In Vitro Transport of Sodium Diclofenac across Rat Abdominal Skin: Effect of Selection of Oleaginous Component and the Addition of Alcohols to the Vehicle

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The in vitro percutaneous transport of sodium diclofenac from various oil vehicles was examined using rat abdominal skin as a model skin membrane. The overall transport of diclofenac through the skin from the oleaginous vehicles was very poor because of a poor solubility of sodium diclofenac in nonpolar oils. To increase the solubility and the permeability of sodium diclofenac, ethanol and n-octanol were added to each oil (designated as the formulated vehicles). The addition of ethanol and n-octanol to the nonpolar vehicles resulted in an extreme increase in drug solubility in each vehicle, with a remarkable increase in the permeation of diclofenac. The effects of oil components in the formulated vehicles on the permeation of diclofenac across the skin were in the following order: squalene ≥ squalene > liquid paraffin > middle chain triglyceride > olive oil > castor oil.

In order to clarify the reason for the differences in permeation of diclofenac from these formulated vehicles, the release of diclofenac and n-octanol from these vehicles in vitro was studied. The release rates of n-octanol from the formulated vehicles were in the following order: liquid paraffin > squalene > squalane > middle chain triglyceride > olive oil > castor oil. On the other hand, a linear correlation was observed between the initial release rate of diclofenac from the formulated vehicle and the in vitro permeation of diclofenac through the rat skin. Thus, the oil component in the formulated vehicle affects the release of the drug and the enhancer from the vehicle to the skin. The transport rate of diclofenac from the formulated vehicle of squalane at the steady state proportionally increased with an increase of drug concentration, and the lag times were not influenced by a change of the drug concentration in the formulated vehicles. Therefore, it may be suggested that the intrinsic permeation of diclofenac through the skin is not influenced by the concentration of sodium diclofenac in the vehicle. From these results, it is considered that the important factors in increasing the skin permeation of a drug from an oil vehicle are to select oils which have a low affinity for the drug and enhancer, and to increase the drug concentration in the oil.

Keywords: percutaneous absorption; sodium diclofenac; oleaginous vehicle; solubility; drug release; primary alcohol; drug content

Introduction

Recently, percutaneous administration of nonsteroidal antiinflammatory drugs has been extensively studied as a drug delivery route promising a systemic efficacy. But the skin forms an effective barrier to the permeation of foreign material, including drugs. Thus, transdermal drug administration is generally restricted to a limited number of drugs.

Sodium diclofenac is a widely used nonsteroidal antiinflammatory drug and has generally been administered orally and rectally but not topically because of its poor absorptivity across the skin.2)

To improve the therapeutic efficacy of a topically applied drug, factors affecting percutaneous drug absorption should be clarified on the basis of both the physicochemical properties of the drug and the skin. In order to improve bioavailability and increase therapeutic efficacy after topical application of a drug, it is necessary to employ a percutaneous absorption enhancer and/or to use an appropriate vehicle.

Many compounds, such as pyrrolidones,3) N,N-diethyl-m-toluamide,4) dimethyl sulfoxide5) and decylmethyl sulf oxide5) have been suggested as penetration enhancers. Further, various surfactants7) and Azone5) have also been reported as penetration enhancers.

The other approach is to modify the formulation of the vehicle. Transdermal absorption of a drug is influenced by the physicochemical properties of both the drug and the vehicle. The selection of an appropriate vehicle is very important in increasing the efficacy of a topically applied drug.9) Important physicochemical factors to improve the vehicle include the solubility of the drug in the vehicle and the transfer of drug from the vehicle to the skin.

In the present study, to obtain fundamental information for transdermal permeation of diclofenac employing simplified oleaginous vehicles, nonpolar oils which have been frequently used as vehicle components in pharmaceutical and cosmetic preparations were examined. The effect of drug concentration in the vehicle on the percutaneous drug permeation was investigated.

