Rat Percutaneous Transport of Diclofenac and Influence of Hydrogenated Soya Phospholipids

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(Received February 4, 1987)

Poor penetration of diclofenac through in vitro rat dorsal skin including subcutaneous tissue was observed. The poor penetration of diclofenac seemed to be predominantly due to the poor permeability of the stratum corneum. Hydrogenated soya phospholipids (phospholipid) in aqueous gel form increased the penetration of diclofenac in the in vitro study, by increasing diclofenac transport through the stratum corneum. In the in vivo percutaneous absorption of diclofenac, the presence of phospholipid in aqueous gel form increased both plasma diclofenac concentration and diclofenac accumulation in the dorsal skin tissue, including subcutaneous tissue. Since a marked accumulation of diclofenac in the subcutaneous tissue after application of the aqueous gel was observed both in vivo and in vitro, percutaneous application of diclofenac in the aqueous gel form, prepared with phospholipid, may be available for topical treatment rather than for systemic treatment.

Keywords—sodium diclofenac; percutaneous transport; in vitro, in vivo study; rat dorsal skin; subcutaneous tissue; hydrogenated soya phospholipid; aqueous gel; plasma concentration

As described by Osterenga et al.,2) the efficacy of topically applied drugs is often dependent on the composition of the vehicle. The ability of a drug in a topical formulation to penetrate the skin and exert its effect is dependent on two consecutive events. The drug must first diffuse out of the vehicle to the skin surface, and then it must penetrate this natural barrier en route to the site of action. These two processes are intimately related, and both are dependent upon the physical properties of the drug, vehicle, and barrier.

Sodium diclofenac is an effective nonsteroidal antiinflammatory drug. There are few reports on the penetration of sodium diclofenac through the skin, though it has been reported3) that sodium diclofenac was absorbed well from the rectum.

In the present study, we investigated the penetration of diclofenac through rat skin and the accumulation of diclofenac in subcutaneous tissue in both in vitro and in vivo experiments. We also examined the effect of hydrogenated soya phospholipids on the penetration of diclofenac. Phospholipids are surfactants, and it has been reported that several surfactants increase the skin permeability.4) Thus, it is of interest to study the effect of phospholipid as a vehicle component on the penetration of sodium diclofenac.

Experimentals

Materials—Sodium diclofenac was supplied by Ciba Geigy Japan (Takarazuka, Japan). Hydrogenated soya phospholipids (phospholipid), which was supplied by Nikko Chemicals Co., Ltd. (Tokyo, Japan), contains about 30% phosphatidycholine and 70% phosphatidylethanolamine, and its iodine value was about 6%. Other reagents used were of analytical grade.

Animals—Male Wistar rats, 200 to 250 g, were fasted for 16 h prior to experiments, but water was given freely. The dorsal hair of rats was shaved with electric clippers. For the in vitro study, the dorsal skin was excised just
before an experiment, but the excised skin included subcutaneous tissue.

**In Vitro Penetration Study**——The in vitro penetration study was performed in an LG-1084-LPC penetration cell (Laboratory Glass Apparatus Inc., Berkely, CA, U.S.A.). Briefly, the fluid volume of the receptor side (subcutaneous tissue side) was 5.5 ml, and that of the donor side (stratum corneum side) was 4 ml in the present study. As a fluid, 0.1 M sodium phosphate buffer (pH 7.4) was used. When phospholipid was added to the test solution on the donor side, the solution after addition of phospholipid was homogenized at 80 °C for 10 min to accelerate the hydration of phospholipid to produce the aqueous gel form.5) The skin membrane surface area exposed to fluid was 530 mm² (26 mm diameter). Fluid on the receptor side was kept at 37 °C.

After settlement of the skin into the apparatus, 4 ml of test solution containing sodium diclofenac at a concentration of 1.25 mg/ml was placed on the donor side, and then 100 µl samples were collected from the receptor side at 1 h intervals for 7 h. Just after collection of fluid, 100 µl of buffer solution was added to keep the volume constant, fluid on the receptor side was agitated with a magnetic stirrer.

In another series of experiments, 5.5 ml of the test solution or the aqueous gel (0.25%(w/w) phospholipid) containing sodium diclofenac was placed on the receptor side (subcutaneous tissue side) to investigate the distribution of diclofenac from the buffer solution to the subcutaneous tissue. After the solution of diclofenac was placed on the subcutaneous side, 20 µl samples were collected from the receptor side at 5, 15, 30, 45, 60 min, and 2 h.

To measure accumulation of diclofenac in the tissue, the tissue was homogenized, following the rinsing of the skin tissue with saline after an in vitro penetration study. After addition of acetonitrile to the homogenate and centrifugation, the supernatant was collected and then dried under a flow of nitrogen gas. The residue was dissolved in a mixed solvent of 25%(v/v) acetonitrile and 75%(v/v) 0.05 M citrate buffer (pH 5.5), which was used as a mobile phase in high-performance liquid chromatography (HPLC).

