Evaluation of the Topical Delivery of a Prednisolone Derivative Based upon Percutaneous Penetration Kinetic Analysis

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Prednisolone (PN) and an esterified derivative (PND) were evaluated in pharmacological and pharmacokinetic studies. The pharmacological study was performed using a rat croton-oil induced ear edema model. The results for the topical effect in skin and the systemic effect through multiple topical applications showed that PN and PND were equally potent in suppressing edema, and that PN caused a reduction in thymus weight, whereas PND had little effect.

The concentration of these steroids in hairless mouse skin was estimated from an in vitro percutaneous absorption study using the computer simulation program MULTI(FILT). PND was found to be poorly absorbed. In fact, the PND concentration in the viable skin remained low (0.79 μg/cm²), even after 7 d. However, the estimated concentration of PND in the viable skin appears to be in excess of the threshold for effective topical effect during the pharmacological evaluation. In contrast, in the case of PN, the estimated PN concentration increased gradually after application and reached a level of 10.22 μg/cm² at day 7, suggesting that this increase in PN concentration in the viable skin could result in a systemic effect.

The difference between PN and PND concentration in the skin during the time course could be due to the metabolism of PND to PN in the viable skin. Consequently, the difference between the pharmacological study is reflected from the results of the pharmacokinetics of PN and PND in the skin.

Key words prednisolone; prednisolone derivative; croton oil-induced ear edema; percutaneous absorption; MULTI (FILT)

Researchers are now seeking a structurally modified steroidal agent which retains local potency without producing any accompanying adverse systemic effects under conditions of multiple topical applications, which will serve as an antedrug. Such a steroidal antedrug would be converted into a mild-acting steroid after its application to the disease site on skin.

The pharmacokinetic evaluation for such a steroidal derivative on skin is very important, since it would be converted into the parent drug at the site of application. Generally, only pharmacological evaluations of topical and systemic effects have been performed for such steroidal agents. This may be due to the difficulty in accurately determining the concentration of the drug in the skin over a time course.

The purpose of this study is to establish a simple procedure for the clarification of the pharmacological potency of a steroidal prodrug by estimating the intradermal steroid concentration in the skin over a time course. In the present study, prednisolone 21-undecanoate, an esterified derivative of prednisolone (PND), was synthesized, and its predicted transdermal kinetic parameters were compared with the parent compound, prednisolone (PN).

MATERIALS AND METHODS

Chemicals PN was purchased from Sigma Co. (St. Louis, MO, U.S.A.). Other reagents were either analytical or HPLC grade. Pure water was prepared using an automatic water distillation apparatus (Advantec, Tokyo, Japan). PND was synthesized using procedures described in a previous paper.

A homogeneous suspension-type ointment containing 0.5% w/w steroidal agent as PN was prepared as follows. Diethyl sevuse (DES) (5% w/w) was added to PN and to PND as a solubilizer. Fluid paraffin (10% w/w) and white Vaseline (79.3–79.5% w/w) was then added, followed by further addition of a surfactant (3% w/w) and wax (2% w/w) to produce a suspension. The resulting mixture was then agitated at 80°C.

Animals Male Sprague-Dawley rats from Charles River (Wilmington, MA, U.S.A.) with body weights between 100 to 200 g were used in the pharmacological study. 7 weeks old female hairless mice (Hr/Kud) were used in the in vitro percutaneous absorption study (Kyudo, Tosu, Japan). After arrival, the animals were fed ad libitum and maintained in conventional cages for one week prior to the commencement of the study.

Analysis of Steroidal Agents Each steroid was assayed using an HPLC system (LC-10A (Shimadzu, Kyoto, Japan) equipped with a CR-6A integrator (Shimadzu, Kyoto, Japan). A 4.6 ID analytical column 150 mm in length was used (ODS-120T (Tohso), Tokyo, Japan) at 40°C. The UV detection wavelength was 245 nm for PN, and 205 nm for PND. An acetonitrile/water mixture solution (35:65) was used as the mobile phase with a flow rate of 1.0 ml/min.

Pharmacological Study Using a Croton Oil-Induced Ear Edema Model According to the method described by Heiman et al., the effects of steroidal agents were examined using a croton oil-induced ear edema model. Before consecutive topical applications of the steroidal ointment to the ears of the Sprague-Dawley rats, the initial ear thickness of each rat was measured using a micrometer. 20 mg of the steroidal ointment was applied to the surface of the ear. A group treated with only base ointment constituted the control group. This treatment was continued once per day for 7 d. In the final treatment at day 7, 25 μl of a solution of croton oil

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in acetone (50 mg/ml) was applied to the surface of the ear, 4 h after the application of the ointment. The ear thickness was remeasured 4 h after the croton oil application. Sufficient blood samples were collected in order to prepare 2 ml of plasma. Plasma corticosterone levels were assayed by RIA using the rat $^{[22]}$ corticosterone assay system (Amersham, Buckinghamshire, England), and the weights of thymus, adrenal and total body were measured.

