Effect of Iontophoresis and Switching Iontophoresis on Skin Accumulation of Ketoprofen

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The effect of iontophoresis and switching iontophoresis on the skin accumulation of drugs was investigated. An acrylic diffusion cell mounted with electrode cells (bore: 2.2 cm) with circular platinum electrodes (diameter: 2.0 cm) was used for the skin accumulation study. The skin accumulation of fluorescein after non-switching and switching iontophoresis was macroscopically compared with that achieved by passive diffusion (control). Intense fluorescence was observed after the application of non-switching and switching iontophoresis. Furthermore, fluorescence was observed just under the electrode cell and hardly spread in the skin beyond the area of the electrode cell. The skin accumulation of ketoprofen after non-switching and switching iontophoresis was also compared with control data. Although non-switching iontophoresis showed the highest amount of ketoprofen accumulated in skin, skin irritation was observed. Among the various switching intervals, switching iontophoresis using 10-min intervals achieved the highest value, and there was no skin irritation. Furthermore, the amount of ketoprofen accumulated was maintained after switching iontophoresis at 10-min intervals up to 180 min. Since the amount of ketoprofen in skin after switching iontophoresis was greater than that after intermittent iontophoresis, switching iontophoresis should increase the amount of ketoprofen due to enhancement of skin penetration by skin hydration. These findings suggest that switching iontophoresis using an optimal switching interval can prevent skin irritation and enhance drug accumulation in the skin.

Key words switching iontophoresis; skin accumulation; ketoprofen; skin hydration

Transdermal therapeutic dosage forms have been applied to the treatment of local and systemic diseases. Since drug delivery through the skin is often limited, physical methods have been used to enhance transdermal drug transport.1—7) Iontophoresis is one of the physical methods used to deliver ionic compounds to the body using an electrorepulsive force. The permeability of nonionic compounds is also enhanced by iontophoresis via electroosmosis and convective water flow.8—10) There are reports that systemic drug delivery is enhanced using iontophoresis.11,12) Iontophoresis has also been applied to topical preparations to increase the local therapeutic effect.13—15) For local pain control, transdermal administration of local analgesics using iontophoresis may be useful as an alternative to injection. Although antifungal agents are orally administered for the treatment of tinea unguium, problems such as systemic side effects and drug interactions have been reported. If the antifungal agent can be delivered effectively to the local lesion using iontophoresis, those problems may be resolved.

In drug delivery through the skin using iontophoresis, skin irritation is a problem. Previously, we reported that switching iontophoresis (switching the polarity of the electrodes periodically) could decrease the skin irritation and enhance the permeation of peptides and compounds through the skin.16—19) This effect is thought to be achieved because switching iontophoresis prevents the skin from polarizing and enhances skin hydration.10,20)

In this study, ketoprofen was used as a model drug, and drug accumulation in rat skin following iontophoresis and switching iontophoresis was investigated. Then, we estimated the optimal conditions for iontophoresis of topical preparations.

MATERIALS AND METHODS

Materials Fluorescein sodium, ketoprofen and acetonitrile (HPLC grade) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were obtained commercially as the purest grade available.

Animals Male Wistar rats weighing 190—240 g were purchased from Tokyo Laboratory Animals Science Co., Ltd. (Tokyo, Japan). The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Hoshi University. The guidelines for animal experimentation of Hoshi University conform to the basic guidelines published by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Diffusion Cell The acrylic diffusion cell used for skin accumulation studies is shown schematically in Fig. 1A. After removing the hair from rats using electric clippers, dorsal skin was excised and mounted on a diffusion cell with the epidermal side facing the electrode cells. The exposed skin area was 4.0 cm × 7.5 cm, and the distance between the edges of the electrode cells was 2.0 cm. The receiver side was filled with phosphate buffer (pH 7.4) and the receiver solution was magnetically stirred and maintained at 37 °C. The electrode cell (bore: 2.2 cm, application area: 3.8 cm 2) made of glass is shown schematically in Fig. 1B. The electrode (diameter: 2.0 cm, thickness: 0.1 mm) used was a circular disk of platinum. The distance between the electrode and skin was 1.5 cm. The electrode was fixed to the cell using 1.5% (w/v) agar gel containing 0.05% (w/v) NaCl, and donor solutions were injected to fulfill the space between the agar gel and the skin.

