Skin permeability of tulobuterol in two transdermal formulations and their followability

Yuichi Takizawa1,*, Takeshi Goto2,3, Shuji Sato2,3, Naoya Ohmori2,3, Kenji Mori3, Yayoi Shimada3, Kuei-Chen3, Takamitsu Miyagi4, Fumio Fukai1

1 Faculty of Pharmaceutical Sciences, Tokyo University of Science, Chiba, Japan; 2 Faculty of Nursing, Josai International University, Chiba, Japan; 3 Kazusa Research Institute for Drug Discovery, Faculty of Pharmaceutical Sciences, Josai International University, Chiba, Japan; 4 Faculty of Pharmaceutical Sciences, Aomori University, Aomori, Japan.

Summary

Various generic transdermal formulations of tulobuterol containing rubber and acrylate base polymers are commercially available in Japan. However, none of the formulations have been compared directly with respect to the skin permeability of tulobuterol and to their follow ability. Tulobuterol Tape Sawai of rubber base and Tulobuterol Tape NP of acrylate base were used to conduct the in vitro 24-hour skin permeability test of tulobuterol at receiver solution temperatures of 32°C, 37°C, and 40°C. Furthermore, the followability of these tapes were examined by measuring the depth of the pores that were formed in their adhesive layer. Consequently, the maximum flux of tulobuterol was greater for Tulobuterol Tape NP. Arrhenius plot analysis revealed that Tulobuterol Tape Sawai was more sensitive to skin surface temperature compared with Tulobuterol Tape NP. Skin abrasion had a greater effect on the skin permeability of tulobuterol in Tulobuterol Tape Sawai than in Tulobuterol Tape NP. Followability was greater for Tulobuterol Tape NP than for Tulobuterol Tape Sawai. These results suggest that a transdermal formulation of acrylate base is preferable to that with a rubber base when skin surface temperature varies or when the skin is abraded. In clinical settings, therefore, a formulation of acrylate base is preferable to a formulation of rubber base when skin surface temperature varies or when the skin is abraded. The formulation needs to be applied to the skin of less asperity for the achievement of better transdermal absorption of tulobuterol.

Keywords: Tulobuterol, transdermal, rubber base, acrylate base, intact skin, abraded skin

1. Introduction

Various transdermal formulations of tulobuterol, including the pioneer transdermal patch, Hokunalin®, are commercially available in Japan (Table 1). Hokunalin® contains a rubber base polymer, in which only a small portion of tulobuterol is dissolved and most of the drug crystals is suspended in the adhesive layer. Thus, the concentration of tulobuterol dissolved in the layer is thus kept constant by a mechanism called
nonpolar functional groups, while acrylate base polymers contain polar functional groups (e.g., amido). The polar functional groups of acrylate base polymers interact with the polar functional groups (e.g., hydroxyl groups) of drugs, resulting in retention of drugs in base polymers (4,5). Hence, the skin permeability of tulobuterol that has the hydroxyl group may be affected by the polarity of base polymers.

Morimoto et al. conducted the release test of drugs containing the amido, carboxyl, or ester group into the 40% aqueous solution of polyethylene glycol (PEG) at 37°C; they calculated the distribution coefficients (logD), glass-transition temperature (Tg), and wavelength of the hydroxyl group and reported the amido group, amino group, carboxyl group, and ester group in decreasing order of the interactions of the function groups with base polymers (4,5).

Kato et al. prepared the transdermal formulations of tulobuterol containing gum, silicon, or acrylate polymers to examine the 24-hour releasability of the drug across rabbit skin (1). Consequently, they reported gum base, silicon base, and acrylate base in decreasing order of drug releasability. These findings led us to hypothesize that drug releasability would be controlled by base polymers, and we conducted an in vitro hairless mouse skin permeability test to confirm our hypothesis.

Transdermal formulations, also called pressure-sensitive agents (PSAs), acquire adhesion between the skin and their adhesive layer by applying a slight pressure when attached to the skin. Transdermal formulations are endowed with the function of PSAs by selecting base polymers with a low Tg to exert adhesion or by adding a tackifier at skin surface temperature (6,7). Tojo et al. reported lower elasticity and greater peeling strength in association with the lower Tg of acrylate base polymers (3). Furthermore, Zhao et al. added isopropyl myristate to the acrylate adhesive to decreases Tg and degree of elasticity (G’) and to increase tack adhesiveness (8).

Another important index of base polymers for the transdermal formulations is followability that enables close contact between the formulation and the skin surface (6). In the present study, we examined the skin permeability of tulobuterol in and the followability of two tulobuterol tapes.

