Mechanism of Enhancement of Percutaneous Absorption of Molsidomine by Oleic Acid

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The mechanism of percutaneous absorption of molsidomine, enhanced by a two-component system consisting of oleic acid and propylene glycol, was studied in vitro and in vivo in rats. About 10-20% of the oleic acid was absorbed from the skin independently of the oleic acid-propylene glycol ratio. In contrast, about 95% of molsidomine and propylene glycol were transdermally absorbed from the two-component system containing 10% oleic acid in 6h. The permeability (percent of dose) of molsidomine through the excised rat skin was comparable to that of propylene glycol (both about 80% in 24h). These results suggest that the molsidomine and propylene glycol permeated simultaneously through rat skin. Our proposed mechanism of percutaneous absorption of molsidomine assumes that oleic acid and propylene glycol penetrate into the stratum corneum and improve the permeability of the skin by dissolving hard lipoidal components, and then molsidomine, dissolved in the propylene glycol, passes through the modified stratum corneum.

Keywords—controlled-release transdermal dosage form; percutaneous absorption mechanism; percutaneous absorption enhancer; molsidomine; oleic acid; stratum corneum; propylene glycol

In designing controlled-release transdermal dosage forms of molsidomine containing percutaneous absorption enhancers, it is necessary to control the release not only of molsidomine but also of the absorption enhancers from the pharmaceutical preparation. Furthermore, it is important to know the mechanism by which such absorption enhancers modify the absorption of molsidomine.

The principal barrier to percutaneous absorption of drugs is said to be the stratum corneum, which is composed of dead cells consisting mainly of keratin and lipids. As a whole, it is considered to possess the nature of a lipid barrier. Mixed systems of fatty acids and alcohols have been examined as effective enhancers of percutaneous absorption, but the mechanism by which the epidermal barrier properties are altered is not known.

We have sought absorption enhancers acting on the lipid barrier to improve the percutaneous absorption of molsidomine, and reported in the previous paper that the two-component system of oleic acid and propylene glycol was remarkably effective; 10% oleic acid gave the maximum effect. Here we report our investigations of the mechanism of action of oleic acid and propylene glycol on the skin permeability to molsidomine.

Experimental

Materials—Molsidomine was produced by Takeda Chemical Industries, Ltd., Japan. Oleic acid, propylene glycol, polyethylene glycol 200, and glycerin were of reagent grade. Oleic acid labeled with $^{14}$C, specific activity 59 mCi/mmol, was supplied by New England Nuclear Co., Boston. The reagents were of analytical grade.

Animals—SD-JCL strain male rats aged 7 weeks and weighing 240 to 280 g, were supplied by Clea Japan Inc., Tokyo.

Test Solutions for Absorption Studies in Vivo—All test solutions to be administered were prepared by
dissolving or suspending 10 mg of molsidomine and making the weight up to 200 mg. Oleic acid labeled with $^{14}$C was used after being appropriately diluted with the non-labeled substance. A single dose (200 mg) contained 10 μCi of the labeled compound. The test solution was administered by the procedure described in the previous paper.5>

**Determination of the Plasma Concentration** The plasma concentration of molsidomine was determined in the manner reported previously.5> The plasma concentration of oleic acid labeled with $^{14}$C was measured by the same method as used for $^3$H-labeled protirelin (TRH) in the previous study.5>

**Determination of the Residual Amount on the Skin** The residual amount of molsidomine or propylene glycol remaining at the site of application was measured by the procedure reported in the previous paper.5) The residual amount of oleic acid was determined by measuring the $^{14}$C-labeled compound in the recovered solution by the same procedure as employed for molsidomine.

**Skin Permeation in Vitro** The diffusion cell used was similar to the apparatus of Hashida5) (Fig. 1), on which a piece of excised skin was mounted. The rats were killed by exsanguination, and their denuded abdominal skin was excised, immediately immersed in saline in a refrigerator kept at about 5 °C, and allowed to stand for 8 to 18 h. No significant difference was observed in the permeation of molsidomine through excised skin allowed to stand for various periods of time up to 24 h.

The test solution, except for specific test samples, contained 50 mg of molsidomine (5%). Oleic acid, an absorption enhancer, was incorporated at a concentration of 10%, unless otherwise stated. One gram of the test solution was applied to the skin. The lower chamber, filled with saline, was stirred vigorously. The temperature was 25±1 °C. Samples, 50 to 200 μl, were taken from the sampling port for assay of molsidomine.