Materials and Methods

Materials Sodium diclofenac was kindly supplied by Ciba Geigy Japan (Takarazuka, Japan). Squalene and middle chain triglyceride (MCT, Triesta-F-801R) were supplied by Nikko Chemicals Co. Ltd. (Tokyo, Japan). Squalane, olive oil, liquid paraffin and castor oil used were commercially available. An ethylene-vinyl acetate copolymer (EVA) membrane (composed with ethylene-vinyl and acetate at 90:10; thickness of 40 μm) was obtained from Tamapoly Co. Ltd., (Tokyo, Japan). A cellophane membrane (seamless cellulose tubing, size: 27/32) was obtained from Viskase Seles Corp. Other reagents used were of analytical grade and were used without further purification.

Preparation of Formulated Vehicles To increase the solubility and permeability of sodium diclofenac in oil, ethanol (10% w/w) and n-octanol (5% w/w) were added to each oil. These mixtures were designated as the formulated vehicles. Twenty-five milligrams of sodium diclofenac was mixed well with 10 g of the mixture until a clear oily fluid was obtained at 23 ± 2°C. The oils used to prepare the formulated vehicles are listed in Table I. These preparations were used in both the in vitro permeation study and in the in vitro release study. When oil alone was used as a single component vehicle, the drug was suspended in the vehicle.

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**In Vitro Permeation Study** After removal of abdominal hair from male Wistar rats (250 to 300 g) with electric clippers, the abdominal skin was excised under pentobarbital anesthesia immediately before the experiment. The excised skin was mounted in a Franz-type diffusion cell. In this study, 10 ml of 0.1 M sodium phosphate buffer (pH 7.2) was used as the receptor medium, and 1 g of the test vehicle was placed on the donor side. The surface area exposed for diffusion was 1.77 cm² (diameter = 1.5 cm). The receptor medium was kept at 37 °C and stirred with a magnetic stirrer at 600 rpm.

Aliquots (0.1 ml) of the receptor medium were withdrawn periodically for 12 h. Immediately after each collection of the medium, 0.1 ml of the fresh buffer was added. The concentration of diclofenac in the sample was determined by high performance liquid chromatography (HPLC).

**In Vitro Release Study** The release of n-octanol and diclofenac from the vehicle was determined using Franz-type diffusion cells. Experiments were carried out following the same method used in the *in vitro* permeation study. An EVA membrane in n-octanol and a cellophane membrane in sodium diclofenac were used as separation membranes. To maintain a sink condition, 10% w/w ethanol–water in n-octanol and a 10% w/w ethanol–buffer solution (0.1 M sodium phosphate buffer, pH 7.2) in sodium diclofenac were employed as the receptor medium.

**Solubility Study** An excess amount of sodium diclofenac was added to each test vehicle. The mixture was then allowed to stand at 23 ± 2 °C for 24 h under agitation. The viscous suspension was filtered through a membrane filter with a pore size of 0.45 μm to obtain a clear fluid, and the final concentration of sodium diclofenac was measured by HPLC.

**Assay** Assay of diclofenac was carried out by HPLC as described by Yaginuma et al. Assay limitation of sodium diclofenac was 50 ng/ml. To determine the degree of presence of n-octanol, gas chromatography was used. The gas chromatograph (Shimadzu GC-7A) was equipped with a flame ionization detector. The carrier gas was nitrogen at a flow rate of 40 ml/min. The column was of coated glass, 1 m × 2 mm i.d., packed with Diasolid ZT (Nihon Chromato, Ltd.). The column temperature was set at 90 °C for 1 min and was programmed to reach 150 °C at a rate of 18 °C/min. Both the injector and the detector temperatures were maintained at 200 °C.

**Results and Discussion**

**Effect of Vehicles on the Percutaneous Permeation of Diclofenac** When each oil alone was used as a simple vehicle, diclofenac was not detected in the receptor medium during the experimental period of 12 h. For example, when 2.5 mg of sodium diclofenac was loaded in 1 g of vehicle, the detected amount of diclofenac in the receptor medium was less than 0.5 μg over a 12 h experimental period. This poor flux is considered to be responsible for the poor solubility of sodium diclofenac in the simple oil vehicles (Table I). To dissolve sodium diclofenac completely in the oil vehicle, ethanol was added to oil. But the steady state flux of diclofenac from the vehicle was below 0.5 μmol/cm²/h. From these results, it is considered that the permeability coefficient of sodium diclofenac is low.

