Passive and Iontophoretic Delivery of Three Diclofenac Salts across Various Skin Types

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The in vitro permeation of three diclofenac salts—diclofenac sodium (DFS), diclofenac potassium (DFP) and diclofenac diethylammonium (DFD)—across skin by both passive and iontophoretic transport was investigated. Various skin types were used as the barriers to elucidate the mechanism controlling transdermal delivery of diclofenac salts. The importance of the intercellular (paracellular) route for both DFS and DFP in passive permeation was elucidated. The transfollicular route constitutes an important permeation pathway for DFS but not for DFP. The route and mechanism for transdermal iontophoresis of DFD across the skin was somewhat different to that of the other salts. Hair follicles may be a more important pathway for DFD than for DFS and DFP under iontophoresis, while the intercellular lipid pathway showed the opposite result. Combination of iontophoresis and a penetration enhancer, cardamom oil, did not show a synergistic effect on diclofenac salt permeation. The results of this investigation suggest that the transdermal mechanism and the route of diclofenac salt uptake via passive and iontophoretic transport can be affected by their counterions.

Key words diclofenac salt; transdermal delivery; iontophoresis; skin; penetration enhancer

Diclofenac has been widely used systemically and locally as an anti-inflammatory agent. It has been reported that orally administered diclofenac undergoes hepatic first-pass metabolism and produces considerable gastrointestinal disturbances. 1,2 Transdermal delivery is suitable for diclofenac, in order to overcome these two major shortcomings of oral therapy. Many diclofenac salts which possess various physicochemical and pharmacokinetic properties have been synthesized.3,4

To deliver drug molecules across skin, transdermal devices based on passive diffusion as well as iontophoresis are most often studied and reported. Transdermal iontophoresis is defined as a method involving transport of ionized or unionized molecules into skin tissues by the passage of direct current and the appropriate electrode polarity. It is often considered in relation to drugs whose transdermal delivery is limited by passive diffusion. Several variables may affect the transdermal iontophoretic permeation of drug molecules, including physicochemical properties of the drug, the vehicle composition, electric factors and the skin barrier properties.5,6

In the present study, three major goals were identified. First, to compare the transdermal characteristics of three diclofenac salts, including diclofenac sodium (DFS), diclofenac potassium (DFP), and diclofenac diethylammonium (DFD), under passive and iontophoretic permeation. Second, to use various skin types as barriers in an in vitro permeation study, including synthetic cellulose membrane, nude mouse skin, stratum corneum (SC)-stripped skin, delipid skin and furry rat skin, to elucidate the transdermal mechanism of these three diclofenac salts. The coupling of iontophoresis with a penetration enhancer may permit the use of lower amounts of drug, enhancer, or current density within the delivery system, thus potentially reducing the adverse effects, toxicity problems, and formulation difficulties.7 In our previous studies, the acetone extract of Ammonium Cardamomum (Zingiberaceae) was found to enhance the passive and iontophoretic permeation of DFS.8,9 Accordingly, the third goal was to investigate if iontophoresis and cardamom oil have a synergistic effect on the permeation of the three salts.

MATERIALS AND METHODS

Materials Diclofenac sodium (DFS, MW=318.13), diclofenac potassium (DFP, MW=334.24), and diclofenac diethylammonium (DFD, MW=369.29) were gifts kindly provided by Novartis Pharmaceutical Co., Switzerland. The extraction method for cardamom oil has been previously reported.10 All other chemicals and solvents were analytical grade and used as received.

Preparation of Skin Membranes The skin of female nude mice (BALB-c-nu, 11—12 weeks old) was used as the model membrane to compare the permeation characteristics of DFS, DFP, and DFD in this study. The mouse was killed with ether and the full-thickness skin was excised from the dorsal region. To obtain the SC-stripped skin, adhesive tape was applied on the nude mouse skin with uniform pressure and removed 20 times. The delipid skin was prepared by immersing the full-thickness skin in chloroform/methanol (2:1 v/v) solution for 60 min. The skin of male Wistar rat (10—12 weeks old) was obtained by sacrificing the rat with ether; the hair of its abdominal region was shaved and the full-thickness skin was then excised. The cellulose membrane (Spectra-por® 2, MW cut-off=12000-14000, Spectrum Co., U.S.A.) was immersed in distilled water for 24 h prior to the in vitro permeation experiments.

