Molecular State of Active Pharmaceutical Ingredients in Ketoprofen Dermal Patches Characterized by Pharmaceutical Evaluation

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The molecular states of ketoprofen and the interaction between ketoprofen and other pharmaceutical excipients in the matrix layer were examined to determine their effect on the pharmaceutical properties of original and generic ketoprofen dermal patches (generic patches A and B). Molecular states of ketoprofen were evaluated using polarized light microscopy, Raman spectroscopy and powder X-ray diffraction. For the original ketoprofen patch, crystalline components were not observed in the matrix layer. However, crystalline ketoprofen was observed in the two generic ketoprofen patches. Moreover, the ketoprofen exhibited hydrogen bonding with the pharmaceutical excipients or patch materials in the generic products. Skin permeation of ketoprofen from the patches was evaluated using hairless mouse skin. Twelve hours after application, the original patch demonstrated the highest level of cumulative skin permeation of ketoprofen. This was followed by generic patch B while generic patch A showed the lowest level of permeation. Fluxes were calculated from the skin permeation profiles. The original patch was approx. 2.4-times faster compared with generic patch A and approximately 1.9-times faster compared with generic patch B. This investigation suggested that pharmaceutical properties such as skin permeability for these types of products are affected by the precipitation of crystalline ketoprofen in the matrix layer and the interaction of ketoprofen with the pharmaceutical excipients or patch materials.

Key words ketoprofen dermal patch; Raman spectroscopy; skin permeability

Ketoprofen is a non-steroidal anti-inflammatory drug (NSAID). This class of drugs is widely used not only in oral dosage forms but also in topical formulations such as dermal patches, creams and gels for treatment of pain associated with rheumatoid arthritis and musculoskeletal pain. In general, compared to oral dosage forms, NSAID topical formulations have many advantages including avoidance of adverse gastrointestinal effects and hepatic pass effects. Ketoprofen is used particularly as the active pharmaceutical ingredients in topical formulations, because of its excellent skin permeability and pain relief properties with moderate plasma concentrations. Since NSAID dermal patches offer the advantages of dose simplicity and easy self-management, these formulations are commonly used for topical administration.

The topical ketoprofen patches fall into two formulation categories. These include poultice-type patches consisting of water-soluble polymers and nonwoven fabrics, and tape-type patches consisting of hydrophobic polymers and textiles comprised of polyethylene terephthalate. Previously, poultice-type patches had been used predominantly because of the beneficial cooling effect caused by the evaporation of water and the excellent drug release properties. However, the disadvantage of poultice patches is that they can be easily peeled off. At present, patch preparations are commonly composed of an acrylic polymer which facilitates excellent solubility of drug and pharmaceutical excipients and also offers excellent skin adhesion properties.

Eleven kinds of ketoprofen patches containing original and generic formulations are currently available. The Japanese market for ketoprofen patches was greater than 1.3 billion dollars in 2013. According to data from CRECON Research & Consulting, the current market share of the original innovator’s patch is approximately 40%. This did not change from 2006 to 2014. As a reason of no permeating of generic patches, the differences of feeling is suggested. Moreover the differences of pharmaceutical properties between original and generic patches were reported. Ohtani et al. reported a significant difference in the permeability of ketoprofen through rat or human skin between the original and generic patches. Based on this, we hypothesized that the differences between these products are caused by the molecular state of ketoprofen and the interaction between ketoprofen and other pharmaceutical ingredients in the patches. In this study, we evaluated the pharmaceutical properties of the ketoprofen patches related to these differences. We used polarized light microscopic analysis, X-ray diffraction measurements and Raman spectroscopy to accomplish this evaluation. The ketoprofen release properties of the two types of generic patches and the original patch were evaluated by dissolution testing and skin permeation testing.