To enhance the permeability of sodium diclofenac, n-octanol was added to the vehicle containing oil and ethanol. It is well known that n-octanol increases the skin permeation of drugs. The solubility of sodium diclofenac in the formulated vehicles containing ethanol and n-octanol increased remarkably compared to oil alone (Table I). The percutaneous permeation of diclofenac from each formulated vehicle yielded the results shown in Fig. 1. After a lag time, a steady state transport of diclofenac occurred in the following order for the oil used in the formulated vehicle: squalane > squalene > liquid paraffin > MCT > olive oil > castor oil (Fig. 1 and Table II). The values of steady state flux, J (μmol/cm²/h), and lag time were calculated and summarized in Table II.

With respect to drug permeation through the skin from the various formulated vehicles, drug molecules and/or ions should first diffuse out from the vehicle matrix to the skin surface. Then, if the drug has a low affinity for the vehicle components, it will readily diffuse out from the vehicle to the skin. From the J values and solubility (Tables I and II), it was revealed that high solubility of sodium diclofenac in the formulated vehicles resulted in slow permeation of diclofenac (Fig. 1). Thus, it may be concluded that a great affinity of sodium diclofenac to the vehicle inhibits the overall transport of diclofenac due to a slow release of drug and/or a poor transfer from the vehicle to the skin.

In comparison to the results of the permeation of diclofenac through rat skin after an application of sodium

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**Table I. Solubility of Sodium Diclofenac in Oils and Formulated Vehicle**

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>Solubility (mm)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squalane</td>
<td>10.1</td>
</tr>
<tr>
<td>Squalene</td>
<td>12.4</td>
</tr>
<tr>
<td>Liquid paraffin</td>
<td>13.7</td>
</tr>
<tr>
<td>MCT</td>
<td>36.6</td>
</tr>
<tr>
<td>Olive oil</td>
<td>35.5</td>
</tr>
<tr>
<td>Castor oil</td>
<td>38.3</td>
</tr>
</tbody>
</table>

(a) Solubility of sodium diclofenac in the oil and vehicle was measured at 23 ± 2 °C.

**Table II. Permeation Parameters and Release Control Factor of Diclofenac from Formulated Vehicles**

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>J (μmol/cm²/h)</th>
<th>Le (h)</th>
<th>k (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squalane</td>
<td>12.7 ± 14.7</td>
<td>2.5 ± 0.5</td>
<td>252.8 ± 10.9</td>
</tr>
<tr>
<td>Squalene</td>
<td>11.4 ± 18.2</td>
<td>3.5 ± 0.4</td>
<td>233.2 ± 16.0</td>
</tr>
<tr>
<td>Liquid paraffin</td>
<td>65.7 ± 13.6</td>
<td>4.4 ± 0.8</td>
<td>163.9 ± 13.3</td>
</tr>
<tr>
<td>MCT</td>
<td>25.5 ± 7.0</td>
<td>3.2 ± 0.5</td>
<td>115.4 ± 10.8</td>
</tr>
<tr>
<td>Olive oil</td>
<td>4.0 ± 1.0</td>
<td>3.2 ± 0.5</td>
<td>72.4 ± 14.1</td>
</tr>
<tr>
<td>Castor oil</td>
<td>1.3 ± 0.3</td>
<td>5.5 ± 0.8</td>
<td>66.3 ± 10.0</td>
</tr>
<tr>
<td>Buffer (pH 7.4)</td>
<td>0.9 ± 0.2</td>
<td>4.9 ± 0.9</td>
<td>--</td>
</tr>
</tbody>
</table>

(a) The values were obtained from the previous study.² b) The values of steady state flux (J) and lag time (Le) were calculated from the straight line in Fig. 1. c) The values of release control factor (k) were calculated from the straight line in Fig. 4B. Each value represents the mean ± S.D. (n = 4 to 6).

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Fig. 1. Effect of the Oil Component in the Formulated Vehicles on the Permeation of Diclofenac through Rat Skin

0, squalane; □, squalene; △, liquid paraffin; □, MCT; ●, olive oil; ▲, castor oil. Each value represents the mean ± S.D. (n = 4 to 6).
diclofenac in a buffer solution obtained by a preliminary experiment, the values of $J$ obtained from the formulated vehicles, except for olive oil and castor oil, were more than 20 times larger than the value obtained from the buffer solution (Table II). From these results, it may be suggested that the lipid route in the skin barrier is mainly responsible for the permeation of diclofenac through the barrier, regardless of the ionized form of diclofenac.

