Enhancement of Percutaneous Absorption of Molsidomine

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Molsidomine, which is effective in treating angina pectoris, was poorly absorbed through the skin from a simple solution in oleic acid or propylene glycol, and its bioavailability was below 1% in rats. However, molsidomine was efficiently absorbed from a two-component system consisting of oleic acid and propylene glycol. Maximum absorption enhancement was observed in the two-component system containing 10% oleic acid; the bioavailability was about 95% within 6 h. Remarkable percutaneous absorption enhancement was also observed in the presence of linoleic acid when a series of unsaturated straight fatty acids with different carbon numbers were substituted for oleic acid. Lauric acid was the most effective in the series of saturated straight fatty acids. Effective percutaneous absorption enhancement also occurred with lauryl alcohol and oleyl alcohol, but little enhancement was observed with any fatty acid ester or sodium oleate. The two-component system is also effective for some water-soluble drugs and poorly water-soluble drugs, besides molsidomine.

Keywords—controlled-release transdermal dosage form; percutaneous absorption enhancer; molsidomine; angina pectoris; oleic acid; propylene glycol; lauric acid; linoleic acid; two-component system; bioavailability

Transdermal preparations have been used with drugs intended principally for local therapy. However, in recent years the development of transdermal dosage forms for systemic therapeutics has been actively pursued by many research groups. Beckett maintained that the major advantages of a transdermal preparation are that it permits long-acting or controlled-release delivery to achieve constant plasma concentration of a drug, thus conveniently enhancing the drug's efficacy and minimizing its side-effects; it permits the use of pharmacologically active agents with short biological half-lives; and it avoids first-pass metabolism and allows drug input to be rapidly terminated by removing the system from the skin surface if side effects develop.

Drugs such as scopolamine, nitroglycerin, and isosorbide dinitrate, which are commercially available as transdermal drug delivery systems, do not present any problem because they are relatively easily absorbed through the skin and their dose is small. Unlike these drugs, molsidomine (N-ethoxycarbonyl-3-morpholinosydononimine) is poorly absorbed through the skin and consequently it is necessary to enhance its percutaneous absorption.

From the viewpoint of safety it is desirable that enhancers are materials conventionally used in pharmaceutical preparations. Various fatty acids and alcohols have been examined as effective enhancers for percutaneous absorption and for rectal absorption. We attempted to find appropriate enhancers for molsidomine from among the fatty acids and alcohols, and found that the use of oleic acid and propylene glycol gave greatly improved percutaneous absorption of molsidomine in rats.

Experimental

Materials—Molsidomine was synthesized by Takeda Chemical Industries, Ltd., Japan. It is weakly basic with
a molecular weight of 242.23, a melting point of 139 to 142 °C, and a pK_a of 3.2. Protirelin tartrate (TRH-T), oxendolone, and TAI-908 (4-(4-methylbenzoyl)-1-indancarboxylic acid) were supplied by Takeda Chemical Industries, Ltd. and indomethacin, diazepam, and nifedipine were obtained commercially.

Propylene glycol, polyethylene glycol 400, glycerin, oleic acid, lauric acid, and all other chemicals were of analytical grade, obtained from Wako Pure Chemical Ind. Ltd., Osaka.

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

Preparation of Test Solutions—The test solutions used for the percutaneous absorption of molsidomine were prepared by dissolving or suspending a constant amount of 10 mg of the drug and making the weight up to 200 mg by adding a vehicle alone or a vehicle containing an enhancer. The test solution of protirelin tartrate was prepared by mixing 10 μCi of 3H-labeled protirelin (TRH) with a specific radioactivity of 100 Ci/mmol and 0.8 mg of TRH-T and adding the mixture to a vehicle to give a total weight of 200 mg.

Percutaneous Absorption—The rats were anesthetized with pentobarbital, and their abdominal hair was clipped with an electric hair clipper without damaging the skin. A test solution of 200 mg was directly administered to a part of the denuded area (20 cm²; 5 x 4 cm) and blood samples of 0.5 ml were taken from the tail vein at 1, 2, 4, and 6 h afterward.