MATERIALS AND METHODS

Materials The Mohrus patch (20mg/1.0g in matrix layer, 70×100mm, 473.7±9.3mm (thickness), Hisamitsu Pharma-
Table 1. Pharmaceutical Ingredients Included in Dermal Patches

<table>
<thead>
<tr>
<th>Pharmaceutical ingredients</th>
<th>Original</th>
<th>Generic A</th>
<th>Generic B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogenated rosin glycerol ester</td>
<td>○</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Styrene-isoprene-styrene-block copolymer</td>
<td>○</td>
<td>—</td>
<td>○</td>
</tr>
<tr>
<td>Polyisobutylene</td>
<td>○</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Alicyclic hydrocarbon resin</td>
<td>—</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Polybutene</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>ε-Menthol</td>
<td>○</td>
<td>—</td>
<td>○</td>
</tr>
<tr>
<td>Liquid paraffin</td>
<td>—</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>4-tert-Butyl-4′-methoxydibenzoylmethane</td>
<td>○</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Butylated hydroxytoluene (BHT)</td>
<td>○</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Crodamol</td>
<td>—</td>
<td>—</td>
<td>○</td>
</tr>
<tr>
<td>Isopropyl myristate</td>
<td>—</td>
<td>○</td>
<td>—</td>
</tr>
<tr>
<td>Other ingredients</td>
<td>5</td>
<td>2</td>
<td>—</td>
</tr>
</tbody>
</table>

○: Included, —: Not included.

daceutical Co., Inc., Japan) was used as the reference original patch marketed by the formulation innovator. The Patell patch (20 mg/1.0 g in matrix layer, 70 × 100 mm, 444.3 ± 7.0 mm (thickness), Kyorin Pharmaceutical Co., Ltd., Japan) and the Teikoku ketoprofen patch (20 mg/0.7 g in matrix layer, 70 × 100 mm, 405.0 ± 2.6 mm (thickness), Teikoku Seiyaku Co., Ltd., Japan) were used as the generic patches for comparison. They were designated as generic patches A and B, respectively. These generic patches having crystalline compounds of ketoprofen, were selected by the differences of crystal state to original patch. The chemical compounds in each patch formulation are shown in Table 1.\(^{18–20}\) All chemicals used for the experiments reported in this paper were reagent grade and used as received.

**Polarized Light Microscopic Observation** Matrix layers in the patch were observed by polarized light microscopy (Nikon ECLIPSE E600W POL, Japan) equipped with a temperature-controlled stage (Linkam, U.K.).

**Powder X-Ray Diffraction (PXRD)** PXRD experiments were performed with Cu-Kα radiation (λ=1.5418 Å) generated by a X’Pert PRO MPD (PANalytical, Japan) operating a 40° to 80° scan range from 5° to 35° at a voltage of 45 kV and a current of 40 mA. Three patches were piled on the goniometer as measurement samples.

**Raman Microscopy** The Raman spectra of the pharmaceutical dosage preparations were acquired with a Raman microscope (Work Station\(^{21}\), Kaiser Optical Systems Inc., MI, U.S.A.) using a 785-nm excitation laser. An exposure time of 10 s and a 50× objective lens were employed.

**In Vitro Dissolution Test** Dissolution tests of the patches were performed by the paddle method using equipment listed in dissolution test \(<6.10\>\) Japanese Pharmacopoeia (JP)\(^{17}\). The tests were performed at 32°C. The dissolution test samples were prepared by the paddle over disk method which is listed in general test \(<724\>\) USP37 (DRUG RELEASE TRANSDERMAL DELIVERY SYSTEMS). Each patch was stamped out (4.15 cm\(^2\)) and inserted in disks (Transdermal Sandwich\(^{21}\) by Hanson Research). The disks were soaked in 900 mL of dissolution medium (phosphate buffered saline (PBS), pH 7.4). A rotational paddle speed of 50 rpm was employed. Five milliliters of dissolution medium was collected during the course of each test at 0.5, 1, 2, 4 and 24 h, and the same volume of pure PBS was added to the dissolution medium immediately after each sampling. The PBS solution composition was 138 mM NaCl, 2.7 mM KCl, 1.43 mM KH\(_2\)PO\(_4\) and 8.57 mM Na\(_2\)HPO\(_4\).