Furthermore, we have already reported that the topical application of a phospholipid vehicle of sodium diclofenac increased the flux of diclofenac in rats by about 20 times in comparison to that obtained after the application of a buffer solution. And it was also suggested that the plasma levels of diclofenac in human subjects by the topical application of the phospholipid vehicles were expected to be within the clinically effective range. Thus, the formulated vehicles with squalene, squalene or liquid paraffin are considered worthy of further study.

**Release of Diclofenac and n-Octanol from Formulated Vehicles** To clarify the reason for the differences in the permeation of diclofenac among the formulated vehicles, the release of $n$-octanol and diclofenac from the vehicles in vitro was studied. As the separation membrane, an EVA membrane as a lipoidal membrane was used in the release study of $n$-octanol. But sodium diclofenac cannot penetrate through the EVA membrane; therefore, a cellophane membrane as a porous membrane was used in sodium diclofenac.

The release rates of $n$-octanol from the formulated vehicles were in the following order: liquid paraffin > squalene > squalene > MCT > olive oil > castor oil (Fig. 2). It has been reported that the enhancing effect of various alcohols on transdermal drug absorption occurs by possibly increasing the permeability of the stratum corneum. In this study, $n$-octanol enhanced the permeation of diclofenac across rat abdominal skin. Thus, it may be considered that a low affinity of $n$-octanol to the vehicle increases the overall transport of diclofenac due to an enhancing effect of $n$-octanol. Iwata et al. demonstrated that the percutaneous absorption of alcohols was influenced by the co-administration of solvents. And they also suggested that, among several oils, squalene was the most effective oil for the percutaneous absorption of alcohols. Indeed, in this study, it appeared that a slow release of $n$-octanol from

![Fig. 2.](image)  
**Fig. 2.** Release Profiles of $n$-Octanol from the Formulated Vehicle into the Aqueous Phase through the EVA Membrane  
$\bigcirc$, squalene; $\Delta$, squalene; $\triangle$, liquid paraffin; $\blacksquare$, MCT; $\bullet$, olive oil; $\blacktriangle$, castor oil. Each value represents the mean $\pm$ S.D. ($n=4$).

![Fig. 3.](image)  
**Fig. 3.** Relationship between the Permeation Rate of Diclofenac through Rat Skin and the Release Rate of $n$-Octanol through the EVA Membrane  
$\bigcirc$, squalene; $\square$, squalene; $\triangle$, liquid paraffin; $\blacksquare$, MCT; $\bullet$, olive oil; $\blacktriangle$, castor oil.

![Fig. 4.](image)  
**Fig. 4.** Release Profiles of Diclofenac from the Formulated Vehicle into the Aqueous Phase through a Cellophane Membrane  
(A) Plots of the released amount of diclofenac against time; (B) Plots of the released amount of diclofenac against the square root of time. $\bigcirc$, squalene; $\square$, squalene; $\triangle$, liquid paraffin; $\blacksquare$, MCT; $\bullet$, olive oil; $\blacktriangle$, castor oil. Each value represents the mean $\pm$ S.D. ($n=4$).

![Fig. 5.](image)  
**Fig. 5.** Relationship between the Permeation Rate through Rat Skin and the Release Control Factor ($k$) through a Cellophane Membrane  
$\bigcirc$, squalene; $\square$, squalene; $\triangle$, liquid paraffin; $\blacksquare$, MCT; $\bullet$, olive oil; $\blacktriangle$, castor oil. $Y = 0.598X - 38.292$ ($r=0.997$).
the vehicles resulted in a slow permeation of diclofenac. But a significant relationship was not observed between
the release rate of n-octanol and the rate of diclofenac permeability (Fig. 3).

