel base of ointment) and Cellophane Tubing-Seamless (36/32 inch) were obtained from Wako Pure Chemical Industry Co., Ltd. All other chemicals used were of reagent grade.

2) Animals: Male Japanese white rabbits, weighing 2.5 to 3.5 kg, were used. The animals had free access to RC4 diet (Oriental Yeast Co.,
and water before the experiment.

Preparation of VPA-Ca — 2.7 g of NaOH was dissolved in a small amount of water (about 3 ml) and 10 g of VPA was gradually added to the solution with cooling. The 45 ml of 30% calcium acetate solution (30% more than stoichiometric amount) was added to the above mixture. This solution was heated to 80 °C and the precipitate formed was immediately filtered with heating. The residue was dissolved in a small amount of water with cooling and the solution was again heated to 80 °C, followed by filtration as mentioned above. The same operation was repeated two more times and the Ca-salt obtained was dried in vacuum.

Preparation of Ointments — VPA was dissolved in dimethylsulfoxide (DMSO) or diethylene glycol monoethyl ether (carbitol) and a mixture of VPA and VPA-Ca was dissolved in carbitol. The solution was separately mixed with a gel base (Hiviswako 104) containing water, disopropyl adipate and diisopropanolamine. Details of the ointment composition are listed in Table I.

Intravenous (i.v.) Administration — VPA-Na was administered intravenously in a 60 mg/kg (VPA equivalent) dose as a saline solution. After the administration, blood samples were collected periodically for 12 h.

Percutaneous (p.c.) Absorption Experiment — The hair of the back area of rabbits was carefully removed with an electric clipper to prevent damage to the stratum corneum 24 h prior to application of the ointment. Three g of a given ointment was uniformly spread over the shaved back skin (5.0 cm × 5.0 cm area, designated by attaching an adhesive tape with a cut-out area) and immediately occluded with a sheet of aluminum foil and adhesive tape. The ointment remained in contact with the skin for 36 h. Blood samples were collected periodically for 36 h after dosing. The plasma was separated immediately by centrifugation and stored frozen until assay.

In Vitro p.c. Penetration Experiment — The in vitro p.c. penetration measurements were made using a Franz diffusion cell with a 1.0 cm i.d. O-ring flange as described by Chien et al. The rabbits were shaved as mentioned above and the animals were sacrificed by i.v. injection of chloroform. Pieces of full-thickness back skin, fat trimmed away, were freshly excised from the rabbits and mounted individually on a diffusion assembly. After soaking the dermal site of the skin in buffer solution (0.9% NaCl-10 mM phosphate buffer, pH 7.4) for 12 h at 37 °C, 0.5 ml of 5% VPA (in DMSO 15.0 g, propylene glycol 15.0 g and ethanol 12.0 g) and 5% VPA. 5% VPA-Ca (VPA equivalent, in carbitol 15.0 g and propylene glycol 10.0 g) were individually applied to the stratum corneum surface and the top of the cell was closed with a glass ball. Aliquots (0.1 ml) of the receptor fluid were withdrawn periodically from the side-arm for 30 h. The average thickness of the skin preparations, measured with a micrometer, was 1.902 ± 0.253 mm (n = 6).

In Vitro Release Experiment — The ointment (2.0 g) packed into the cellophane tubing was suspended in 0.9% NaCl-10 mM phosphate buffer (50 ml) at 37 °C, with stirring at 150 rpm. Aliquots (0.1 ml) of the solution were removed periodically for 6 h. The in vitro release rate (mg/ml·h1/2) of VPA from the ointment was calculated from the slope of the straight line obtained.