2. Materials and Methods

2.1. Materials, animals, and devices

Tulobuterol Tape Sawai (2 mg; 32 × 32 mm, Sawai Pharmaceutical Co., Ltd., Osaka, Japan) and Tulobuterol Tape NP (2 mg; 32 × 32 mm, Nipro Corporation, Osaka, Japan) were purchased from the market. Male Hos:HR-1 hairless mice aged 7-8 weeks (17-25 g in body weight) were purchased from Japan SLC Co., Inc. (Shizuoka, Japan). Animals were handled in accordance with the rules established by the Institutional Animal Care and Use Committee at Josai International University.

In an in vitro 24-hour hairless mouse skin permeability test of tulobuterol, a vertical diffusion cell (LGA-1084-CL, Laboratory Glass Apparatus, Berkeley, CA) was used to diffuse tulobuterol across the resected hairless mouse skin and an autosampler (FOXY200, Nikkaki Bios Co., Ltd., Tokyo, Japan) to collect the sample solution.

2.2. In vitro 24-hour hairless mouse skin permeability test of tulobuterol

The skin was resected from mice after cervical dislocation. The resected skin was inverted to remove subcutaneous fat, followed by standing of the dermis placed downward onto the filter paper that was impregnated with saline.

Tulobuterol Tape Sawai and Tulobuterol Tape NP were punched out into 15 round pieces of 24 mm in diameter, and the protective liner was peeled off and then attached to the resected skin. Test material was set onto the vertical diffusion cell, and the upper and lower rims of the cell were fixed with a metallic pinch clamp. The
Subsequently, the adhesive surface of the sample was applied to the grinding surface of the sandpaper, a sheet of paper towel (Kim Towel®, Nippon Paper Crecia Co., Ltd., Tokyo, Japan) was put onto each sample, and a 1-kg cylinder weight was then placed onto the sample for 1 min. Subsequently, the weight, the paper towel, and the sandpaper were removed, and a laser microscope (LS-5040, Keyence Co., Ltd., Osaka, Japan) was used to measure the depth of the pores that were formed in the adhesive layer of the examined tapes.

2.5. Statistical analysis

Welch’s t-test was conducted to test differences in flux between the intact and abraded skin in the skin permeability test of tulobuterol and differences in pore depth before and after treatment in the followability test by using Microsoft Excel for Windows (Microsoft, Tokyo, Japan). A value of \( p < 0.05 \) was considered statistically significant. All values are expressed as mean ± SE.

3. Results

3.1. Skin permeability of tulobuterol at 3 temperatures

Tulobuterol Tape Sawai and Tulobuterol Tape NP have rubber and acrylate base polymers, respectively. Time-course changes in the flux of tulobuterol from both formulations at 32°C, 37°C, and 40°C are shown in Figure 1. At all temperatures, the \( J_{\text{max}} \) values were higher in Tulobuterol Tape NP than in Tulobuterol Tape Sawai. Both formulations showed an increase in \( J_{\text{max}} \) in association with temperature elevations (Figure 1). Tulobuterol Tape Sawai exhibited a greater rate of increase than did Tulobuterol Tape NP (Table 2). Namely, the \( J_{\text{max}} \) of Tulobuterol Tape Sawai increased 1.19- and 1.29-fold at 37°C and 40°C, respectively.

2.3. Preparation of the abraded skin and measurement of water content in the skin surface

The resected skin was placed onto an aluminum tray, followed by the stripping of the stratum corneum (SC) 7 times with Scotch Brand BookTape 845 (3M Japan Co., Ltd., Tokyo, Japan) as described previously (9). A water content meter (Corneometer® CM 825; Courage + Khazaka Electronic GmbH, Cologne, Germany) was used to measure water content in the skin surface. The probe was cleaned with absorbent cotton impregnated with ethanol before application onto the skin surface. Water content in the skin surface was measured six times to calculate the mean value.

2.4. Followability test of 2 transdermal formulations

At a room temperature of 25°C, a sandpaper (Fuji Star #120, Sankyo Rikagaku Co., Ltd., Saitama, Japan) was cut into 2 sheets (3 × 4 cm in size), and each sheet was placed on a table, with the grinding surface upward.

Subsequently, the adhesive surface of the sample was applied to the grinding surface of the sandpaper, a sheet of paper towel (Kim Towel®, Nippon Paper Crecia Co., Ltd., Tokyo, Japan) was put onto each sample, and a 1-kg cylinder weight was then placed onto the sample for 1 min. Subsequently, the weight, the paper towel, and the sandpaper were removed, and a laser microscope (LS-5040, Keyence Co., Ltd., Osaka, Japan) was used to measure the depth of the pores that were formed in the adhesive layer of the examined tapes.