In Vitro Permeation Experiments The in vitro permeation study was performed by using side-by-side glass diffusion cells. 8 ml of citrate-phosphate buffer (pH 7.4; 0.06 M) was used as the medium for both donor and receptor compartments. The drug concentration in the donor compartment was 12.5 mM. The drug was more than 99.9% ionized in the donor compartment because of its pKa of 4.16. 11 The available diffusion area between cells was 0.785 cm2. The stirring rate and temperature were kept at 600 rpm and 37 °C. At appropriate intervals, 200 μl aliquots of the receptor medium were withdrawn and immediately replaced by an

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equal volume of fresh buffer. The amount of DFS, DFP or DFD was analyzed by the HPLC method. ③

In the study of penetration enhancer pretreatment, 150 μl of cardamom oil was deposited onto the SC surface after mounting the nude mouse skin in a device. Skin samples were pretreated with the oil for 60 min. After pretreatment, the enhancer solution was removed, and the skin was rinsed with distilled water and the permeation experiments were then started.

**Application of Iontophoresis** A pair of Ag/AgCl wires with an effective working length of 15 mm were immersed in the buffer solution as electrodes, with the cathode in the donor compartment and the anode in the receptor compartment. The cathode and anode were each positioned 3 cm from the side of skin. The electrodes were connected to a constant current power supplier (Model 7651, Yokogawa Co., Japan) and the current density was set at 0.3 mA/cm². The value of $E_{skin}$ and $E_{cont}$ was calculated after determination of flux. $E_{skin}$ represents passive flux across treated skin/passive flux across hairless mouse skin. $E_{cont}$ is iontophoretic flux across skin/passive flux across skin.

**RESULTS**

**Passive Permeation of Diclofenac Salts** With respect to drug permeation across the skin from aqueous solution, a drug should first diffuse out from the vehicle to the skin surface. To clarify the mechanism of the passive permeation of diclofenac, the release rate of diclofenac salts from the vehicles was studied. A cellulose membrane with a molecular weight cut-off value of 12000—14000 was used as the barrier. The cut-off value suggests that there are water-filled pores or channels for drug molecules to diffuse freely. The release profiles of diclofenac salts across the porous membrane are shown in Fig. 1. The three diclofenac salts showed differences in the release rates with the trend of DFS>DFP>DFD (Table 1).

The cumulative amount-time profiles of diclofenac salts across full-thickness nude mouse skin are shown in Fig. 2. The slopes of the resulting plots were computed and the fluxes (nmole/cm²/h) were calculated from the slopes (Table 1). These profiles fit well to the pseudo zero-order kinetics. Although the release rate of DFS was the lowest among the three salts, the flux of DFP across skin was comparable (ANOVA test, p>0.05) to that of DFS and DFD (Table 1). The data in Table 1 shows that the flux of diclofenac salts across SC-striped skin was much higher than that across intact skin. A lower enhancement effect in flux for DFS ($E_{skin}=12.12$) and DFD ($E_{skin}=17.85$) relative to DFP ($E_{skin}=27.68$) after removal of SC was observed.