Evaluation of Skin Accumulation of Fluorescein Since fluorescein sodium is dissociated and charges negatively in water, fluorescein aqueous solution (1 mg/ml) was injected to the cathodal electrode cell as a donor solution,

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Fig. 1. Schematic Representation of the Diffusion Cell (A) and Electrode Cell (B) Used for Skin Accumulation Studies

and normal saline was injected to the anodal electrode cell. Then, direct electric current of 0.76 mA (0.2 mA/cm²) was applied for 60 min. Switching iontophoresis was also performed. The direct electric current of 0.76 mA was applied for 10 min, then the polarity of the electrodes was switched for 10 min. Switching of the polarity was performed repeatedly for 60 min. Furthermore, we also performed switching iontophoresis using 10-min intervals where the distance between the electrode cells was 1 mm. After non-switching and switching iontophoresis, the skin under the electrode cells was washed with purified water, and the electrode cells were removed. Then, the skin was observed under ultraviolet irradiation, and the skin accumulation of fluorescein was compared with that by passive diffusion where no electric current was applied.

**Evaluation of Skin Accumulation of Ketoprofen** Three percent ketoprofen suspension in 0.05 mol/l Tris–HCl buffer (pH 8.0) was prepared as a donor solution. Since ketoprofen charges negatively in alkaline solution, the ketoprofen suspension was injected to the cathodal electrode cell. Normal saline was injected to the anodal electrode cell. Then, a direct electric current of 0.76 mA (0.2 mA/cm²) was applied for 60 min. Switching iontophoresis using 1, 2.5, 5, 10 and 15 min intervals was also performed for 60 min. After non-switching iontophoresis and switching iontophoresis using a 10-min interval, passive diffusion using the same ketoprofen suspension was continued for 120 min, and ketoprofen concentration in skin and receiver solution were determined at the predetermined time. Furthermore, irregularly switched iontophoresis was also performed. Direct current was applied for 9 min, followed by switching the polarity of the electrodes for 1 min, and repeated for 60 min. As a control, passive diffusion of ketoprofen to the skin was carried out.

Further, to examine the effects of switching iontophoresis on the skin, the skin resistance was measured 60 min after non-switching iontophoresis, switching iontophoresis and passive diffusion using a YX-360TR tester (Sanwa Electric Instrument Co., Tokyo, Japan).

**Determination of Ketoprofen Concentration in Skin and Receiver Solution** At the end of the skin accumulation study, skin under the cathodal electrodes was washed using 5 ml of phosphate buffer (pH 7.4) and excised to determine the skin concentration of ketoprofen. Skin specimens were weighed, and homogenized with phosphate buffer (pH 7.4), then stored at 5 °C for 24 h to allow adequate extraction. After centrifugation (2000 rpm×5 min), a 200 μl aliquot of supernatant was obtained. Hydrochloric acid (1 mol/l, 100 μl) was added to the supernatant, and ketoprofen was extracted with ether (5 ml). The ether layer was separated by centrifugation (3000 rpm×10 min) and evaporated to dryness under a nitrogen stream. The mobile phase (500 μl) was added to dissolve the residue. A 20-μl aliquot of the final preparation was injected into the HPLC system. HPLC was carried out using an LC-6AD pump and a C-R7A plus chromatopac (Shimadzu, Kyoto, Japan) equipped with a Neopack C18 column (4.6 mm×250 mm, Nishio Industry Co., Ltd., Tokyo, Japan) and an SPD-10A V UV detector (Shimadzu) set at 256 nm. The mobile phase was a mixture of 5 mmol/l phosphoric acid–acetonitrile–methanol (40 : 30 : 30, v/v/v). The flow rate was 0.8 ml/min. Chromatography was carried out at room temperature.

Ketoprofen concentration in the receiver solution was determined by HPLC after filtration using Ekicrodisc® 13, 0.45 μm (Nihon Pall Ltd., Tokyo, Japan). A 20-μl aliquot of filtrate was injected into the HPLC system. HPLC analysis was performed in the same way as determination of the skin concentration.