Figure 1. Time-course changes in \( J \) at various receiver solution temperatures. The flux of tulobuterol released from Tulobuterol Tape Sawai and Tulobuterol Tape NP was determined at 32°C (a), 37°C (b), and 40°C (c). Values are expressed as mean ± SE (\( n = 3 \)). SE, standard error.
against at 32°C. On the other hand, the \( J_{\text{max}} \) of Tulobuterol Tape Sawai and Tulobuterol Tape NP increased 0.09- and 1.07-fold at 37°C and 40°C, respectively, against at 32°C. Arrhenius plot analysis disclosed a steeper slope by linear approximations for Tulobuterol Tape Sawai compared to Tulobuterol Tape NP (Figure 2), suggesting that the former is more prone to be influenced by skin surface temperature.

### 3.2. Skin permeability of tulobuterol across the intact and abraded skin

The \( J \) values of tulobuterol in the intact and abraded skin at 32°C were compared between Tulobuterol Tape Sawai and Tulobuterol Tape NP (Figure 3). Namely, the \( J_{\text{max}} \) of tulobuterol in Tulobuterol Tape Sawai increased 1.46-fold in the abraded skin against the intact skin, while that of Tulobuterol Tape NP increased 1.29-fold (Table 2).

Water content of the skin was measured because of its importance for skin permeability. The water contents of the intact (n = 6) and abraded (n = 6) skin were 26.83 ± 10.72 a.u. and 75.5 ± 8.5 a.u., respectively. A statistically significant difference (\( p < 0.05 \)) was found between these two types of skin, indicating that the water content of the abraded skin had increased as a consequence of SC stripping. In the test using Tulobuterol Tape Sawai, a statistically significant difference (\( p < 0.05 \)) was found in the water contents of the intact and abraded skin (32.3 ± 13.1 a.u. and 77.5 ± 10.1 a.u., respectively). In the test using Tulobuterol Tape NP, a statistically significant difference (\( p < 0.05 \)) was also found in the water contents of the intact and abraded skin (26.5 ± 8.6 a.u. and 75.2 ± 11.9 a.u., respectively).

### 3.3. Followability test of 2 transdermal formulations

A statistically significant difference (\( p < 0.05 \)) was found in the depths of the pores that had been formed in the adhesive layer of Tulobuterol Tape Sawai and Tulobuterol Tape NP (33.9 ± 4.6 µm and 78.0 ± 8.5 µm, respectively). This result suggests that followability is greater for Tulobuterol Tape NP than for Tulobuterol Tape Sawai (Figure 4).

---

### Table 2. \( J_{\text{max}} \) values of tulobuterol in tulobuterol Tape Sawai and tulobuterol Tape NP in an in vitro, 24-hour hairless mouse skin permeability test

<table>
<thead>
<tr>
<th>Items</th>
<th>Temperature</th>
<th>Tulobuterol Tape Sawai (( n = 3 ))</th>
<th>Tulobuterol Tape NP (( n = 3 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( J_{\text{max}} ) (( \mu g/cm^2/h ))</td>
<td>( J_{\text{max}} ) (( \mu g/cm^2/h ))</td>
</tr>
<tr>
<td></td>
<td>32°C</td>
<td>14.37 ± 0.29</td>
<td>23.34 ± 0.52</td>
</tr>
<tr>
<td></td>
<td>37°C (vs. 32°C)</td>
<td>17.10 ± 0.19 (1.19 fold)</td>
<td>21.07 ± 0.33 (0.90 fold)</td>
</tr>
<tr>
<td></td>
<td>40°C (vs. 32°C)</td>
<td>18.50 ± 2.28 (1.29 fold)</td>
<td>24.99 ± 0.33 (1.07 fold)</td>
</tr>
<tr>
<td>Skin</td>
<td>Intact skin</td>
<td>10.73 ± 0.99</td>
<td>22.44 ± 0.76</td>
</tr>
<tr>
<td></td>
<td>Abraded skin (vs. intact skin)</td>
<td>15.66 ± 0.40 (1.46 fold)</td>
<td>28.94 ± 0.11 (1.29 fold)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE. SE, standard error. \( J_{\text{max}} \), maximum value of flux.
4. Discussion