**In Vivo Absorption Study**——A rat was anesthetized with sodium pentobarbital (30 mg/kg, i.p., for the first injection, and then 15 mg/kg at 2.5 h intervals during the experimental period), and placed on a hot surface at 38 °C. The administration of the test solution was performed with a cylinder (26 mm inner diameter and 20 mm high), which was fixed perpendiculary on the rat dorsal skin surface with glue (Aron Alpha®, obtained from Toa Chemicals Co., Ltd., Tokyo, Japan). After the administration, 200 µl of blood was collected from the right femoral vein via a canuula at designated time intervals for 12 h. After 12 h, the skin tissue including subcutaneous tissue on which the test solution had been applied was excised to measure the accumulation of diclofenac in the tissue. The intravenous administration of diclofenac solution was performed via the jugular vein.

**Assay**——Assay of sodium diclofenac was carried out by HPLC as described by Yaginuma et al.6) The lowest concentration of diclofenac that could be determined was 40 ng/ml.

**Statistical Analyses**——Statistical analyses were performed by means of Student's t-test.

**Results and Discussion**

**In Vitro Penetration of Diclofenac through Rat Skin Including Subcutaneous Tissue**

Penetration of diclofenac through the skin was poor when the test solution on the donor side contained sodium diclofenac at a concentration of 1.25 mg/ml (Fig. 1A). After 7 h, only about 0.015% of the diclofenac was recovered on the receptor side. The application of the aqueous gel, prepared with phospholipid at various contents, increased the penetration of diclofenac, in proportion to phospholipid content (Fig. 1A). However, contents of phospholipid above 0.5% (w/v) did not further increase diclofenac penetration.

Since the accumulation of diclofenac in the subcutaneous tissue is an important factor for topical therapy, accumulation of diclofenac in the tissue was investigated at 2, 5 and 7 h after placing the test solution on the stratum corneum (Fig. 1B). The amount of diclofenac accumulated in the tissue increased with increasing incubation time. The accumulation of diclofenac also increased in the presence of the aqueous gel of phospholipid. The apparent penetration rate of diclofenac at 2, 5 and 7 h was determined by applying the following equation:

\[
\text{apparent penetrate rate} = \frac{[\text{increase of diclofenac on the receptor side}]}{[\text{from (t) h to (t + \Delta t) h}]/[\text{[(t + \Delta t) h]}]
\]

where t and t + \Delta t are times (h) after starting the experiments. In the present study, the values of the apparent penetration rate were determined from the increase of diclofenac amount on the receptor side from 1 to 3 h for the rate at 2 h, from 4 to 6 h for the rate at 5 h, and from 6 to
7 h for the rate at 7 h. As shown in Fig. 2, there was a good relationship between the apparent penetration rate and the total amount of diclofenac accumulated in the skin tissue.

These results indicate that an increase of the penetration rate of diclofenac through the skin occurred along with an increase of diclofenac accumulation in the subcutaneous tissue. In another series of experiments, the distribution of diclofenac to the subcutaneous tissue was examined. When test solution containing sodium diclofenac at various concentrations was placed on the subcutaneous tissue side, distribution of diclofenac into the tissue occurred rapidly, as represented by the decrease of diclofenac concentration in Fig. 3A. This result
indicates that slow uptake of diclofenac into the skin tissue from the stratum corneum side is due to the low permeability of stratum corneum to diclofenac. When the aqueous gel, prepared with phospholipid at 0.25% (w/v), was placed on the subcutaneous side, the distribution of diclofenac into the subcutaneous tissue did not change greatly in comparison with that in the absence of phospholipid (Fig. 3). The ratio of amount of diclofenac accumulated at 2 h to initial diclofenac concentration was not affected by the initial concentration of diclofenac or by the presence of phospholipid.

These observation seem to indicate that the increase of diclofenac accumulation in the tissue from the stratum corneum in the presence of phospholipid (Fig. 1B) occurs through an acceleration of diclofenac penetration through the stratum corneum by phospholipid, since phospholipid did not influence the distribution of diclofenac into the subcutaneous tissue. Although we did not investigate in detail how phospholipid increased the permeability of the stratum corneum to diclofenac, it may be supposed that a surfactant effect of phospholipid is involved, since it has been reported that extraction of lipid from the stratum corneum increased the permeability and several surfactants could extract the lipid. Recently, Natsuki et al. have reported that indomethacin gel ointment containing egg lecithin gave enhanced transdermal absorption of indomethacin from rat dorsal skin. Thus, it is considered
that phospholipids in the aqueous gel increase the permeability of the stratum corneum of rat dorsal skin.