**In Vitro Percutaneous Absorption Study** In vitro percutaneous absorption study in the excised hairless mouse skin was performed as described in a previous paper. 50 mg of either the PN or PND ointment was applied to the donor phase. The perfusion solution was collected at intervals, and the PN or PND levels in the perfusion solution were determined by HPLC.

**Analysis of Data from the in Vitro Percutaneous Absorption Study** According to the method of Yamashita et al., the drug concentration in the stratum corneum and in the viable skin ($C_{sc}(x)$, $C_{d}(x)$) versus thickness ($x$) was simulated using the following formulas:

$$C_{sc}(x) = K_d C_d D_v V_d \sinh d_v \cosh d(x/L_v)$$

$$-K_v D_v V_v \cosh d_v \sinh d(x/L_v)/x/s ks (x)$$

$$C_{d}(x) = K_v K_d C_v D_v V_v \sinh d_v (1-x/L_v) / s ks (x)$$

Herein, $k(s)$, $d_v$ and $d_d$ represent the values described below:

$$k(s) = K_v D_v V_v \sinh d_v \cosh d_v + K_v D_v V_v \cosh d_v \sinh d_v$$

$$d_v = L_v s D_v (x)^{1/2}$$

$$d_d = L_d s D_d (x)^{1/2}$$

$s$ is the Laplace operator with respect to time, $K_v$, $D_v$, $V_v$ and $L_v$ represent the partition coefficient of a vehicle in the stratum corneum, the diffusion coefficient in the stratum corneum and the volume and thickness of the layer. $K_{d}$, $D_{d}$, $V_{d}$ and $L_{d}$ represent the partition coefficient into the viable skin, the diffusion coefficient in the viable skin, and the volume and thickness of the layer.

The partition and diffusion coefficients are obtained by applying a MULTI(FILT) based two-membrane model for an infinite dose to the in vitro percutaneous absorption study. The thickness of the stratum corneum for the hairless mouse is 10 $\mu$m, and of the viable skin is 390 $\mu$m.

**RESULTS AND DISCUSSION**

The topical and systemic effects of each steroid ointment (PN and PND) in a croton oil-induced ear edema model were examined. The results are summarized in Table 1. Both steroid ointments significantly suppressed ear edema formation compared with the control group, and the extent of the suppression of inflammation was similar for the two steroid ointments. Interestingly, PN induced a significant decrease in the weight of the thymus, compared with the control and showed a tendency to cause a decrease in adrenal weight as well. Moreover, PN tended to suppress the body weight. However, no significant differences were observed between the PND and control groups with respect to these systemic effects. Therefore, PND influences systemic effects to a lesser degree than PN. In addition, PN suppressed the edema induced by the croton-oil on the opposite site of the ear which represents a non-treatment site in the pharmacological experiment, compared with the control group (PN: 4.30±0.11 mm in the thickness, PND: 4.67±0.27 mm, control: 5.02±0.12 mm) while no significant difference between the PND and the control groups was observed. These results clearly indicate that PN diffuses into the systemic circulation, as compared with PND.

<table>
<thead>
<tr>
<th>Steroidal agent</th>
<th>Swelling ratio$^a$ (%)</th>
<th>Thymus weight (mg)</th>
<th>Adrenal weight (mg)</th>
<th>Body weight (g)</th>
<th>Plasma corticosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>63.9±15.9</td>
<td>553.8±71.18</td>
<td>22.27±2.12</td>
<td>147.80±8.67</td>
<td>249.2±48.7</td>
</tr>
<tr>
<td>PN</td>
<td>35.0±22.9$^*</td>
<td>416.95±33.10$^{**}$</td>
<td>19.75±2.78</td>
<td>139.78±7.89</td>
<td>227.4±59.5</td>
</tr>
<tr>
<td>PND</td>
<td>36.4±12.1$^*</td>
<td>461.40±63.85</td>
<td>22.28±2.75</td>
<td>146.62±8.54</td>
<td>262.9±42.6</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.D. ($n=6$). $a$ The swelling ratio was obtained from the ear thickness after the treatment for 7 d to the initial ear thickness. $*$, $**$: Significant differences to the control group were calculated at $p<0.05$ and $p<0.01$, respectively (Tukey's test).