**Determination of Ketoprofen in Tape-Stripped Stratum Corneum** The skin under the electrodes was washed using 100 ml of purified water at the end of the skin accumulation study in order to properly remove residual ketoprofen from the skin surface. Residual moisture on the skin surface was removed with filter paper. Each treated skin site was subsequently tape stripped with tape discs measuring 2.2 cm in diameter (Filmolux 609, Filmolux Co., Ltd., Tokyo, Japan). A tape disc was applied to the skin under the electrode, and a weight of 150 g was put on the tape disc for 20 s. Then, the tape disc was removed from the skin surface. Each subsequent tape disc applied to the same skin site removed another skin layer. This tape stripping procedure was repeated 11 times. The first of the 11 tape discs used was discarded because of potential contamination by residual drug on the skin surface. The remaining tape discs were combined and immersed in the mobile phase (4 ml). The mobile phase was a mixture of 5 mmol/l phosphoric acid–acetonitrile–methanol (40 : 30 : 30, v/v/v). Then, ketoprofen in the stripped skin layer was extracted for 24 h at 5 °C. The extract was filtrated using Acrodisc® LC13, 0.45 μm (Nihon Pall Ltd., Tokyo, Japan) and a 20-μl aliquot of the filtrate was injected into the HPLC system. HPLC analysis was performed.
in the same way as determination of the skin concentration.

**Evaluation of Skin Irritation**  After the skin accumulation study, the skin under the cathodal electrode (ketoprofen site) was excised, and fixed by immersion in 10% formalin for at least 24 h. Each skin sample was embedded in hard paraffin after dehydration using ethanol. Then, slices (thickness: ca. 3 μm) were prepared using a microtome, and stained with hematoxylin–eosin. The samples were observed under an optical microscope (X100). A vertical section of the magnified skin was divided into five categories, i.e. epidermis, subepidermis, dermis, hypodermis and skin appendages. Seven items (liquefaction of the epidermis; edema of the subepidermis; collagen fiber swelling of the dermis and hypodermis; inflammatory cell infiltration of the dermis and hypodermis; degeneration of skin appendages) were each ranked into five stages (0—4) symptomatically and were estimated as an irritation score by a histopathologist.

**Statistical Analysis**  Variance in a group was evaluated by the $F$-test. Student’s $t$-test or Welch’s $t$-test was used for the evaluation of differences depending on equal or unequal variance. Data were considered significantly different when the $p$-value was less than 0.05.

**RESULTS AND DISCUSSION**

**Effect of Iontophoresis on Skin Accumulation of Fluorescein**  In Figs. 2A—C, photographs of skin 60 min after non-switching iontophoresis, switching iontophoresis using 10-min intervals and passive diffusion (control) are shown. Intensive fluorescence was observed after application of non-switching and switching iontophoresis as compared with control. Based on these findings, it was confirmed that fluorescence was observed just under the cathodal electrode and fluorescein hardly spread in the skin beyond the area of the electrode cell. Figure 2D shows a photograph of the skin after switching iontophoresis using 10-min intervals, where the distance between the electrode cells was 1 mm. In clinical practice, the possibility that the cathodal electrode and the anodal electrode would be closely applied is considered. Hence, we brought the electrode cells closer to 1 mm. Based on these findings, it was confirmed that fluorescein diffused under the cathodal electrode and was not attracted toward the anodal electrode even if the electrode cells were placed closely together.

**Effect of Iontophoresis on Skin Accumulation of Ketoprofen**  In Fig. 3A, the amounts of ketoprofen accumulated in skin 60 min after non-switching iontophoresis, switching iontophoresis and passive diffusion (control) are shown. The amounts of ketoprofen in skin were increased by non-switching and switching iontophoresis compared with that in the control. Non-switching iontophoresis showed the highest value and switching iontophoresis with a 10-min interval showed the second highest value. The amounts of ketoprofen permeated through skin after 60 min of non-switching iontophoresis, switching iontophoresis and passive diffusion are shown in Fig. 3B. Higher values were shown for switching iontophoresis using 1- and 2.5-min intervals, and for non-switching iontophoresis. Figure 4 shows the ratio of skin resistance after non-switching iontophoresis, switching iontophoresis using 10-min intervals and passive diffusion for 60 min to that prior to application. Skin resistance was reduced by the application of switching iontophoresis. Pikal and Shah reported that skin resistance was altered by hydration when iontophoresis was applied.24) Further, Burnette and Ongpipattanakul reported that (1) skin resistance was decreased by hydration, (2) conductance was increased and (3) the flux of ions was increased linearly by hydration.25) Previously, Ishikawa et al. reported that skin resistance was reduced after application of iontophoresis, and remarkable