**In Vitro Skin Permeation Test** Skin permeabilities were evaluated by an automated transdermal evaluation system (Microette plus, Hanson Research Co., CA, U.S.A.) at 32°C. Test patches were produced by punching out 12-mm disks (1.1 cm\(^2\)) for the donor phase. These were placed on the top of hairless mouse skin\(^{21–23}\) and a 500 g weight was applied for 10 s. The excised skin of hairless mice (Laboskin\(^{2}\), HOS: HR-1 Male, 7 weeks, Hosino Laboratory Animals, Inc., Ibaraki, Japan) was used as a permeation membrane for the in vitro skin permeation test. PBS (7.0 mL), which was stirred at 600 rpm, was used as the receiver phase. One (1) mL of the incubated PBS was collected and replaced with an equal volume of fresh PBS each time. Each collected sample was centrifuged at 9000×g for 10 min. Quantification of the released ketoprofen in the supernatant was performed using HPLC.

**Extraction of Ketoprofen from Patch** Ketoprofen was extracted from the patches before and after the skin permeation tests by using an acetonitrile–methanol 80:20 (v/v) solution.\(^{17}\) The concentrations of ketoprofen in the extract were determined by HPLC.

**Quantification of Ketoprofen Using HPLC** Quantification of ketoprofen was performed using an HPLC system equipped with a C18 analytical column (Cosmosil 5C18-Ar-II, Nakalai Tesque, Japan). The flow rate of the mobile phase (0.01 M phosphate buffer (pH 2.2)–acetonitrile=60:40 (v/v)) was 1.2 mL/min and the temperature was maintained at 40°C. The eluent was monitored with a UV detector at 261 nm.\(^{26}\)

**Statistical Analysis** Tukey–Kramer were used to test the statistical differences in the permeation parameters of ketoprofen among the different patches.

**RESULTS AND DISCUSSION**

**Evaluation of Crystalline Components in the Patches** The molecular states of ketoprofen in the matrix layers of the original patch and the two generic patches were observed by polarized light microscopy. Figure 1a shows the polarized light image of the matrix layer in patches at room temperature (r.t.). Only network structures from the fiber were observed in the original patch. By contrast, birefringence between the network structures in generic patch A suggested crystalline components were present. Crystalline components were also
observed in generic patch B, but were not observed in all the fields of view.

If the observed crystalline components were ketoprofen, they should melt at or near the melting point (94–97°C). Therefore, polarized light microscopic observation was performed at temperature controlled conditions from 80–94°C (Fig. 1b). For the original patch, there were no changes observed even when heating was extended to near 100°C. For generic patches A and B, the putative crystalline components continued to be observed under 90°C. However, the presumed crystalline components disappeared above 90°C. Because the temperature at which these components disappeared corresponded to the melting point of ketoprofen, it was postulated that the observed birefringence was due to the presence of crystalline ketoprofen.

To evaluate the detailed information of the crystalline components in the patches, Raman spectroscopy and PXRD measurements were performed. Raman spectra and PXRD patterns of each component in the patches are shown in Figs. 2 and 3. From Fig. 2, the Raman spectrum of ketoprofen raw material exhibits prominent peaks at 1597 cm$^{-1}$ and 1652 cm$^{-1}$ (data g) which are attributed to aromatic ring and C=O stretching modes, respectively. Neither of these peaks attributed to ketoprofen were observed in spectra from the original patch (data b). On the other hand, in the spectra for crystalline components of matrix layer in generic patch A and generic patch B, a shoulder peak at 1600 cm$^{-1}$ (data c and e) and a shoulder peak at either 1654 cm$^{-1}$ (data c) or 1658 cm$^{-1}$ (data e) were observed. These peaks were not observed if spectra were collected in a region of the patch where there was no birefringence observed (data d and f). The Raman spectra of the patch fibers exhibited peaks at 1613 and 1724 cm$^{-1}$ (data a). Liu et al. have reported that Fourier transform (FT)-IR spectra of APIs in adhesive patches were found to shift due to interactions with the patch materials when deposited on these types of matrices. In particular, the COOH group of ketoprofen was found to form hydrogen bonds with the OH group of the pressure-sensitive adhesive. Thus, they observed a shift in the peak maximum for the C=O group in ketoprofen. This finding is consistent with the observations reported in this paper where small shifts were noted for this peak from 1652 cm$^{-1}$ (data g) to 1654 cm$^{-1}$ (data c) or 1658 cm$^{-1}$ (data e) in the two generic patches.