![Fig. 1. Percutaneous Absorption Profiles of PN and PND through Intact and Stripped Hairless Mouse Skin](image1.png)

Each value represents the mean±S.D. of three determinations.
Table 2. Partition and Diffusion Coefficients of PN and PND in Excised Hairless Mouse Skin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PN</th>
<th>PND</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_s$</td>
<td>502.2</td>
<td>0.138</td>
</tr>
<tr>
<td>$D_s$ (cm$^2$ h$^{-1}$)</td>
<td>1.43x10$^{-8}$</td>
<td>1.15x10$^{-7}$</td>
</tr>
<tr>
<td>$K_d$</td>
<td>3.52</td>
<td>0.026</td>
</tr>
<tr>
<td>$D_d$ (cm$^2$ h$^{-1}$)</td>
<td>2.92x10$^{-5}$</td>
<td>2.92x10$^{-5}$</td>
</tr>
</tbody>
</table>

Fig. 2. Simulated Concentrations of PN and PND in Excised Hairless Mouse Skin at Different Time Periods

To interpret the above pharmacological result, an in vitro percutaneous absorption study was performed. The intradermal concentration in the hairless mouse was simulated based on the partition and diffusion coefficient obtained from the absorption profile. In vitro percutaneous absorption studies in the intact and the stripped skin are shown in Fig. 1. In this absorption study, the absorption parameters $K_s$, $K_d$, $D_s$, and $D_d$ were calculated by using a nonlinear regression program combined with a fast Laplace transform algorithm (MULTI(FILT)). Each parameter is shown in Table 2.

In the case of PND, $D_d$ of PND is thought to be equal to that of PN. In fact, PND was undetectable in the receptor solution under these experimental conditions, indicating that PND was rapidly metabolized to PN in the viable skin. The other parameters for PND were subsequently calculated by using $D_d$ of PN. The saturated solubility of PN and PND in DES, which was used as a solubilizer in the ointment base, were 3522 and 204 μg/ml respectively. These values were used as the concentration of steroidal agent in the ointment ($C_0$) for purposes of this simulation. Using these parameters, the concentrations of PN and PND in both the stratum corneum and the viable skin were calculated using Eqs. 1 and 2 at different time periods (Fig. 2).

To obtain the concentration per unit area of the skin, the AUC of the curve was calculated at different time periods from the simulation result in Fig. 2. The simulation results suggest that the partition of PND into the stratum corneum was fairly small and that its concentration in the layer was maintained at a low level. However, the concentration of PND in the viable skin increased for a short period, and reached equilibrium about 48 h after application (Table 3). The saturation of PND in stratum corneum and viable skin for a short period as estimated by the simulation results can be explained by the small $K_s$ value and the large $K_d/K_s$ value (partition coefficient of drug between stratum corneum and viable skin). Consequently, the amount of PND in the viable skin appears to depend on the percutaneous absorption rate.

The swelling ratio was obtained by measuring the thickness of the inflamed ear which has been induced with croton oil 4 h after a single application of PN or PND ointment to rats. Both ointments showed a swelling ratio of about 40%, and the suppression effects were nearly the same (data not shown). The simulated concentration of PN in the viable skin 8 h after the application of the steroid ointment was 0.31 μg/cm$^2$, and 0.26 μg/cm$^2$ for PND. Both ointments showed similar estimated concentration ranges in the viable skin. Therefore, the simulated viable skin concentration for PN and PND indicated that equivalent topical effects were obtained.

Table 3. Estimated Concentration of PN and PND per Unit Area in Skin at Different Time Periods

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Stratum corneum (μg/cm$^2$)</th>
<th>Viable skin (μg/cm$^2$)</th>
<th>Stratum corneum (μg/cm$^2$)</th>
<th>Viable skin (μg/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>38.34</td>
<td>0.31</td>
<td>0.29</td>
<td>0.26</td>
</tr>
<tr>
<td>24</td>
<td>62.72</td>
<td>3.83</td>
<td>0.32</td>
<td>0.52</td>
</tr>
<tr>
<td>48</td>
<td>78.27</td>
<td>7.59</td>
<td>0.34</td>
<td>0.76</td>
</tr>
<tr>
<td>168</td>
<td>88.60</td>
<td>10.22</td>
<td>0.34</td>
<td>0.79</td>
</tr>
</tbody>
</table>

The above values were obtained by integration of the Eqs. 1 and 2.

The suppression effect of the single application was similar to that of the consecutive 7-d application. Although the estimated concentrations of PN and PND after 7 d were 10.22 and 0.79 μg/cm$^2$ respectively, the topical action for both compounds could be equivalent because these 2 concentrations were considered to have attained the threshold for effective topical action. Moreover, in the case of consecutive application of PN, an increase of the viable skin concentration would lead to an increase in the plasma concentration, hence giving rise to thymus atrophy. In addition, since the perfused PND concentration would be kept low, the systemic effect would therefore be smaller than for the case of PN.

The excised hairless mouse skin after the application of steroids for 24 h, which had been used in the in vitro percutaneous absorption study was further treated using a method which involved the preparation of stripped skin, in order to measure the concentrations of PN and PND in the viable skin using HPLC. The viable skin concentration for PN was 3.06±2.01 μg/cm$^2$ while the concentration for PND was 0.57±0.13 μg/cm$^2$. These data are consistent with the simu-
lated results of PN and PND concentrations in viable skin (see Table 3).

In conclusion, the estimated concentration of the topical steroidal agent in skin, obtained by analyzing the data from an in vitro percutaneous absorption study using the computer simulation program MULTI(FILT) appears to be useful for the convenient interpretation of pharmacological evaluation.

REFERENCES