In Vitro Percutaneous Transport of Sodium Diclofenac and Diclofenac from Oleaginous Vehicle

Koichi TAKAHASHI, a, b Satoko TAMAGAWA, a Toyoshi KATAKI, a Hironori YOSHIKOH, a Akira KAMADA, a J. Howard RYTTING, a Toshiaki NISHIHATA, a and Nobuyasu MIZUNO a

Faculty of Pharmaceutical Sciences, Mukogawa Women's University, a 1, 68 Koshien Kyubancho, Nishinomiya, Hyogo 663, Japan, Faculty of Pharmaceutical Sciences, Fukuyama University, b 585 Sanzen, Higashimuracho, Fukuyama, Hiroshima 727-02, Japan, Faculty of Pharmaceutical Sciences, Osaka University, c 1-16 Yamadaokas, Suita, Osaka 565, Japan, and Pharmaceutical Chemistry Department, University of Kansas, d 2065 Constant Avenue, Lawrence, Kansas 66046, U.S.A. Received May 28, 1990

The penetration enhancement of sodium diclofenac and diclofenac by alcohols with various alkyl chains (C \textsubscript{1} to C \textsubscript{10}) was evaluated by the steady state flux of diclofenac through rat abdominal skin. Decanol showed the greatest effect in this series. A more remarkable enhancing effect of the alcohols was observed in sodium diclofenac than in diclofenac. Diclofenac can penetrate through the ethylene–vinyl acetate membrane as a lipid model membrane, but sodium diclofenac cannot. Decanol enhanced the penetration of phenol red being dependent on its concentration in the vehicle. Therefore, decanol may interact with lipid components of the skin and increase the aqueous pathway in the skin. These results indicate that sodium diclofenac and diclofenac may be penetrated through partially different pathways.

Keywords: percutaneous permeation; sodium diclofenac; diclofenac; enhancer; primary alcohol; oleaginous vehicle; phenol red

The stratum corneum provides the principal barrier to the percutaneous penetration of topically applied substances. Only a few materials which tend to be extremely lipophilic and have low melting points are easily transported to the underlying viable aqueous tissue. It is important to enhance the penetration of many drugs through the stratum corneum. A number of compounds, such as surfactants, organic solvents, and azone, have been reported as penetration enhancers.

Sodium diclofenac, which is a strong anti-inflammatory agent, has been reported as not easily absorbed by transdermal application. In the present study, we investigated the effects of primary alcohols on the transdermal penetration of this drug from an oleaginous vehicle. We also compared the transport of sodium diclofenac with diclofenac, because it is generally considered that an ionized form with greater lipophilicity is easily transported in comparison with an ionized form. As this study was performed as a preliminary study for the transdermal formulation of diclofenac, either sodium diclofenac or diclofenac was dissolved in the oleaginous vehicle to avoid a possible change of the crystal form of the drug in the formulation used.

Experimental

Materials: Sodium diclofenac and diclofenac were supplied by Ciba-Geigy Japan (Takarazuka, Japan). Squalane was supplied by Nikko Chemicals Co., Ltd., (Tokyo, Japan). An ethylene–vinyl acetate copolymer (EVA) membrane (composition, ethylene: vinyl acetate = 90:10; thickness, 40 μm) obtained from Tamapoly Co., Ltd., (Tokyo, Japan) was used. Other reagents used were of analytical grade.

Preparation of Test Vehicles: 25 mg of sodium diclofenac or 23 mg of diclofenac was mixed with the 10 g of the combined solvent composed of 10% ethanol, 5% primary alcohol and 85% squalane. The mixture was agitation until a clear solution was obtained. The resulting vehicle solution was used in the in vitro penetration study. In the experiment using phenol red, 25 mg of phenol red was mixed in a suspension form with 10 g of the combined solvent which was composed of squalane containing 10% ethanol and various concentrations of decanol (Table II).

In Vitro Percutaneous Study: After the removal of hair by an electric clipper, abdominal skin was excised from a rat (male Wistar, 250 to 300 g) just prior to the experiment. The excised skin was placed on a Franz type diffusion cell. In the present study, 10 ml of 0.1 M sodium phosphate buffer (pH 7.2) was used as a receptor medium and 1 g of the test vehicle was placed on the donor side.

Quoting (0.1 ml in diclofenac or 0.2 ml in phenol red) of the receptor medium were collected periodically for 12 h. Just after each collection, 0.1 or 0.2 ml of the buffer was added to the receptor medium. During the experiments, the medium in the receptor was agitated by a magnetic stirrer at 37°C.