Determination of Molsidomine in Plasma—A venous blood sample (0.5 ml) was centrifuged (3000 g x 10 min), and 0.2 ml of the resulting plasma was taken into a test tube containing 1 ml of water and 5 ml of chloroform. This mixture was shaken for 10 min, and molsidomine was extracted into the chloroform layer; then 4 ml of the chloroform layer was pipetted out, and the solvent was evaporated off. The residue was taken up in 0.2 ml of a mixed solution of 0.05 M sodium acetate, acetonitrile, and tetrahydrofuran (a volume ratio of 70 : 30 : 0.2), and 541 of the resulting solution was injected into a liquid chromatograph (LC-4A: Shimadzu Seisakusho Ltd., Kyoto), equipped with a pBondapak C18 (i.d. 4 mm x 300 mm) column, and a ultraviolet (UV) detector (313 nm). The flow rate of the mobile phase was 0.8 ml/min. This method gave adequate separation of molsidomine and its metabolite cyanoethyleneaminomorpholine (retention time of molsidomine, 5.8 min; retention time of the metabolite, 6.7 min).

Molsidomine was extremely stable; 99.8% was recovered intact after incubation in rat plasma at 37 °C for 3 h.

Bioavailability of Molsidomine—An aliquot of saline solution (0.5 ml) containing 4 mg of molsidomine was administered intravenously to rats. At 10, 20, 30, 45, 60, 90, 120, and 180 min afterward, venous blood samples were taken to determine the plasma concentration of molsidomine. The area under the plasma concentration—time curve (AUC) was calculated, and found to be 13.858 (μg·h/ml). The bioavailability (absolute bioavailability) through the percutaneous absorption of molsidomine was determined by applying Eq. 1.

\[
bioavailability(\%) = \frac{AUC_{0\rightarrow\infty}^{\text{transdermal}} (\mu g \cdot h/ml)}{AUC_{0\rightarrow\infty}^{\text{intravenous}} (\mu g \cdot h/ml)} \times 100 \tag{1}
\]

Solubility of Molsidomine—An excess of molsidomine was added to each vehicle and the mixture was shaken at 25 ± 1 °C for 2 h. The undissolved molsidomine was collected on a filter paper and the molsidomine concentration in the filtrate was determined by liquid chromatography.

Measurement of the Residual Amount of Molsidomine on the Skin—The residual amount of molsidomine remaining at the site of application on the skin was determined by washing the skin 10 to 15 times with absorbent cotton soaked with a solution of chloroform and ethanol (1 : 1); the amount of molsidomine in the recovered solution was measured by liquid chromatography. The recovery was investigated in a separate experiment. First, 200 mg of 10% oleic acid–propylene glycol solution containing 10 mg of molsidomine was applied to the denuded abdomen (20 cm²) of a rat, then 5 min later, the area was washed and the amount of molsidomine recovered was measured. The average recovery for 5 rats was 97.2%. The residual percent (%) found when a test sample was applied was corrected by applying Eq. 2.

\[
corrected \text{ residual percent (\%)} = \frac{\text{residual percent found (\%)} \times 97.2 \text{ (\%)}}{97.2 \text{ (\%)}} \tag{2}
\]

Determination of TRH-T—At a fixed time after application of the test solution, a 0.12 ml blood sample was taken from the tail vein, and centrifuged. The resulting plasma (0.05 ml) was pipetted into a polyethylene vial containing 5 ml of a scintillator based on toluene, and the contents of the vial were mixed. The mixture was allowed to stand, then the whole radioactivity was measured with a β-ray scintillation counter to determine the protirelin equivalent concentration (μg·eq/ml) in the plasma.

Determination of Applied Drugs—The plasma concentrations of diazepam and TAI-908 were determined by liquid chromatography in accordance with the procedure for determining molsidomine; benzene was used as an extraction solvent for diazepam. Oxendolone, indomethacin and nifedipine were determined by the methods of Itakura, Misaki et al., and Pietta et al., respectively.

Results and Discussion

Percutaneous Absorption of Molsidomine from Single Vehicles

The results of the percutaneous absorption of molsidomine from single lipid-soluble and
water-soluble vehicles are presented in Table I. Molsidomine was best absorbed percutaneously from octyl-decyl oil (medium chain triglyceride) among lipid-soluble vehicles and from dimethyl sulfoxide (DMSO) among water-soluble vehicles; the bioavailability in both instances was about 4%. With all other vehicles the bioavailability was not more than 1%. These values were consistent with the absorbed amounts of molsidomine as determined from the residual amounts remaining at the application sites. However, no vehicle was found that specifically enhanced the percutaneous absorption of molsidomine.