**In Vivo Percutaneous Absorption of Diclofenac in Rat**

In the *in vivo* percutaneous absorption study, two application forms were examined; the solution and the aqueous gel containing phospholipid at 0.5% (w/v). After intravenous administration of diclofenac into rat jugular vein, elimination of diclofenac occurred rapidly, as shown in Fig. 4B.

After the administration of diclofenac solution or diclofenac aqueous gel, diclofenac appeared in the plasma, and the plasma concentration of diclofenac was maintained roughly

![Fig. 4. Plasma Diclofenac Concentration in Rats as a Function of Time after the Percutaneous Administration (A) of 4 ml of the Solution (Open Circles) or 4 ml of the Aqueous Gel (Closed Circles, 0.5% (w/v) Phospholipid) at a Dose of 5 mg of Sodium Diclofenac (1.25 mg of Sodium Diclofenac/ml in Test Sample) and after Intravenous Administration (B) of Diclofenac at a Dose of 0.5 mg/rat](image)

Each value represents the mean ± S.D. (n=4). a) p < 0.05 versus the solution.

**Table 1. Area under the Curve of Plasma Diclofenac Concentration (AUC) for 12 h after Administration and Total Amount (AMOU) of Diclofenac Accumulated in the Dorsal Skin Tissue Including Subcutaneous Tissue, after Intravenous Administration or Percutaneous Administration of Sodium Diclofenac in Rats**

<table>
<thead>
<tr>
<th>Administration</th>
<th>Dose (mg/rat)</th>
<th>AUC (µg h/ml)</th>
<th>BA (%)</th>
<th>AMOU (µg)</th>
<th>Wet weight of tissue (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous administration</td>
<td>0.5</td>
<td>4.24 ± 0.47</td>
<td>100</td>
<td>Undetectable</td>
<td>2.52 ± 0.41</td>
</tr>
<tr>
<td>Percutaneous administration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solution</td>
<td>5.0</td>
<td>1.82 ± 0.56</td>
<td>4.29</td>
<td>11.6 ± 6.2</td>
<td>2.61 ± 0.37</td>
</tr>
<tr>
<td>Aqueous gel (d)</td>
<td>5.0</td>
<td>10.86 ± 3.77a</td>
<td>25.61</td>
<td>96.2 ± 31.7a</td>
<td>2.29 ± 0.24</td>
</tr>
</tbody>
</table>

a) The amount of diclofenac accumulated in the tissue was measured at 0.5 h after intravenous administration, and at 12 h after percutaneous administration. b) BA represents bioavailability, which was determined as follows:

\[ BA = \frac{(AUC)_{\text{i.v}} \cdot \text{(dose)}}{(AUC)_{\text{p.c.}} \cdot \text{(dose)}}_{\text{skin}} \]

where skin and i.v. represent percutaneous administration and intravenous administration, respectively. c) Wet weight of the skin tissue excised to measure the accumulation of diclofenac. d) Aqueous gel containing 0.25% (w/v) phospholipid. Each value represents the mean ± S.D. (n = 4). e) p < 0.05 versus solution.
from 2 to 12 h during the experimental period (Fig. 4A). The plasma diclofenac concentration obtained by the application of aqueous gel was significantly greater than in the case of the solution. The appearance of diclofenac in plasma seems to be rapid in comparison with the in vitro penetration study. Since it is considered that blood flow systems in the rat reach close to epidermis, as in humans,9) diclofenac after penetration through the stratum corneum passes into the blood flow system after diffusion through the thin epidermis layer. However, the skin including subcutaneous tissue in the in vitro study represents a long diffusion layer before reaching the receptor solution; i.e., a long lag time was observed in the in vitro study before appearance of diclofenac in the receptor solution.

The amounts of diclofenac accumulated in the skin tissue region including subcutaneous tissue were greater when the aqueous gel was applied than in the case of the solution (Table I). This result is consistent with the in vitro study; i.e., marked accumulation of diclofenac in the tissue caused a higher concentration of diclofenac in plasma. Thus, it is considered that the amount of diclofenac accumulated in the tissue under the skin can be related to plasma concentration of diclofenac. In terms of intravenous administration of diclofenac, when the plasma diclofenac concentration was more than 2 µg/ml (Fig. 4B), accumulation of diclofenac in the subcutaneous tissue of the skin was not detectable (Table I).

The bioavailability of diclofenac in percutaneous absorption, which was determined evaluating the area under the curve (AUC) of plasma diclofenac concentration for 12 h, in comparison with that after intravenous administration, was 5% for the solution and 25% for phospholipid aqueous gel (Table I). It should be noted that a marked accumulation of diclofenac in the skin tissue was observed (Table I), in spite of the low bioavailability of diclofenac in plasma, after application of the aqueous gel form seems to be available for topical treatment rather than for systemic treatment.

References and Notes

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