Transport of the drug across the EVA membrane was also performed according to the method described above.

Solubility Study: An excess amount of sodium diclofenac, diclofenac or sodium phenol red was added to the test vehicle and was agitated for 24 h at 23 ± 2°C. The suspension was filtered through a membrane filter (0.45 μm) to obtain a clear solution, and the concentration of sodium diclofenac and diclofenac was measured by high performance liquid chromatography (HPLC). Phenol red was photometrically measured at 560 nm after extraction with 0.01 N NaOH solution.

Assay: The determination of diclofenac was carried out by HPLC as described by Yaguma et al. The concentration of phenol red was photometrically determined at 560 nm after the addition of 0.01 N NaOH solution.

Results and Discussion

Effect of Primary Alcohols on Percutaneous Transport of Diclofenac: Figure 1 shows the transport profiles of diclofenac and its sodium salt through the excised skin from the vehicle containing various primary alcohols. After a lag time, a steady state transport of diclofenac after application of either sodium diclofenac or diclofenac was observed. The steady state flux and apparent lag time can be calculated from the straight line plotted in Fig. 1 and summarized in Table I. The flux rates of sodium diclofenac and diclofenac were affected with a primary alcohol, and the apparent lag time seemed to increase with the decrease of flux. When the vehicle prepared with squalane and 10% ethanol without other primary alcohol was applied, the flux rates were below 0.5 mmol/cm²/h in sodium diclofenac and 25.89 ± 0.80 mmol/cm²/h in diclofenac. These results suggested that diclofenac easily penetrates from the vehicle uncontaminated enhancer through the rat skin, but sodium diclofenac does not.

As shown in Fig. 2, the marked increase in the steady state flux of diclofenac was observed in sodium diclofenac when the alcohol with a carbon number of C\textsubscript{10} was added to the vehicle. Thereafter, an increase in the carbon number of the alcohol in the vehicle decreased the steady state flux of diclofenac. On the other hand, in diclofenac, the same fluxes of diclofenac were observed with C\textsubscript{8} and C\textsubscript{10}. The greater flux in diclofenac was observed when sodium
diclofenac was used, in comparison to that when only diclofenac was used.

The primary alcohols may play two possible roles in the permeation enhancement observed in this study. First, the primary alcohols may affect the release of sodium diclofenac or diclofenac from the vehicle. Second, the primary alcohols may interact with the lipid components in the stratum corneum and act as a plasticizer. In this study, sodium diclofenac did not penetrate through the rat skin without the enhancer. Also, the solubility of sodium diclofenac and diclofenac to the vehicle containing 5% primary alcohol (C₈ to C₁₄) was almost constant (its value is about 10 mm in sodium diclofenac and 17 mm in diclofenac). From these results, it is considered that the effect of primary alcohols in increasing permeability of skin epithelium is mainly due to their action as a plasticizer. The low enhancing effect of alcohol with an increase in carbon number (C₁₂ and C₁₄) may be due to the low action as a plasticizer.

There are many compounds, such as surfactant³ in-

cluding fatty acid and azone,⁵,⁶ which have been reported as penetration enhancers. The transport of water soluble compounds was enhanced to a greater extent by these enhancers compared with the lipophilic compounds.¹⁰ In this study, the greater enhancing effect of primary alcohols was observed in sodium diclofenac rather than in diclofenac as described above.

Percutaneous penetration may occur in two possible pathways; an aqueous pathway which allows for the penetration of water soluble compounds and a lipoidal pathway which allows for the penetration of lipophilic compounds.¹¹ An EVA membrane which is used as a model membrane for the transdermal study by Kondo et al.¹² is a lipophilic membrane and is known to be permeable to

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**Fig. 1. Permeation Profiles of Sodium Diclofenac and Diclofenac through Rat Skin from Vehicles Containing Various Primary Alcohols**

- ○, sodium diclofenac; ●, diclofenac. Each value represents the mean ± S.D. (n = 4 to 6).