The observations from the PXRD experiments were, in
part, consistent with the findings from the Raman data. The PXRD patterns (Fig. 3) from generic patch A showed specific diffractions (14.5, 18.5, and 23.0°) attributed to ketoprofen. Interestingly, however, these diffractions were observed neither for the original patch nor generic patch B.

From the Raman and PXRD results, it was concluded that the birefringence observed in the microscopic examinations of both generic patches (Fig. 1), could be attributed to crystalline ketoprofen. In generic patch B, evidence of crystalline ketoprofen was not found by PXRD, even though it was detected by Raman spectroscopy. It is postulated that the reason for this was that the Raman measurement was more sensitive with respect to the detection of ketoprofen compared to the PXRD measurement. Moreover, microscopic analysis suggested that the level of crystalline formation in generic patch B was lower than that of patch A. This would be consistent with the fact that a PXRD diffraction peak was not observed for patch B. It is also worth noting that, while there appeared to be a greater level of ketoprofen crystallization in generic patch A, the shift in the C=O stretching band was greater for generic patch B. Hence, generic patch B may exhibit less crystallization compared to generic patch A but display a stronger interaction with excipients or patch materials. It cannot be conclusively stated that there is an inverse correlation between these two phenomena as suggested by this single observation, however, both factors would be expected to affect product performance.

**Drug Release Study of Original and Generic Patches**

The investigation outlined above suggested that the molecular states of ketoprofen and the interactions between ketoprofen and other pharmaceutical excipients or materials in the matrix layers were different in the original patch and the generic patches. We therefore investigated the effect of these differ-
The ketoprofen remaining in the skin permeation profiles of ketoprofen from each of the patches. Significant differences on ketoprofen dissolution from patches. Figure 4 shows the dissolution profiles of ketoprofen from each of the patches (all patches including 1.19 mg/4.15 cm² ketoprofen). After 4 h, the extent of the dissolution of ketoprofen was greatest for the original patch, followed by generic patches A and B. However, significant differences in the general dissolution pattern were not observed for the three products. The dissolution amount of ketoprofen was equivalent in all patches after 24 h.

Table 2 shows the skin permeation parameter and the skin permeation profiles of ketoprofen from each of the patches (all patches including 0.282 mg/1.0 cm² ketoprofen). The flux was calculated from the slope of the straight-line portion in the skin permeation profiles. Significant differences between the original patch and the generic patches were observed, and the flux of the original patch (14.6±2.8 µg/cm²/h) was approximately 2.4-times faster compared with generic patch A (6.2±1.5 µg/cm²/h) and approximately 1.9-times faster compared with generic patch B (7.7±2.6 µg/cm²/h). After 24 h, the cumulative skin permeations of ketoprofen from the original patch, generic patches A and B were 132.0±11.3, 114.9±16.8, and 97.8±11.9 µg/cm², respectively. The ketoprofen levels remaining in the patch matrices were 10.0, 19.5, and 23.4% in the original patch, generic patches A and B, respectively. The ketoprofen remaining in the skin permeation profiles of ketoprofen from each of the patches. Significant differences on ketoprofen dissolution from patches. Figure 4 shows the dissolution profiles of ketoprofen from each of the patches (all patches including 1.19 mg/4.15 cm² ketoprofen). After 4 h, the extent of the dissolution of ketoprofen was greatest for the original patch, followed by generic patches A and B. However, significant differences in the general dissolution pattern were not observed for the three products. The dissolution amount of ketoprofen was equivalent in all patches after 24 h.