**Determination of Molsidomine and Solvent** Molsidomine was determined by liquid chromatography in the manner described in the previous paper.5) Propylene glycol and glycerin were determined by the method of Lambert and Neish.7) A 1 ml aliquot of a buffer (NH$_3$ + NH$_4$Cl) at pH 6 and 0.1 ml of 0.05 M metaperiodic acid as an oxidizing agent were added to 50 μl of the sample, and the mixture was allowed to stand at 37 °C for 15 min. Then 2.0 ml of acetyl acetone was added as a coloring reagent. The resulting mixture was allowed to stand at 37 °C for 40 min and cooled with water; the absorbance was measured at $A_{max}$ 410 nm. The concentration of propylene glycol or glycerin in the sample was determined from a standard curve prepared previously. Polyethylene glycol 200 was determined by the phosphomolybdic acid method.8) Three drops of HCl (1→5), two drops of BaCl$_2$·HCl, and two drops of phosphomolybdic acid were added to 5 ml of the sample solution. The resulting precipitate was separated by centrifugation, the supernatant solution was removed, and 5 ml of distilled water was added to the remaining precipitate. The mixture was shaken and centrifuged again, and the supernatant solution was eliminated. The precipitate was dissolved in 1.2 ml of concentrated sulfuric acid, then 5 ml of distilled water, 1.0 ml of ammonium thiocyanate, and 0.5 ml of SnCl$_2$·HCl were added to the solution; the absorbance was measured at 470 nm. The amount of polyethylene glycol 200 was determined from a standard curve prepared separately.

**Skin Treated with 10% Oleic Acid–Propylene Glycol** The treated skin was obtained as follows. A 10% oleic acid–propylene glycol solution (200 mg) free of molsidomine was applied to the denuded abdominal skin. The rats were killed by exsanguination 1 and 6 h later and the treated areas were excised, then cleaned 20 times with adsorbent cotton soaked with 50 ml of 50% aqueous ethanol without damaging the skin. The skin was mounted in the diffusion cell, and propylene glycol or glycerin solution (1 g) containing molsidomine (50 mg) was applied to it. The permeation proportions of molsidomine and vehicles were measured 10 h after the application. As a control, the same sample preparations were applied to non-treated skin, and the permeation proportions of molsidomine and vehicles were determined 24 h after the application.

**Skin Treated with Oleic Acid** A 50 μl aliquot of ethanol containing 10 mg (corresponding to 1%) of oleic acid was applied to the excised skin (7 cm$^2$), which was allowed to stand for 1 h. Then the skin was mounted in the diffusion cell and 1 g of propylene glycol containing 50 mg of molsidomine was applied to it. The amounts of molsidomine that permeated were measured with time. In another experiment, 1 g of 1% oleic acid–propylene glycol solution containing 50 mg of molsidomine was applied to the skin coated with 50 μl of ethanol, and the amounts
of molsidomine that permeated were measured.

**Stripping of the Stratum Corneum**—The stratum corneum was stripped 15 times with a commercially available cellophane adhesive tape.

**Histological Examination of the Stratum Corneum**—Histological changes in the stratum corneum were examined by applying 10% oleic acid–propylene glycol to denuded abdominal skin, which was excised 1 h later, fixed in formalin by the conventional procedure, stained with hematoxylin-eosin, and examined under a microscope. Untreated skin served as a control.

### Results and Discussion

#### Percutaneous Absorption of Oleic Acid

Figure 2 shows values (ml⁻¹) obtained by dividing the plasma concentrations of oleic acid by the dose of oleic acid when either 200 mg of oleic acid alone or a 10% oleic acid (20 mg)-propylene glycol solution was applied. In both cases, oleic acid was absorbed nearly proportionally to the dose. The residual amount of oleic acid remaining on the skin after 6 h was 77% for the former system and 90% for the latter system (Table I). These results indicate that about 10 to 20% of oleic acid was absorbed percutaneously in 6 h. It is interesting that only about 10% of the oleic acid was absorbed from the two-component system that allowed the absorption of nearly 95% of the molsidomine.

#### Percutaneous Absorption of Propylene Glycol

The residual amounts of propylene glycol on the skin were measured at 6 h after application of the two-component system containing various proportions of oleic acid. At the same time, the amounts of molsidomine were measured. These results are summarized in

![Fig. 2. Percutaneous Absorption of C¹⁴-Oleic Acid through the Abdominal Skin of the Rat](image)

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**TABLE 1. Percutaneous Absorption of Vehicles in Rats**

<table>
<thead>
<tr>
<th>OA Proportion (%)</th>
<th>Percent remaining on the skin (%)</th>
<th>Bioavailability of molsidomine (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>PG</td>
<td>OA</td>
</tr>
<tr>
<td>0</td>
<td>95</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>90</td>
</tr>
<tr>
<td>20</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>50</td>
<td>45</td>
<td>—</td>
</tr>
<tr>
<td>95</td>
<td>—</td>
<td>77</td>
</tr>
</tbody>
</table>

OA, oleic acid; PG, propylene glycol. Bioavailability = \([AUC_{tr}^{\text{oral}} (\mu g \cdot h/ ml) / AUC_{tr}^{\text{oral}} (\mu g \cdot h/ ml)] \times 100.\)
The greatest enhancement of absorption of propylene glycol occurred at about 10% oleic acid. The residual percent of propylene glycol on the skin was 3%, which is close to the residual percent for molsidomine. The same tendency was observed in the other systems tested. Therefore, the percutaneous absorptions of propylene glycol and molsidomine were assumed to be very similar.