Tulobuterol containing the hydroxyl group in its molecule interacts with the polar function group of the acrylate base polymer with the amido group, while Tulobuterol Tape Sawai with a rubber base that does not contain the polar function groups exhibits no such interaction. Kato et al. conducted a 24-hour rabbit skin releasability test of the transdermal formulations of tulobuterol of gum, silicon, and acrylate bases and reported gum base, silicon base, and acrylate base in decreasing order of drug releasability (1). Furthermore, Kokubo et al. prepared the transdermal formulations of di(propyl)phthalate, ketoprofen, ampicillin, and lidocaine that contained acrylate, gum, and silicon base polymers to examine drug releasability (11). Consequently, they reported no effect of gum and silicon base polymers on drug releasability and a decrease in drug releasability in the transdermal formulations of acrylate base due to the interactions of carboxyl group-containing drugs and acrylate base polymers. Therefore, the skin permeability of tulobuterol was predicted to be greater in Tulobuterol Tape Sawai. Surprisingly, however, the skin permeability of tulobuterol was greater for Tulobuterol Tape NP than for Tulobuterol Tape Sawai; the result was probably attributable to the greater followability of Tulobuterol Tape NP compared with Tulobuterol Tape Sawai.

The skin permeability of tulobuterol in both Tulobuterol Tape Sawai and Tulobuterol Tape NP increased in association with elevations in receiver solution temperature. Similar results were obtained in an in vitro skin permeability test of nonsteroidal anti-inflammatory drugs in the transdermal formulations of rubber base (12). Drug solubility into the skin increased exponentially in association with elevations in receiver solution temperatures, resulting in higher skin permeability at higher temperatures (12,13). We speculate that a similar mechanism is responsible for higher skin permeability at higher receiver solution temperatures in the present study. Arrhenius plots analysis suggested that Tulobuterol Tape Sawai is more sensitive to changes in skin surface temperature compared to Tulobuterol Tape NP. The interaction of the polar functional groups of acrylate base polymers with tulobuterol probably reduces the skin permeability of tulobuterol at 37°C and 40°C where the skin permeability of tulobuterol is increased.

The skin is composed of the epidermis containing the stratum corneum (SC) and dermis. The SC is a biobarrier that prevents water evaporation and the penetration of foreign matter from the exterior into the body and also functions as a biomembrane that controls drug diffusion into the skin (14-17). Both Tulobuterol Tape Sawai and Tulobuterol Tape NP showed a higher \( J_{\text{max}} \) in abraded skin than in intact skin. We consider that these findings are attributable to the fact that the thinner SC of the abraded skin lost its function as the drug release-controlling membrane. Furthermore, the increase rate of \( J_{\text{max}} \) in the abraded skin was higher for Tulobuterol Tape Sawai than for Tulobuterol Tape NP. Again, we speculate that the interaction of the polar functional groups of the acrylate base with tulobuterol inhibits the skin permeability of tulobuterol which is enhanced by skin abrasion.

The followability of Tulobuterol Tape NP was greater compared with Tulobuterol Tape Sawai, suggesting that the former is more flexible than the latter. The skin surface has asperity. The transdermal formulations of drugs that do not follow the asperity produce a less effective area of contact between the adhesive surface and the skin and cause concerns about a decrease in the skin absorbability of the drugs.

Miyazaki et al. used the transdermal formulations of acrylate base with different storage elastic moduli to examine the relationships between the followability of the formulations and the severity of SC detachment (6). Consequently, they reported the better followability to skin surface asperity with respect to the transdermal formulations of adhesive bases that had lower storage elastic moduli and that these formulations homogeneously detached the SC. Tojo et al. reported lower storage elastic moduli and greater peeling strengths in acrylate base polymers with lower Tg values (7). Thus, in general, base polymers with a lower Tg value and/or a lower storage elastic modulus exhibit greater followability at skin surface temperature (6-8).

Tulobuterol Tape Sawai has styrene-isoprene-styrene (SIS) block copolymers that contain polyisoprene as a soft segment and polystyrene as a hard segment. On the other hand, Tulobuterol Tape NP has acrylate 2-ethylhexyl as a soft segment and diacetone-acrylamide, acetooctoxyethyl methacrylate, and methyl methacrylate copolymers as a hard segment (18-20). SIS block copolymers, which contain polystyrene, have a higher Tg compared with diacetone-acrylamide, acetooctoxyethyl methacrylate, and methyl methacrylate copolymers; therefore, Tulobuterol Tape Sawai would have a higher Tg compared with Tulobuterol Tape NP, resulting in lower followability.

In conclusion, a formulation of acrylate base is clinically preferable to a formulation of rubber base when skin surface temperature varies or when the skin is abraded. The formulation needs to be applied to the skin of less asperity for the achievement of better transdermal absorption of tulobuterol.

Acknowledgements

The authors thank Satoshi Sakima, MD, for his critical review of the manuscript and are grateful to Josai International University for the provision of study material and physical space for this study.
References


(Received September 20, 2017; Revised September 23, 2017; Accepted September 24, 2017)