Delipid skin was also used as the barrier for diclofenac salts. The delipidation process in this present study can extract the lipid content of the whole skin. Extraction of lipid greatly increased the passive permeation of diclofenac salts, especially for DFS ($E_{skin}=28.62$) and DFP ($E_{skin}=29.49$). Table 1 also demonstrates that the flux of diclofenac salts across furry Wistar rat skin increases in the order of DFS>DFD>DFP. This trend was quite different to that across nude

![Fig. 1. Cumulative Amount of Diclofenac Salts Released per Unit Area (nmol/cm²) versus Time Profiles across Cellulose Membrane under Passive Diffusion](image1)

**All data represent the means of three experiments ± S.D.**

![Fig. 2. Cumulative Amount of Diclofenac Salts Permeated per Unit Area (nmol/cm²) versus Time Profiles across Nude Mouse Skin under Passive Diffusion](image2)

**All data represent the means of three experiments ± S.D.**

**Table 1. The Data for in Vitro Passive Permeation of DFS, DFP and DFD across Various Skin**

<table>
<thead>
<tr>
<th>Skin types</th>
<th>DFS  a)</th>
<th>DFP  b)</th>
<th>DFD  c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flux (nmol/cm²/h)</td>
<td>$E_{skin}$</td>
<td>Flux (nmol/cm²/h)</td>
</tr>
<tr>
<td>Cellulose membrane</td>
<td>1102.86±84.62</td>
<td>1.00</td>
<td>995.93±12.86</td>
</tr>
<tr>
<td>Nude mouse</td>
<td>23.31±2.94</td>
<td>12.12</td>
<td>282.53±45.10</td>
</tr>
<tr>
<td>SC-striped</td>
<td>667.06±97.61</td>
<td>24.89</td>
<td>688.94±75.54</td>
</tr>
<tr>
<td>Delipid</td>
<td>14.95±3.02</td>
<td>7.54</td>
<td>8.31±2.38</td>
</tr>
<tr>
<td>Rat</td>
<td>175.79±55.24</td>
<td>5.43</td>
<td>236.36±57.02</td>
</tr>
<tr>
<td>Cardamom oil-treated</td>
<td>14.95±3.02</td>
<td>7.54</td>
<td>8.31±2.38</td>
</tr>
</tbody>
</table>

a) DFS, diclofenac sodium.  b) DFP, diclofenac potassium.  c) DFD, diclofenac diethylammonium.  d) $E_{skin}$, passive flux across treated skin/passive flux across hairless mouse skin. Each value represents the mean ± S.D. (n=3).
mouse skin.

Pretreatment of nude mouse skin by cardamom oil as a skin penetration enhancer for diclofenac salts was considered. The major constituents of cardamom oil are cyclic monoterpenes, which showed good enhancing effect for diclofenac permeation. As shown in Fig. 3, the passive permeation of diclofenac salts was significantly enhanced by cardamom oil pretreatment.

**Iontophoretic Permeation of Diclofenac Salts**  The iontophoretic delivery of diclofenac salts was investigated and compared in this study since the efficiency of drug delivery can be affected by the drug counterions employed in an iontophoretic system. Application of 0.3 mA/cm² current density significantly enhanced the release rate of all salts relative to their passive transport across cellulose membrane (Figs. 1 and 4). The same effect is observed for diclofenac salts permeating across hairless mouse skin (Figs. 2 and 5). However, the magnitude of permeation enhancement achieved by iontophoresis appears to be relatively low in the case of cellulose membrane.

The iontophoretic flux and enhancement ratio of diclofenac salts across nude mouse skin increased in the order of DFS > DFP > DFD as shown in Table 2. Identical to the result of the passive permeation experiment, DFS showed the lowest enhancement effect ($E_{\text{ext}} = 3.33$) under iontophoresis after the removal of SC (Table 2). The iontophoretic enhancement of DFD was significantly higher than that of DFS and DFP after the removal of SC or lipid matrix (Table 1). DFS showed the highest enhancement effect among the three salts ($E_{\text{ext}} = 5.83$) after iontophoretic permeation across furry rat skin (Table 2). However, this value did not exceed the enhancement ratio of DFS across nude mouse skin ($E_{\text{ext}} = 6.50$). The same result was observed for DFP.