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Table 2. Permeation Parameters of Ketoprofen from Each of the Three Patches

<table>
<thead>
<tr>
<th>Patches</th>
<th>Flux (µg/cm²/h)</th>
<th>Cumulative Amount (24 h) (µg/cm²)</th>
<th>Ketoprofen Remaining in the Patches (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>14.6±2.8</td>
<td>132.0±11.3</td>
<td>10.0</td>
</tr>
<tr>
<td>Generic A</td>
<td>6.2±1.5</td>
<td>114.9±16.8</td>
<td>19.5</td>
</tr>
<tr>
<td>Generic B</td>
<td>7.7±2.6</td>
<td>97.8±11.9</td>
<td>23.4</td>
</tr>
</tbody>
</table>

Each data represents the mean±S.D. (n=10: original and generic patch B, n=8: generic patch A). The asterisk are statistically significant difference, (***) indicating p<0.01.

The differences might influence the time to start to work. The data of flux correlated qualitatively with the rough level of crystallization observed in the three patches. It was concluded that the differences in skin permeability might be related to the differences in the molecular states of ketoprofen in the three patches as well as the interactions of ketoprofen with the pharmaceutical excipients or materials used for the matrix layers.

Inoue and Sugibayashi reported that the transdermal absorption of the amorphous form of ketoprofen was enhanced compared to that of the crystalline form. Because of the supersaturation of ketoprofen that was created by the amorphous content in patches, transdermal absorption of ketoprofen was enhanced. It was reported that both the miscibility of the drug in the polymer phase and the crystallization from the amorphous solid dispersion were related to the physical stability of the drug-polymer mixture and the thermodynamically controlled process of crystallization. In our study, the molecular states of ketoprofen between the original and the generic patches were likely different because of the varying miscibility of ketoprofen in the respective polymers. Ketoprofen appeared to form hydrogen bonds with the pharmaceutical excipients or materials used for the matrix layers in the generic patches. These interactions appeared to inhibit the drug release and also skin permeation of ketoprofen. Moreover, the interaction between the matrix and the skin surfaces might influence the ketoprofen permeability. All patches include L-menthol as a pharmaceutical excipient. Wu et al. reported that the penetration rate of ketoprofen from gels increase when L-menthol is added to the formulation until 5%, and become constant more than 5%. The temporal decreasing of L-menthol is assumed to influence to the penetration rate of ketoprofen too. We would like to examine the issues in the future about the effect of L-menthol concentration of patches on the penetration rate of ketoprofen and the time cause of concentration of L-menthol in each patches.

It is interesting that there were no significant changes in the release of ketoprofen in the dissolution test. This was thought
to be due to the rapid disruption of hydrogen bonding that takes place in PBS. The dissolution test data are generally assumed to reflect the skin permeation profile. However, the observations noted from this work suggest that the results of these two tests could be different. Hence, it can be stated that, when the pharmaceutical properties of patches are evaluated, multiple tests should be employed.

CONCLUSION

The molecular states of ketoprofen in the matrix layers were different among the original patch and the two generic patches examined in this study. This was determined by polarized light microscopy, Raman microscopy and X-ray diffraction studies. For the original ketoprofen patch, crystalline components were sparsely observed in the matrix layer. On the other hand, crystalline components were more prominent in both generic patches. Based on the skin permeability test, it was shown that the flux and the cumulative permeation of ketoprofen in the generic patches were lower than the original patch. This was noted in spite of the fact that the dissolution profiles of the three were not significantly different. We presumed that the differences in skin permeability among the three patches occurred due to the differences of 1) the miscibility of ketoprofen in the matrix layer and 2) the interaction between ketoprofen and the pharmaceutical excipients or materials such as polymers and l-menthol that used for the patch formulations.

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Conflict of Interest The authors declare no conflict of interest.

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