![](image-url)
reduction was observed after application of switching iontophoresis. Furthermore, skin resistance was decreased as the switching interval shortened. Hence, as a reason for the absence of correlation between the amounts of ketoprofen in skin and the amounts of ketoprofen permeated through skin after switching iontophoresis using 1- and 2.5-min intervals, greater skin hydration was considered. As the methods of evaluating the extent of skin hydration, high frequency electric conductivity and transepidermal water loss have been performed. We should confirm the extent of skin hydration after switching iontophoresis using those methods in the future. Since a large amount of ketoprofen was observed in skin after non-switching iontophoresis, it was considered that the application of a continuous electrorepulsive force was important to increase the amount of ketoprofen in skin. Switching iontophoresis using 10-min intervals enhanced skin hydration to some extent and applied continuous electrorepulsive force. Hence, the amount of ketoprofen in skin might be increased after switching iontophoresis using 10-min intervals. In contrast, although continuous electrorepulsive force was applied by switching iontophoresis using 15-min intervals, skin hydration might hardly be enhanced. Figure 5 shows the amount of ketoprofen that accumulated in skin after intermittent iontophoresis, i.e., direct electric current was applied for 10 min, then the electric current was turned off for 10 min, and the cycle was repeated in this manner for 60 min. Since the amount of ketoprofen after switching iontophoresis was greater than that after intermittent iontophoresis, the amount of ketoprofen accumulated should be increased due to skin hydration by switching iontophoresis. These findings suggest that penetration of the drug is enhanced by skin hydration and electrorepulsive force during switching iontophoresis using an optimal switching interval (10-min interval in this study). However, the skin hydration should be lower with shorter switching intervals (1- and 2.5-min intervals in this study). Hence, the amount of the drug permeated through the skin after switching iontophoresis using 10-min intervals is suppressed compared with that after switching iontophoresis using 1- and 2.5-min intervals. Figure 6A shows the skin accumulation profiles of ketoprofen following iontophoresis applied for 60 min and then passive diffusion for 120 min. The amount of ketoprofen accumulated was decreased gradually after non-switching iontophoresis. In contrast, the amount of ketoprofen accumulated was maintained until 180 min after switching iontophoresis. The skin permeation profiles of ketoprofen are also shown in Fig. 6B. Flux was calculated from the slope of the linear portion of the cumulative amount permeated-time plots for a zero-order model and expressed as the mass of ketoprofen passing across 1 cm² of skin over time. The flux on non-switching and switching iontophoresis was 17.0 µg/cm²/h and 7.20 µg/cm²/h, respectively. The higher value was observed for non-switching iontophoresis. These findings suggest that ketoprofen should be distributed at a high concentration in skin from the epidermis side to the dermis side after non-switching iontophoresis, and then the drug concentration gradient might gradually change to a state of passive diffusion. Hence, the drug concentration in skin decreased with increase in the amount of the drug permeated through the skin. In contrast, the drug concentration gradient after switching iontophoresis using 10-min intervals might be similar to that after passive diffusion. After switching iontophoresis, enhancement of
penetration of the drug into the skin might be maintained for a while by the influence of skin hydration. Hence, it was considered that the amount of the drug accumulated was maintained for 120 min after switching iontophoresis.

The amounts of ketoprofen accumulated in the stratum corneum 60 min after non-switching iontophoresis, switching iontophoresis and passive diffusion are shown in Fig. 7. Greater values were observed after passive diffusion and non-switching iontophoresis. Since the amounts of the drug in the stratum corneum after passive diffusion and non-switching iontophoresis were consistent, the possibility that the drug can not be pooled in the stratum corneum is considered. After switching iontophoresis, the amount of ketoprofen decreased with increases in the switching interval. It was considered that ketoprofen was charged negatively and pulled back by the switched electric current just before the end of the experiment. However, since switching iontophoresis showed that a greater amount of drug accumulated in skin compared with that following intermittent iontophoresis, only the drug in the surface of skin could be pulled back during the application of switched electric current. Furthermore, a possibility that ketoprofen in the stratum corneum might permeate into the dermis side according to convective-flow by electroosmosis during the application of switched electric current was considered. Thus, during the application of switched electric current, enhancement of drug penetration by skin hydration, drawing out of the drug from the skin surface and permeation of the drug to the dermis side according to convective-flow by electroosmosis might affect transdermal drug delivery.