Determination of VPA — VPA concentrations in samples were determined by the method of Moolenaar et al. with slight modifications. To 0.1 ml of plasma sample or the solution, 0.1

<table>
<thead>
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<th>Table I. Formula of VPA and VPA-Ca Ointments</th>
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<td>Rp.</td>
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<tr>
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</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>3</td>
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</table>

a) Content as VPA. b) Propylene glycol. c) Dimethylsulfoxide. d) Diethylene glycol monoethyl ether. e) Hiviswako 104. f) Diisopropanolamine. g) Disopropyl adipate. The remainder was water.
ml of 3 N sulfuric acid and 0.2 ml of internal standard (cyclohexanecarboxylic acid 150 μg/ml in chloroform) were added and VPA was extracted with 2.0 ml of chloroform. After centrifugation the chloroform layer was concentrated in vacuum. The concentrated sample was injected into a gas-liquid chromatograph (Shimadzu, model GC-4MC, with a hydrogen flame ionization detector, 10% diethyleneglycol succinate with 1% H₃PO₄ on Uniport WHP 100—120 mesh, 3 mm × 2 m). The detector and column temperatures were 200 and 140 °C, respectively. The flow-rates of nitrogen, hydrogen and air were 60, 30 and 150 ml/min, respectively.

**Analysis of Data** — Kinetic parameters were calculated by using the least-squares fit program, MULT1, by a personal computer (PC-9801VM2, NEC). The area under the plasma concentration-time curve (AUC) was determined by the trapezoidal rule. The absolute bioavailability was calculated by the following equation:

\[
\text{Bioavailability (\%) = } \frac{AUC_{\text{p.c.}} \cdot \text{Dose}_{\text{i.v.}}}{AUC_{\text{i.v.}} \cdot \text{Dose}_{\text{p.c.}}} \times 100
\]

where \(AUC_{\text{p.c.}}\) and \(AUC_{\text{i.v.}}\) are AUC after p.c. and i.v. administrations, respectively.

The in vitro penetration parameters were calculated from the penetration data by using the following equations:\(^{11}\)

\[
\tau = \frac{\delta^2}{6D}
\]

\[
J_s = \frac{(K_m \cdot D \cdot C_s)}{\delta} = K_p \cdot C_s
\]

where \(J_s\) is the penetration rate, \(K_m\) is the skin/solution partition coefficient, \(D\) is the diffusion constant within the skin, \(\tau\) is the lag time, \(\delta\) is the thickness of skin and \(C_s\) is the drug concentra-

![Graph showing plasma concentration of VPA after Intravenous Administration of VPA-Na](image)

**FIG. 1.** Plasma Concentration of VPA after Intravenous Administration of VPA-Na

Each point represents the mean observed value with S.D. Dose, 60 mg/kg (VPA equivalent).

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 a p-value of 0.05 or less was considered to be significant.

**RESULTS**

**I. v. Administration**

The plasma concentrations after i.v. administration of VPA-Na (60 mg/kg, VPA equivalent) are shown in Fig. 1. The plasma levels declined in an apparently biexponential manner but the presence of the slower elimination phase was doubtful because the plasma levels were very low 7 to 9 h after dosing and were not detected at 10 h. Additionally, the decline is described as monophasic in human subjects\(^{5}\) and dogs.\(^{12}\) Therefore, the data were analyzed according to the one-compartment open model. Pharmacokinetic parameters calculated by using the model are listed in Table II. The elimination rate constant, \(k_e 0.954 \pm 0.016 \text{ h}^{-1}\), indicated that the elimination of drug was relatively rapid.

**In Vivo p.c. Absorption Studies**

The plasma VPA concentrations after the topical application of VPA and VPA · VPA-Ca ointments are shown in Fig. 2. The kinetic

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (Mean ± S.D.)</th>
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<tr>
<td>(k_e) (h⁻¹)</td>
<td>0.954 ± 0.016</td>
</tr>
<tr>
<td>(t_{1/2}) (h)</td>
<td>0.726 ± 0.013</td>
</tr>
<tr>
<td>(V_d) (1. kg⁻¹)</td>
<td>0.192 ± 0.014</td>
</tr>
<tr>
<td>(AUC) (μg · h/ml)</td>
<td>353.1 ± 38.3</td>
</tr>
<tr>
<td><strong>Dose, 60 mg/kg (VPA equivalent). Each value represents the mean ± S.D.</strong></td>
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parameters obtained are listed in Table III. After application of formulation 1 (Rp. 1, 5% VPA-DMSO ointment), the rapid elevation of plasma VPA concentration was observed at the initial time stage and consequently the peak plasma concentration ($C_{\text{max}}$) occurred 2 h after application of the ointment. On the other hand, the VPA penetration through the skin from Rp. 2 (5% VPA - 5% VPA-Ca ointment) was slower than that from Rp. 1, and the $C_{\text{max}}$ (41.31 ± 10.98 µg/ml) was attained at 4 h after dosing. The VPA absorption from Rp. 3 (5% VPA-carbitol ointment) was also rapid, but the $C_{\text{max}}$ was considerably lower than those of Rp. 1 and 2 and the low bioavailability (about 60%) of Rp. 3 being obtained.