**Permeation of Molsidomine and Water-Soluble Polyhydric Alcohols**

In the *in vivo* experiment described above, propylene glycol was percutaneously absorbed in larger amounts than expected from the system in which oleic acid was simultaneously used. A more detailed investigation was carried out *in vitro* on the permeation of molsidomine and propylene glycol. Similar investigations were performed with other water-soluble polyhydric alcohols, polyethylene glycol 200 and glycerin. An attempt was also made to clarify what roles these vehicles play in enhancing the percutaneous absorption of molsidomine.

Three rats were used to measure the permeation (percent permeated with respect to the dose) of molsidomine and of propylene glycol from the 10% oleic acid–propylene glycol

![Figure 3. Permeation of Molsidomine (Left) and Propylene Glycol (Right) through Excised Rat Skin (10% Oleic Acid–Propylene Glycol)](image)

**Table II. Permeation of Molsidomine and Polyethylene Glycol 200 through Excised Rat Skin**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Molsidomine (%)</th>
<th>PEG 200 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>1.1 ± 0.1</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>16</td>
<td>9.7 ± 0.9</td>
<td>9.3 ± 1.8</td>
</tr>
<tr>
<td>24</td>
<td>25.1 ± 2.3</td>
<td>21.6 ± 4.1</td>
</tr>
</tbody>
</table>

* a) PEG 200, polyethylene glycol 200. All values represent mean ± S.E. (n = 3).

![Figure 4. Permeation of Molsidomine and Glycerin through Excised Rat Skin (10% Oleic Acid–Glycerin)](image)
In both cases, permeation began after a lag time of 2 h, and about 30 to 40% of each substance permeated over a 10 h period. There was adequate consistency in the permeation curves for both substances among the three samples.

Polyethylene glycol 200 showed a greatly lowered permeation (Table II) and molsidomine and polyethylene glycol 200 were found to permeate in equal proportions. As shown in Fig. 4, glycerin exhibited a slower rate of permeation than did polyethylene glycol 200, whereas molsidomine and glycerin also permeated in equal proportions.

These results are summarized in Table III. As was the case with the investigations\(^5\) conducted in vivo, the ability of molsidomine to permeate the skin was greatly influenced by the type of water-soluble polyhydric alcohol used as a vehicle. Molsidomine and the polyhydric alcohols were found to permeate in equal proportions in each case; this suggests that molsidomine permeates through the skin dissolved in the polyhydric alcohol. The use of a vehicle that is capable of more readily dissolving molsidomine, and that permeates through the skin more effectively, results in better permeation of the drug.

### Permeation of Molsidomine and Vehicles through Skin Treated with 10% Oleic Acid–Propylene Glycol

The permeation of molsidomine, propylene glycol, and glycerin through rat skin treated with 10% oleic acid–propylene glycol was investigated in vitro (Table IV). Molsidomine dissolved in propylene glycol showed the same permeation behavior irrespective of the duration of treatment with the absorption enhancer; at both 1 and 6 h the permeation was increased markedly over that of the control. Thus, treatment with the absorption enhancer for 1 h was sufficient. A glycerin solution of molsidomine did not affect the permeation. Although the reason for this is not clear, it is presumed that the stratum corneum adsorbs or absorbs oleic acid and permeation of glycerin is prevented. Actually, it was found that oleic acid is poorly compatible with glycerin. The reduced permeation of glycerin through the skin treated with the absorption enhancer suggested that the enhancer did not damage the skin.

### Permeability of Molsidomine from Skin Treated with Oleic Acid

To evaluate the effect of oleic acid on the percutaneous absorption of molsidomine, an investigation was carried out in vitro on the permeation of molsidomine through excised skin.
Pretreated with oleic acid (Fig. 5). The rate of permeation of molsidomine was about twice that through the non-treated skin. The lag time was also shortened by the pretreatment. Perhaps the oleic acid adsorbed on the surface or absorbed inside the stratum corneum permits the molsidomine together with propylene glycol to permeate comparatively easily.