**Table I. Effect of Alcohol on the Transport Parameter of Sodium Diclofenac (DCNa) and Diclofenac (DC) through Rat Skin**

<table>
<thead>
<tr>
<th>Carbon number</th>
<th>DCNa Lag time (h)</th>
<th>DCNa Flux (nmol/cm²/h)</th>
<th>DC Lag time (h)</th>
<th>DC Flux (nmol/cm²/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₈</td>
<td>2.2 ± 0.4</td>
<td>106.0 ± 10.2</td>
<td>2.2 ± 0.4</td>
<td>71.7 ± 6.9</td>
</tr>
<tr>
<td>C₁₀</td>
<td>2.8 ± 0.2</td>
<td>158.4 ± 18.7</td>
<td>2.8 ± 0.3</td>
<td>68.8 ± 4.2</td>
</tr>
<tr>
<td>C₁₂</td>
<td>2.7 ± 0.4</td>
<td>89.8 ± 2.8</td>
<td>3.7 ± 0.3</td>
<td>41.0 ± 1.2</td>
</tr>
<tr>
<td>C₁₄</td>
<td>3.4 ± 0.8</td>
<td>52.9 ± 6.1</td>
<td>No experiment</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. (n = 4 to 6). The steady state flux and lag time were calculated from the straight line plotted in Fig. 1.

**Fig. 2. Relationship between Permeation Rate and Carbon Number of Alcohols**

○, sodium diclofenac; ●, diclofenac.

**Fig. 3. Permeation Profiles of Sodium Diclofenac and Diclofenac through the EVA Membrane**

○, sodium diclofenac; ●, diclofenac.

**Table II. Effect of Decanol Concentration on the Transport Parameter of Phenol Red through Rat Skin**

<table>
<thead>
<tr>
<th>Concen. (%)</th>
<th>Solubility (µg/mL)</th>
<th>Flux (µg/cm²/h)</th>
<th>Pe (cm²/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>12.0</td>
<td>4.0 ± 1.3</td>
<td>0.33 ± 0.09</td>
</tr>
<tr>
<td>10</td>
<td>13.3</td>
<td>4.6 ± 1.1</td>
<td>0.37 ± 0.08</td>
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<tr>
<td>20</td>
<td>19.4</td>
<td>16.2 ± 1.6</td>
<td>0.84 ± 0.08</td>
</tr>
<tr>
<td>40</td>
<td>30.0</td>
<td>36.9 ± 1.2</td>
<td>1.23 ± 0.04</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. (n = 4 to 6). a) Solubility of phenol red in the vehicle was measured at 23 ± 2°C. b) The permeability rate constant (Pe) was calculated from the flux and initial concentration in the donor.
nonionic molecules. Declofenac penetrated across an EVA membrane from an oily vehicle, but sodium diclofenac did not (Fig. 3). This observation suggests that different pathways through the skin epithelium are involved in the transport of both diclofenac and sodium diclofenac.

**Effect of Decanol Concentration in the Vehicle on the Percutaneous Transport** Because a decanol provided a greater effect on the transport of both diclofenac and sodium diclofenac than other alcohols, the effect of decanol concentration on percutaneous transport was investigated.

To investigate the effect of decanol on the aqueous pathway, the effect of decanol concentration in the vehicle on the transport of phenol red was examined. It is known that phenol red is a water-soluble compound and is not absorbed from the gastrointestinal tract without an enhancer. Also, phenol red is often used as an indicator for measurement of water flux. As shown in Table II, decanol enhanced the transport of phenol red along with an increase in decanol concentration in the vehicle. Since the solubility of phenol red also increased with the concentration of decanol in the vehicle, the permeability rate constant \((Pe)\) was calculated from the flux and the initial concentration in the donor. Since the value of \(Pe\) for phenol red increased along with an increase in the decanol concentration (Table II), it is possible that the aqueous pathway is accentuated by interactions of the alcohol with the lipid components of the skin epithelium.

However, as shown in Table III, the penetration rate of sodium diclofenac which was markedly enhanced from the vehicle containing 5% decanol, decreased with an increase in decanol concentration. On the other hand, the penetration of diclofenac was almost constant (perhaps a slight decrease with an increase of the concentration) in all concentrations of decanol in the vehicle examined. The solubilities of sodium diclofenac and diclofenac increased along with an increase in the decanol concentration (Table III). From these results, the decrease in the sodium diclofenac (and diclofenac) transport along with the increase in the decanol concentration may be due to a change in physicochemical properties of the vehicle with respect to the drug rather than an effect of decanol on the transport route for the drug across the skin epithelium; i.e., the release of sodium diclofenac might be reduced by an increase in decanol concentration. However, a greater enhancing effect of alcohol was observed when sodium diclofenac was used, in comparison to that when diclofenac was used. To clarify the detailed mechanism of alcohol on the permeation of the drug further studies are needed. But, from the results of this study, it is considered that sodium diclofenac and diclofenac are penetrated through partially different pathways, i.e., a lipoid pathway for diclofenac and an aqueous pathway mainly for sodium diclofenac.

**References**