Plasma VPA concentrations higher than 30 µg/ml were maintained for 4 and 6 h after application of Rp. 1 and 2, respectively. The bioavailability (> 97%) after dosing of Rp. 1 and 2 ointments was almost the same as that after i.v. administration. The elimination of VPA from plasma after ointment application was slow compared with that after i.v. administration, probably due to the continuous release into the systemic circulation in skin.

In Vitro p.c. Penetration Studies

The in vitro penetration experiment was performed by using VPA or VPA - VPA-Ca solution corresponding to Rp. 1 and 2. The penetration profiles of 5% VPA and 5% VPA - 5% VPA-Ca solutions through the skin are shown as a function of time in Fig. 3 and the penetration parameters calculated are shown in Table IV. The

<table>
<thead>
<tr>
<th>Rp.</th>
<th>VPA (mg)</th>
<th>$C_{\text{max}}$ (µg/ml)</th>
<th>$AUC$ (µg · h/ml)</th>
<th>Bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>150</td>
<td>61.69 ± 15.01</td>
<td>355.9 ± 74.1</td>
<td>102.5 ± 4.3</td>
</tr>
<tr>
<td>2</td>
<td>300</td>
<td>41.31 ± 10.98</td>
<td>532.8 ± 50.1</td>
<td>97.3 ± 2.6</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>20.55 ± 3.10</td>
<td>160.6 ± 20.7</td>
<td>60.8 ± 6.5</td>
</tr>
</tbody>
</table>

(a) Applied ointment 3.0 g/25 cm². (b) VPA equivalent. (c) $p < 0.05$ compared with Rp. 1.

**FIG. 2. Plasma Concentration of VPA after Application of VPA and VPA-Ca Ointments**

The applied dose of each ointment was 3.0 g/25 cm². Each point represents the mean ± S.D. ●, Rp. 1; ○, Rp. 2; △, Rp. 3.

**FIG. 3. Penetration Profiles of VPA through Rabbit Skin**

The points are expressed as the mean total amount of drug penetrated into receptor solution. Each point represents mean ± S.D. ●, 5% VPA solution; ○, 5% VPA - 5% VPA-Ca solution.
TABLE IV.  Percutaneous Penetration Parameters of VPA through Rabbit Skin

<table>
<thead>
<tr>
<th>Rp.</th>
<th>Lag time (h)</th>
<th>$J_s$ (mg/h · cm²)</th>
<th>$K_p$ (10⁻³ cm/h)</th>
<th>$K_m$ (10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.90 ± 1.45</td>
<td>0.107 ± 0.025</td>
<td>2.14 ± 0.51</td>
<td>46.1 ± 13.5</td>
</tr>
<tr>
<td>2</td>
<td>7.71 ± 1.06</td>
<td>0.017 ± 0.005 a)</td>
<td>0.167 ± 0.053 a)</td>
<td>4.17 ± 1.74 a)</td>
</tr>
</tbody>
</table>

*Each value represents the mean ± S.D. a) p < 0.01 compared with Rp. 1.*

penetration rate, $J_s$, of VPA in the 5% solution was dramatically rapid, while the $J_s$ value in 5% VPA · 5% VPA-Ca solution was much smaller.

Although there was no statistically significant difference in lag times between two solutions, the $K_m$ for VPA in 5% VPA solution was significantly higher than that in 5% VPA · 5% VPA-Ca solution ($p < 0.01$). These results might explain the rapid absorption of VPA after *in vivo* dosing of 5% VPA ointment.