Permeability of Molsidomine through Damaged Skin

The stratum corneum, which functions to prevent the invasion of foreign material, naturally constitutes a major barrier to the permeation and absorption of drugs administered percutaneously. To evaluate the magnitude of this barrier to percutaneous absorption of molsidomine, an investigation was performed in vitro on the permeability of skin from which the stratum corneum had been stripped (Fig. 6). The rates of permeation of molsidomine from 10% oleic acid–propylene glycol and a simple vehicle consisting exclusively of propylene glycol were 714 (µg/cm²/h) and 641 (µg/cm²/h), respectively; this difference is not significant. Stripping of the stratum corneum resulted in a 900-fold increase in the rate of permeation; the rate of permeation from propylene glycol in the intact skin was 0.7 (µg/cm²/h). The

<table>
<thead>
<tr>
<th>Drug and vehicle</th>
<th>Permeation (% of dose)</th>
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<tbody>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>No treatment</td>
</tr>
<tr>
<td></td>
<td>(Control)</td>
</tr>
<tr>
<td>Molsidomine in propylene glycol</td>
<td>0.1</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>1</td>
</tr>
<tr>
<td>Molsidomine in glycerin</td>
<td>0.1</td>
</tr>
<tr>
<td>Glycerin</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Fig. 5. Effect of Skin Treatment with Oleic Acid on the Permeation of Molsidomine through Excised Rat Skin

Fig. 6. Permeation of Molsidomine through Stripped Skin and Intact Skin of Rats

- ▲—, stripped skin, 10% oleic acid–propylene glycol.
- ⋅⋯, stripped skin, propylene glycol alone.
- △—, intact skin, 10% oleic acid–propylene glycol.
- ⋅○⋯, intact skin, propylene glycol alone.
apparent rate of permeation of molsidomine from the absorption-enhancing system in the intact skin was 399 (μg/cm²/h). This striking difference from the control value demonstrates how effectively the absorption enhancer altered the barrier of the stratum corneum.

Histological Examination of the Stratum Corneum
Investigations were carried out to determine whether 10% oleic acid-propylene glycol brings about a change in the stratum corneum. The photomicrographs shown in Fig. 7 indicate that the treatment with the absorption enhancer does not cause changes as exfoliation or appreciable damage to the stratum corneum.

Mechanism of Percutaneous Absorption of Molsidomine
The rate-determining step in the percutaneous absorption of molsidomine is the stratum corneum, as is obvious from the finding that the rate of absorption from skin stripped of the stratum corneum is 900 times faster than that from the intact skin. Consequently, to enhance the percutaneous absorption of molsidomine, it is necessary to alter the permeability of the stratum corneum. The stratum corneum consists mainly of keratin and lipid, and as a whole, is considered to be a lipid membrane. In general there is great interest in how this lipid layer can be altered to allow the permeation and absorption of drugs. We have found that the 10% oleic acid-propylene glycol system caused remarkable enhancement of molsidomine absorption. The mechanism of this effect is discussed below on the basis of the results of in vivo and in vitro studies in rats.

It is presumed that oleic acid, alone or together with propylene glycol, might act to dissolve the hard sebum on the surface of or within the stratum corneum to lower its viscosity and thus alter its permeability. This is supported by the results of the study carried out in vitro on the permeability of molsidomine through skin treated with oleic acid. Thus, it is further assumed that improving the permeability with oleic acid facilitates the permeation of molsidomine and propylene glycol. Over a 6 h period, the absorption of oleic acid was as low as 10 to 20% of the dose, whereas molsidomine and propylene glycol were almost entirely absorbed. As shown in the study carried out in vitro, molsidomine and propylene glycol permeated in the same proportions with respect to the dose. This indicates that molsidomine, dissolved in propylene glycol, permeates through the stratum corneum as if it were subject to solvent drag. A number of reports have appeared on drugs whose percutaneous absorption is consistent with the lipid theory, but it became clear that molsidomine was absorbed from the oleic acid–propylene glycol system by a different mechanism. The marked decrease in the absorption-enhancing effect exhibited when glycerin replaced propylene glycol is explained by the fact that since glycerin is less compatible with oleic acid in the stratum corneum, the segregated oleic acid constitutes a barrier which inhibits the permeation of glycerin. It is still not established whether the main pathway of the percutaneous permeation
or absorption of molsidomine is intercellular or transcellular permeation through the stratum corneum. The absorption enhancement of molsidomine from the oleic acid-propylene glycol system is not due to damage to the stratum corneum. This is clear from the result of the experiment on drug administration with glycerin on skin treated with the absorption enhancer, and from photomicrographs of the stratum corneum.

The findings obtained in this study are expected to be of help in designing controlled-release transdermal dosage forms.

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References

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