It is known that percutaneous absorption of a drug varies with its physico-chemical properties, such as molecular weight, melting point, solubility, and partition coefficient between oil and water, and also factors associated with the pharmaceutical preparation, such as the type of vehicle or base employed. Higuchi has shown that the absorption rate of a fairly water-soluble drug in the steady state can be expressed by Eq. 3.

\[
\text{rate of percutaneous absorption} = \frac{\text{partition coefficient (skin/vehicle)}}{\text{[drug concentration in pharmaceutical preparation]}} \\
\times \frac{\text{[drug diffusibility in skin][applied area][thickness of skin]}}{AUC_{\text{vehicle}}^{25}}
\]

This equation indicates that to enhance percutaneous absorption, it is necessary to increase the drug concentration in the vehicle and the partition coefficient of the drug from the vehicle to the skin, etc.

No particular relation was found between the percutaneous absorption of molsidomine \((AUC_{\text{vehicle}}^{25})\) and its solubility or partition coefficient in the vehicles shown in Table I.

**Enhanced Percutaneous Absorption of Molsidomine**

To enhance the percutaneous absorption of molsidomine, a screening test of possible absorption enhancers was carried out. Incorporation of either oleic acid (10%, w/w) or lauric acid (10%, w/w) into propylene glycol dramatically enhanced the percutaneous absorption of molsidomine (Fig. 1). In the case of oleic acid, the absorption, in terms of \(AUC_{\text{vehicle}}^{25}\), was enhanced 140 times over that of the control (propylene glycol). The plasma concentration of molsidomine rose sharply from 30 min after application of the drug, reached the maximum \((C_{\text{max}})\) in about 2 h, and thereafter decreased rapidly. The bioavailability was as high as 97%, and molsidomine was mostly absorbed within 6 h after it was administered. The residual amount of the drug at the site of application was found to be 3%, which was in good agreement with the bioavailability. When lauric acid was used, the absorption of molsidomine

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>(C_{\text{max}}) ((\mu\text{g/ml}))</th>
<th>(AUC_{\text{vehicle}}^{25}) ((\mu\text{g} \cdot \text{h/ml}))</th>
<th>Solubility at 25°C (%)</th>
<th>Partition coefficient at 25°C</th>
<th>Bioavailability(^a) (%)</th>
<th>Percent(^b) remaining on skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycol salicylate</td>
<td>0.06</td>
<td>0.16</td>
<td>15.1</td>
<td>42.7(^c)</td>
<td>0.46</td>
<td>—</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>0.11</td>
<td>0.43</td>
<td>1.37</td>
<td>0.43</td>
<td>1.2</td>
<td>98.0</td>
</tr>
<tr>
<td>Octyl-decyl oil</td>
<td>0.81</td>
<td>1.42</td>
<td>0.36</td>
<td>0.21</td>
<td>4.1</td>
<td>94.8</td>
</tr>
<tr>
<td>Isopropyl myristate</td>
<td>0.03</td>
<td>0.12</td>
<td>0.09</td>
<td>0.07</td>
<td>0.35</td>
<td>—</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>0.04</td>
<td>0.24</td>
<td>6.37</td>
<td>0.48(^d)</td>
<td>0.69</td>
<td>98.9</td>
</tr>
<tr>
<td>Polyethylene glycol 400</td>
<td>0.03</td>
<td>0.09</td>
<td>5.23</td>
<td>—</td>
<td>0.26</td>
<td>—</td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>0.47</td>
<td>1.31</td>
<td>2.60</td>
<td>—</td>
<td>3.8</td>
<td>94.4</td>
</tr>
<tr>
<td>Glycerin</td>
<td>0.02</td>
<td>0.08</td>
<td>1.80</td>
<td>0.79</td>
<td>0.52</td>
<td>—</td>
</tr>
</tbody>
</table>

\(a\) Bioavailability = \((\text{AUC}_{\text{vehicle}}^{25}/\text{AUC}_{\text{control}}^{25})\) × 100. \(b\) Percent of molsidomine at 6 h after administration. Each value is the mean of three animals. \(c\) Oily vehicle-water. \(d\) Benzene/vehicle.
was slower, but was still markedly enhanced over the control. The maximum absorption occurred at 4 h after administration, and the bioavailability within 6 h was 64%. If the bioavailability had been determined over a more prolonged period, however, a higher value would have been obtained. It is interesting that the percutaneous absorption of molsidomine from a single vehicle of oleic acid or propylene glycol is low, whereas the absorption from the two-component system is strikingly enhanced.