**In Vitro Release of VPA from Ointment**

The *in vitro* release profiles of VPA from ointments are shown in Fig. 4. When the data for the amount of VPA released was plotted *versus* $t^{1/2}$, where $t$ is the time, the resulting curve approximated a straight line.

The release rates for Rp. 1 and 2 were 0.585 ± 0.044 and 0.781 ± 0.071 mg/ml · h$^{1/2}$ ($n = 3$), respectively. The difference in the rates between these prescriptions seemed to be based on the different concentration of drug; the VPA content in Rp. 2 was two times higher than that in Rp. 1. Both formulations did not show appreciable lag time, suggesting rapid release from these ointments.

**DISCUSSION**

Pharmacokinetic data of antiepileptic drugs are of importance because they can define the therapeutic plasma concentration range, give a clear clinical evidence of toxicity as well as insufficient dosing and show intersubject variations in plasma concentration. The monitoring of plasma concentration of antiepileptic drugs has become a routine clinical practice and knowledge of their pharmacokinetics is essential to make appropriate interpretation of plasma concentration data and therapeutic effect. Generally, antiepileptic drugs such as phenytoin, phenobarbital, carbamazepin, VPA and primidone have been used in oral dosage forms. The development of a dosage form for treatment of patients with severe gastro-intestinal diseases (one of the adverse reactions to VPA-Na is gastro-intestinal irritation) and status epilepticus which does not allow oral administration of drugs would be of great benefit. The p.c. dosage form of VPA could be very useful because the dosage form is simple to administer and can be well received.

The p.c. absorption of VPA 5% VPA ointment was relatively rapid. The rapid absorption of VPA from the ointment may be due to its hydrophobicity and to the small molecular weight in addition to the gel base. On the other hand, the absorption, after the application of 5% VPA · 5% VPA-Ca combination ointment, was slower than with that after 5% VPA ointment dosing and the $C_{max}$ (41.31 ± 10.98 µg/ml) was observed 4 h after dosing. The relatively slow absorption of VPA from the combination ointment may be attributed to slow skin penetration, especially through the stratum corneum, due in part, probably, to the increase in the mass size caused by the hydrophobic interaction of VPA with VPA-Ca. This supposition is strengthened by the fact that the rate ($J_s$) of *in vitro* skin penetra-

![FIG. 4. Release of VPA from Ointments](image-url)
tion from VPA solution was much faster than from VPA - VPA-Ca solution (Fig. 5). Although there is a possibility that the difference between the solvents used in these two ointments may affect the drug release from the solvents, the data shown in Fig. 4, that release rates from both ointments were not dramatically different, suggested that the difference in solvents was not involved in the release rate.

The reported therapeutic ranges of VPA are 50 to 100 µg/ml,13 about 80 µg/ml,14 and 25 to 150 µg/ml.15 It seems that the range of 50 to 100 µg/ml has become enshrined as the optimum range of plasma concentration for VPA in most laboratories. It has been clarified that the effective plasma concentrations at the start of treatment periods are much higher than those in the stable phases to maintain patients completely free of seizure.16,17 The concentrations between 42 and 50 µg/ml18 and below 50 µg/ml18 are reported to give better clinical responses for period of several months following the institution of VPA-Na monotherapy. Therefore, we assumed the therapeutic plasma concentration in the stable phase to be above 30 µg/ml. The period to maintain the concentration was 4 h for Rp. 1 and 6 h for Rp. 2, while for Rp. 3 the concentration was not maintained during the entire application period. Consequently, the 5% VPA - 5% VPA-Ca ointment may be successfully used for the treatment of epileptic patients.

In this study the bioavailability after application of Rp. 1 and 2 was approximately 100%. These values thus suggest that the proposed dosage form (VPA - VPA-Ca ointment) is very effective similar to oral and i.v. administrations.

In conclusion, the present results lead us to postulate that the p.c. application of VPA - VPA-Ca ointment resulted in a sufficient absorption of VPA into systemic circulation to maintain the therapeutic VPA concentrations for several hours. It was also clarified that VPA, a small and a lipophilic molecule, was easily absorbed from skin without absorption enhancers.

REFERENCES