The effect of iontophoresis on cardamom oil-pretreated skin is summarized in Fig. 6. The enhancement effect of permeation after pretreatment of skin under iontophoresis was significantly lower than that under passive diffusion. It may be that the enhancement due to iontophoresis is so large that the enhancement due to penetration enhancer is negligible in comparison. There is either no effect or a negative effect on

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![Fig. 3. Passive Flux of Diclofenac Salts across Intact Nude Mouse Skin and Cardamom Oil-Pretreated Skin](image)

All data represent the means of three experiments ± S.D.

![Fig. 4. Cumulative Amount of Diclofenac Salts Released per Unit Area (nmol/cm²) versus Time Profiles across Cellulose Membrane under Iontophoresis](image)

All data represent the means of three experiments ± S.D.

![Fig. 5. Cumulative Amount of Diclofenac Salts Permeated per Unit Area (nmol/cm²) versus Time Profiles across Nude Mouse Skin under Iontophoresis](image)

All data represent the means of three experiments ± S.D.

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**Table 2. The Data for in Vitro Iontophoresis Permeation of DFS, DFP and DFD across Various Skin**

<table>
<thead>
<tr>
<th>Skin types</th>
<th>DFS</th>
<th>DFP</th>
<th>DFD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flux (nmol/cm²/h)</td>
<td>$E_{\text{skin}}$</td>
<td>$E_{\text{ext}}$</td>
</tr>
<tr>
<td>Cellulose membrane</td>
<td>1504.98 ± 70.11</td>
<td>1.36</td>
<td>1488.85 ± 55.80</td>
</tr>
<tr>
<td>Nude Mouse</td>
<td>151.54 ± 13.83</td>
<td>1.00</td>
<td>139.90 ± 27.27</td>
</tr>
<tr>
<td>SC-stripped</td>
<td>497.92 ± 163.18</td>
<td>1.76</td>
<td>547.25 ± 235.22</td>
</tr>
<tr>
<td>Delipid</td>
<td>540.09 ± 120.12</td>
<td>0.81</td>
<td>1073.05 ± 57.65</td>
</tr>
<tr>
<td>Rat</td>
<td>87.23 ± 18.38</td>
<td>0.58</td>
<td>34.44 ± 2.87</td>
</tr>
<tr>
<td>Cardamom oil-treated</td>
<td>184.98 ± 6.04</td>
<td>1.22</td>
<td>136.54 ± 27.76</td>
</tr>
</tbody>
</table>

a) DFS, diclofenac sodium.  b) DFP, diclofenac potassium.  c) DFD, diclofenac diethylamide.  d) $E_{\text{skin}}$ = iontophoretic flux across skin/iontophoretic flux across hairless mouse skin.  e) $E_{\text{ext}}$ = iontophoretic flux across skin/passive flux across skin. Each value represents the mean ± S.D. ($n$ = 3).
the iontophoretic transport of DFS and DFP after pretreatment of cardamom oil.

DISCUSSION

Passive Permeation of Diclofenac Salts The three diclofenac salts showed differences in the release rate across cellulose membrane with the trend of DFS > DFP > DFD (Table 1). This indicates that the counterions may play an important role in the release of diclofenac. Although the release rate of DFD was the lowest among the three salts, the flux of DFD across intact skin was comparable (ANOVA test, \( p > 0.05 \)) to that of DFS and DFP (Table 1). According to the data from artificial membranes and intact skin, the possibility of that counterions influence the skin permeation of diclofenac was elucidated. There are three routes for a drug to permeate the skin: (1) intracellular (transcellular); (2) intercellular (paracellular); and (3) transappendageal (shunt). Once into the SC, drug flux branches to these multiple pathways. Various types of skin were used as the barriers in this study to obtain mechanistic information for the delivery of diclofenac salts. The data in Table 1 shows that the flux of diclofenac salts across SC-stripped skin was much higher than that across intact skin, suggesting the rate-limiting properties of SC in passive permeation of diclofenac salts. The lower enhancement effect in flux for DFS and DFD relative to DFP after removal of SC indicates that the isolated dermis, lacking the skin's naturally impermeable tissue layer, still had a pronounced barrier effect on DFS and DFD.