**Evaluation of Skin Irritation after Iontophoresis** Figure 8 shows microphotographs of vertical sections of rat skin 60 min after non-switching iontophoresis, switching iontophoresis and passive diffusion. Skin sections examined histopathologically were scored for skin irritation as shown in Table 1. There was no apparent skin irritation after switching iontophoresis using 10-min intervals or after passive diffusion. In contrast, after non-switching iontophoresis edema of the subepidermis was observed in all rat skin specimens, and liquefaction of the epidermis was observed in one rat skin specimen. These findings suggest that switching iontophoresis can suppress skin irritation by preventing the skin from polarizing. Furthermore, in the previous study, it was reported that pH of a donor solution in the cathodal electrode cell after switching iontophoresis using 5-, 10-, 20-min intervals did not change significantly, although pH of a donor solution after non-switching iontophoresis changed significantly.

![Fig. 7. Amount of Ketoprofen Accumulated in Stratum Corneum after Iontophoresis for 60 min](image)

![Fig. 8. Microphotographs of Vertical Section of Skin after Iontophoresis for 60 min](image)

**Table 1. Evaluation of Skin Irritation by Histopathological Findings**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Control (passive)</th>
<th>Non-switching</th>
<th>Switching (10 min)</th>
<th>Switching (9—1 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquefaction</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Subepidermis</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Edema</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Dermis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen fiber swelling</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Inflammatory cell infiltration</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hypodermis</td>
<td></td>
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<tr>
<td>Collagen fiber swelling</td>
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<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Inflammatory cell infiltration</td>
<td>—</td>
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<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Skin appendages</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Degeneration</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total irritation score</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Score 0, no change; 1, very slight; 2, slight; 3, moderate; 4, marked. The irritation scores were estimated by a histopathologist (n=1).
cantly.16) Hence, in this study, a possibility that a cause of skin irritation after non-switching iontophoresis was alteration of pH of the donor solution was also considered. Since switching iontophoresis effectively prevented skin irritation, we investigated whether irregularly switched iontophoresis would increase drug accumulation in the skin, while suppressing skin irritation. Figure 5 also shows the amounts of ketoprofen accumulated in skin 60 min after irregularly switched iontophoresis, i.e. direct electric current was applied for 9 min, followed by switching the polarity of the electrodes for 1 min. The accumulated amount of the drug increased after irregularly switched iontophoresis compared with that after switching iontophoresis using 10-min intervals. However, liquefaction of the epidermis was observed in all rat skin specimens after irregularly switched iontophoresis as shown in Fig. 8 and Table 1. Based on these findings, 1 min of switched electric current might not be sufficient for depolarization of the skin following 9 min of electric current application.

CONCLUSIONS

Previously, the effectiveness of switching iontophoresis for systemic treatment was investigated. In this study, we tried to investigate the effectiveness and optimal condition of iontophoresis for local treatment. From the observation of skin after the application of fluorescein, it was confirmed that fluorescein hardly spread in the skin beyond the area of the electrode cell. Thus, there is a potential for drug administration targeting the focal site of local diseases by iontophoresis. Although non-switching iontophoresis induced the highest amount of ketoprofen accumulation in skin, skin irritation was also observed. Among the various switching intervals examined, switching iontophoresis using 10-min intervals showed the highest amount of ketoprofen accumulation in skin without causing skin irritation. Furthermore, the amount of ketoprofen accumulated was maintained until 180 min after switching iontophoresis. Since switching iontophoresis showed a greater amount of drug accumulation in skin compared with that by intermittent iontophoresis, an enhancement of drug penetration by skin hydration was suggested. Although the amount of ketoprofen accumulated in the stratum corneum decreased after switching iontophoresis, this reduction might indicate enhanced drug permeation in the stratum corneum. Since switching iontophoresis using the optimal switching interval could prevent skin irritation and enhance drug accumulation in skin, the effectiveness of switching iontophoresis for local treatment was suggested.

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