The percutaneous absorption of molsidomine in relation to the amount of oleic acid or lauric acid in propylene glycol was next investigated (Fig. 2). In the oleic acid–propylene glycol system, the absorption rose rapidly as the amount of oleic acid incorporated was increased. Maximum enhancement was observed with an incorporation of about 10%, and decreased gradually with further addition. When more than 50% oleic acid was added, the drug hardly dissolved in the two-component system; consequently, the decrease of absorption at higher proportions of oleic acid is at least partially attributable to a reduction in the amount of molsidomine dissolved. Lauric acid was found to behave similarly. In the two-component system, oleic acid or lauric acid is thought to act as an absorption enhancer, whereas propylene glycol is assumed to act as an auxiliary agent for absorption enhancement. Few cases are known where a specific ratio of two components provides the maximum percutaneous absorption-enhancing effect. In a study by Nelson Research and Development maximum permeation through the skin of triamcinolone acetonide was attained with a 10% Azone®–ethanol solution. Such a specific two-component system is considered to be of use in designing percutaneous dosage forms; alteration in the ratio of the two components permits selection of systems giving arbitrary percutaneous absorption of molsidomine. Furthermore, the percutaneous absorption can be adjusted by diluting the system with any vehicle or base.

The effect of an enhancer on the percutaneous absorption of molsidomine was also examined using saturated or unsaturated fatty acids having different numbers of carbon atoms (Fig. 3). The saturated fatty acids, except lauric acid (having 12 carbon atoms), showed only minor enhancement. Although the reason why lauric acid enhances the absorption of molsidomine has not been clarified, it is assumed that, together with propylene glycol, it may
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Linoleic acid, an unsaturated fatty acid, resulted in a bioavailability of 78% and produced an absorption-enhancing effect nearly equal to that of oleic acid.

The effect of the type of functional groups of aliphatic compounds on the percutaneous absorption of molsidomine was also investigated (Table II). When the functional group was replaced by a hydroxyl group, the enhancing effect was still present. Lauryl alcohol was as effective as lauric acid, whereas oleyl alcohol was only half as effective as oleic acid. In contrast, replacement of the functional group with a methyl ester group brought about a marked decrease in the enhancing effect. A small enhancing effect was seen with sodium oleate. Okada et al. reported that citric and succinic acids were exceptionally effective as vaginal absorption enhancers for leuprolide, whereas their sodium salts were not. Similar results were observed in the present percutaneous absorption system.

Effects of Vehicles on Absorption Enhancement

Propylene glycol, a basic vehicle component, was replaced by water-soluble polyhydric alcohols or oily vehicles in combination with oleic acid (Table III). The polyhydric alcohols and lipid-soluble vehicles, except ethylene glycol, all produced results similar to those obtained with the control and did not show enhancement. It was confirmed that oleic acid, combined with propylene glycol, gave the most striking enhancement. This combination is considered to be most effective to dissolve the sebum or to act on the whole stratum corneum to alter the permeability to molsidomine, but the precise mechanism is unknown.

Dose Dependence on the Plasma Concentration of Molsidomine

Plasma concentrations of molsidomine were measured after application of 5 and 10 mg of molsidomine to rats (Fig. 4). The $AUC$ from 0—6 h after the 5 mg administration was approximately half that after the 10 mg administration and the plasma concentration was
found to be nearly proportional to the dose.

**Pharmacokinetics of Percutaneous Absorption of Molsidomine**

The rate of percutaneous absorption of molsidomine in rat was determined using the 10% oleic acid-propylene glycol system. To determine the rate of absorption, an analysis based on the following compartment theory was used. Assuming that the behavior of a drug in vivo follows the one-compartment model and that the process of absorption is zero- or first-order, the following two kinds of model equations can be derived:

**zero-order model;**

\[
C_p = \frac{K_0}{K_e K_d} [1 - e^{-K_e t_{1}}] \quad (t_0 \leq t \leq t_1) 
\]

\[
C_p = \frac{K_0}{K_e V_d} [1 - e^{-K_e t_{1}}] e^{-K_e (t-t_1)} \quad (t > t_1)
\]

**first-order model;**

\[
C_p = \frac{K_e F D}{V_d (K_d - K_e)} [e^{-K_d t_{1}} - e^{-K_e t_{1}}]
\]

wherein;