Skin is known to consist of lipids (15–20%), proteins (40%, mostly keratin), and water (40%). There is significant evidence for transdermal drug delivery being by the intercellular lipid route. In order to verify this mechanism, delipid skin was used as the barrier for diclofenac salts. Extraction of lipid greatly increased the permeation of diclofenac salts especially for DFS and DFP, suggesting the importance of the intercellular route for both drugs in passive permeation (Table 1). This result should not be interpreted so as to entirely exclude the participation of pathways other than intercellular route since the flux of diclofenac salts across delipid skin was still lower than the release rate across cellulose membrane, through which the drug molecules can diffuse freely.

Nishihata et al. suggest that dithiothreitol and ascorbate increase the permeation of diclofenac across excised rat dorsal skin by increasing the protein thiol content in keratinized tissue of the skin. Takahashi et al. also suggest the flux of diclofenac from vehicles containing urea was higher than that from the vehicles without urea, possibly due to the hydration enhancement of cornified skin. The above results indicate the possible pathway of diclofenac salts via the intracellular route.

Hair follicles and sweat glands have also been postulated to provide a shunt pathway, by which ionic molecules can traverse the skin without being exposed to the lipid environment of the skin. The flux of diclofenac salts across furry Wistar rat skin increases in the order of DFS > DFD > DFP. This trend was quite different to that across nude mouse skin. In skin with a high density of hair follicles such as furry rat skin, shunt transport prevailed. This observation indicates that the transfollicular route constitutes the important permeation pathway for DFS but not DFP in passive diffusion. This result may reflect the less important route of SC for passive permeation of DFS than DFP according to the data of SC-stripped skin in Table 1.

The passive permeation of diclofenac salts was significantly enhanced by cardamom oil pretreatment of skin. In a histological study of skin structure, cardamom oil enhanced the permeability of skin by disturbing the lipid matrix in SC layer. Both the SC and lipid matrix in skin had important roles in the passive permeation of DFP according to the data in Table 1, resulting in the highest enhancement of DFP permeation after pretreatment of cardamom oil. On the other hand, the lowest \( E_{\text{skn}} \) value of DFD after cardamom oil pretreatment may have been due to the less important role of lipid for passive permeation of DFD than the other salts (Table 1).

Iontophoretic Permeation of Diclofenac Salts The magnitude of permeation enhancement achieved by iontophoresis appears to be relatively low in the case of cellulose membrane. This is due to the relatively high hydrophilic nature of the membrane used in this study which was shown to result in a relatively high passive diffusion of drug molecules. Therefore, the cellulose membrane may not reflect the actual enhancement of diclofenac salts permeation by iontophoresis.

The trend of iontophoretic permeation of the three diclofenac salts across intact skin is somewhat different to that of passive permeation (Fig. 5). The iontophoretic flux and enhancement ratio of diclofenac salts across skin increases in the order of DFS > DFP > DFD. Yoshida and Roberts have suggested that the iontophoretic behavior of anionic solutes including diclofenac can be best described by the free volume model. According to this model, the ion sphere mobility has been assumed to be proportional to the fractional volume of the space that is accessible to the ion sphere. Therefore, the molar volume as well as solute radius have been shown to be inversely related to iontophoretic mobility. In addition to these ionselective properties, the skin also shows size-selective effects in iontophoretic transport. Application of iontophoresis may increase the porosity and create pores with effective radii in the lipid matrix. Although the diclofenac anion was dissociated in the donor compartment...
during iontophoresis, the radius and mobility of diclofenac anion can be affected by its counterion.\textsuperscript{3} The previous studies show that the radius of diclofenac anion increased with the increase of the molecular weight and radius of its counterion.\textsuperscript{2,22} Therefore the radius of diclofenac showed the trend of DFD > DFP > DFS, which has been shown to be inversely related to the iontophoretic enhancement effect of diclofenac salts (Table 2).