- \(K_0\): zero-order absorption rate
- \(K_e\): first-order absorption rate constant
- \(K_d\): first-order elimination rate constant
- \(V_d\): distribution volume
- \(t_1\): completion time of absorption
- \(t\): time
- \(t_0\): lag time
- \(D\): dose
- \(F\): fraction absorbed
- \(C_p\): plasma concentration

Further, the plasma concentration of the drug after intravenous administration is given by the following equation:

\[
C_p = \frac{D_0}{V_d} e^{-K_e t}
\]

**TABLE III. Effect of Vehicles on the Percutaneous Absorption of Molsidomine in Rats**

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>(C_{max}) ((\mu g/\text{ml}))</th>
<th>(AUC_{\infty}^o) ((\mu g \cdot \text{h}/\text{ml}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oily vehicle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycol salicylate</td>
<td>0.14 ± 0.01</td>
<td>0.65 ± 0.05</td>
</tr>
<tr>
<td>Methyl salicylate</td>
<td>0.40 ± 0.02</td>
<td>1.96 ± 0.12</td>
</tr>
<tr>
<td>Olive oil</td>
<td>0.65 ± 0.11</td>
<td>2.06 ± 0.28</td>
</tr>
<tr>
<td>Liquid paraffin</td>
<td>ND(^o)</td>
<td>ND</td>
</tr>
<tr>
<td>Polyhydric alcohol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>2.40 ± 0.16</td>
<td>5.12 ± 1.81</td>
</tr>
<tr>
<td>Polyethylene glycol 400</td>
<td>0.42 ± 0.05</td>
<td>1.23 ± 0.14</td>
</tr>
<tr>
<td>Glycerin</td>
<td>0.31 ± 0.02</td>
<td>0.71 ± 0.05</td>
</tr>
</tbody>
</table>

ND: Not detected. Each test solution contained 10% oleic acid. Each value is the mean ± S.E. of three rats.

Fig. 4. Dose Dependence in the Percutaneous Absorption of Molsidomine in Rats

—○—, 10 mg of molsidomine/rat; —●—, 5 mg of molsidomine/rat. Each point represents the mean ± S.E. of four to six rats.
where $D_{iv}$ is the dose administered intravenously. By analyzing plasma concentration data obtained after intravenous injection and percutaneous administration in terms of these model equations, the pharmacokinetic parameters for each can be determined. The calculations were performed using MULTI,14) a program for a personal computer. The results of the calculations are shown in Fig. 5 and Table IV. A comparison of the zero-order model with the first-order model indicates that the former yields a smaller AIC15) (Akaike's information criterion to select the optimum statistical model). Therefore, in this case the plasma concentration change of molsidomine after percutaneous administration is consistent with the zero-order absorption model. As is evident from Fig. 5, the zero-order model fits the data better. In addition, it is convenient for investigating to what extent the data obtained in vivo and in vitro are consistent with each other, because the steady-state flux obtained from the permeation experiment in vitro has a zero-order permeation rate. The absorption rate of molsidomine from the 10% oleic acid–propylene glycol system is 5.103 (mg/h) (Table IV). The absorption rate per unit surface area $J$ and the availability were calculated as 255 ($\mu g/cm^2/h$) and 97.14 (%), respectively.

**Application of the Percutaneous Absorption Enhancer to Other Drugs**

These studies were extended to determine whether the 10% oleic acid–propylene glycol system is effective for drugs other than molsidomine (Table V). The drugs selected were those that require sustained release for systemic administration. The absorptions of indomethacin...
and its homologous compound, TAI-908, were enhanced 50 to 100 times over the control, with 70 to 90% availability. The absorptions of diazepam and nifedipine were enhanced 2- to 7-fold over the control, with about 40% availability. Oxendolone, a lipid-soluble steroid derivative, was absorbed least among the drugs investigated.

The stratum corneum consists of a dense layer of dead cells filled mainly with a polymerized keratin matrix and lipids, and is regarded as a lipid barrier to drug permeation. Many reports have described the comparative advantage of lipid-soluble drugs for percutaneous absorption; this is the so-called lipid theory of percutaneous absorption.

However, the absorption of TRH-T, a water-soluble peptide compound, was enhanced 7-fold over the control, with about 30% availability. The two-component system was thus confirmed to exhibit absorption enhancing action for water-soluble drugs as well. On the other hand, insulin, with a molecular weight of about 6000, was not absorbed. Percutaneous absorption of drugs with a molecular weight of more than 1000 seems to be difficult and presumably insulin was not absorbed because of its high molecular weight.

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