The skin permeability may be temporarily altered during iontophoresis by: (1) an expansion of the existing channels or creation of new pores; (2) the fluidization of lipid matrix in the intercellular spaces; or (3) the rearrangement of proteins in SC.\textsuperscript{24,25} The route and mechanism for iontophoretic permeation of drugs across the skin may be different to that for passive permeation. Table 2 shows the flux and enhancement of diclofenac salts across various skin types under iontophoresis. The $E_{\text{skin}}$ value of diclofenac salts across SC-stripped skin is lower than that across intact skin. Moreover, the $E_{\text{skin}}$ value is much lower for iontophoretic permeation than for passive diffusion after the removal of the SC layer. These data indicate that the importance of SC as a rate-limiting barrier was reduced for the permeation of diclofenac salts across skin during iontophoretic application. That is, the rate-limiting characteristics of SC in passive permeation could be overcome by iontophoresis. Similar to the results of the passive permeation experiment, DFS showed the lowest enhancement effect under iontophoresis after the removal of SC. This indicates that the outermost layer of skin represents a less important barrier for DFS than the other salts in both passive and iontophoretic delivery.

The importance of the intercellular route in transdermal iontophoresis has also been proposed.\textsuperscript{15,21} The mechanism of this route can be a fluidization of the lipids induced by an electric field. The lipids would then become more mobile and molecular-sized pores may be formed in the lipid matrix.\textsuperscript{26,27} This may result in the lipid interior becoming more accessible to diclofenac salts. The iontophoretic enhancement of DFD was significantly higher than that of DFS and DFP after the removal of SC or lipid matrix. We supposed that DFS and DFP with smaller radii transport via the intercellular route in SC more easily than DFD because of the formation of molecular-sized pores in the lipid matrix by iontophoresis. Extraction of lipid from the skin would diminish this effect, resulting in the lower enhancement of DFS and DFP across SC-stripped and delipidated skins than DFD after iontophoretic application. It may also suggest that the lipid matrix in SC constitutes the important pathway for DFS and DFP, and pathways other than the intercellular route should be elucidated for DFD. This result is similar to that of passive permeation, which showed that the lipid pathway was less important for DFD than the other salts.

The effect of hair follicle distribution on iontophoretic permeation was also studied. DFS showed the highest enhancement effect after iontophoretic permeation across furry rat skin among the three salts (Table 2). However, this value did not exceed the enhancement ratio of DFS across nude mouse skin ($E_{\text{skin}} = 6.50$). The same result was observed for DFP. This indicates that the permeation of DFS and DFP via the transfollicular route can not be activated by an electric field. The furry rat skin provided a higher enhancement for DFD than nude mouse skin after applying iontophoresis, suggesting the advantage of hair follicles for iontophoretic transport of DFD. Another possible pathway for diclofenac salts under iontophoresis is the transcellular route, in which drugs are transported directly through cornecyes comprising the SC. However, this pathway is expected to be less important in the presence of low-resistance appendages.\textsuperscript{22} Further investigations on the mechanism and role of the transcellular route under iontophoresis are in progress in our laboratory.

The effect of iontophoresis on cardamom oil-treated skin is summarized in Fig. 6. The enhancement effect of permeation after pretreatment of nude mouse skin under iontophoresis was significantly lower than that under passive diffusion. It may be that the enhancement due to iontophoresis is so large that the enhancement due to penetration enhancer is negligible in comparison. There is either no effect or a negative effect on the iontophoretic transport of DFS and DFP after pretreatment of cardamom oil. It was found that the main mechanism of action of cardamom oil is disordering of the lipids in SC.\textsuperscript{10} This effect may close up the intercellular route for drugs after iontophoretic application.\textsuperscript{28} According to the results discussed earlier, the intercellular route constitutes the important pathway for iontophoretic transport of DFS and DFP, resulting in the poor efficiency of combining iontophoresis and a penetration enhancer. This explanation can also be used to rationalize the result which showed that the combination further increased the flux of DFD. Iontophoresis can facilitate the transfollicular route of DFD, which is distinct from the intercellular route in skin disordered by cardamom oil.

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