The release profiles of diclofenac from the formulated vehicles are presented in Fig. 4. When the amounts of
diclofenac released were plotted against the square root of time, a linear relationship was obtained for each vehicle.
From these observations, in the early stages, a leaching-type drug release process as proposed by Higuchi\(^{14}\) may
be applied for the release of diclofenac from the vehicles studied. The value of \( k \) in Table II was calculated from
the slope of the initial straight line in Fig. 4B. This value (\( k \)) may be considered an apparent rate constant factor for
the release of diclofenac from the vehicles.

A linear relationship was observed between the release rate of diclofenac from the cellophane membrane and
the rate of drug permeability across rat abdominal skin (Fig. 5). This good correlation may indicate that release control
factor of the drug from the vehicle is an important factor for the formulation of the vehicle to facilitate drug trans-
port across the skin.

**Table III. Effect of Diclofenac Content on the Permeation Parameter of Diclofenac**

<table>
<thead>
<tr>
<th>Content of diclofenac (mg/g vehicle)</th>
<th>0.5</th>
<th>1.0</th>
<th>1.5</th>
<th>2.0</th>
<th>2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>( L_e^{\alpha} ) (h)</td>
<td>2.4 ± 0.4</td>
<td>2.9 ± 0.7</td>
<td>3.1 ± 0.4</td>
<td>2.8 ± 0.5</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>( p_i )</td>
<td>25.4 ± 3.1</td>
<td>45.3 ± 7.0</td>
<td>65.6 ± 2.2</td>
<td>92.0 ± 6.2</td>
<td>112.6 ± 14.7</td>
</tr>
<tr>
<td>( P_e^{\beta} ) (mmol/cm²/h)</td>
<td>16.2 ± 2.0</td>
<td>14.0 ± 2.2</td>
<td>13.9 ± 0.5</td>
<td>14.6 ± 1.0</td>
<td>14.3 ± 1.9</td>
</tr>
<tr>
<td>( x \times 10^{-3} ) (cm/h)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\( \alpha \) The values of steady state flux \( J \) and lag time \( L_e^{\alpha} \) were calculated from the
straight lines in Fig. 6. \( \beta \) Permeability coefficient \( P_e \) was calculated from the
steady state flux and the initial concentration of sodium diclofenac in the donor compartment. Each value represents the mean ± S.D. (n = 4 to 6).

**Effect of Sodium Diclofenac Content in the Formulated Vehicle** Among oils used in the present study, squalane
was the most appropriate oil for the formulated vehicles as well as for the simple oelagineous ones. In order to study
the effects of diclofenac content in the vehicle on drug permeation through the skin, a vehicle formulated with
squalane was used.

An increase in the drug concentration in the vehicle re-
sulted in an increase in the amount of diclofenac which
permeated the skin (Fig. 6). The penetration flux, \( J \),
of diclofenac at a steady state was nearly proportional to
the drug concentration in the vehicle (Fig. 7). But, the values
of the permeability coefficient \( P_e \) which was calculated
from the flux and initial concentration of sodium diclofenac
in the donor compartment) and lag time were not influenced
by the concentration of the drug in the formulated vehicles
(Table III). Similarly, the intrinsic permeability for the skin
epithelium was not influenced by drug concentration in the
vehicle, suggesting that the mechanism involved in the
transport process is passive diffusion.

In summary, the steady-state flux of diclofenac from an
oil alone or a vehicle containing ethanol and oil was low.
To enhance the permeability of sodium diclofenac, n-octanol
was added to the vehicle. The solubility and steady-state
flux increased dramatically with the formulated vehicles in
squalane, squalene, or liquid paraffin. These results appear
to be due to the low affinity (high release rate) of n-octanol
and sodium diclofenac to these oil components. In the
castor oil vehicle, however, a low release rate of n-octanol
and sodium diclofenac and low steady-state flux were
observed. This may be due to the low affinity of n-octanol
and sodium diclofenac to the castor oil vehicle. Also, the
steady-state flux of diclofenac was nearly proportional to
the drug concentration in the vehicle. From these results, it
is considered that the important factors for increasing skin
permeation of a drug from an oil vehicle are: to select an
oil which has a low affinity for the drug and enhancer; and
to increase the drug concentration in the oil. And squalane,
squalene or liquid paraffin may be useful oil components
for the transdermal administration of sodium diclofenac.

**References and Notes**

1) A part of this work was presented at the 109th Annual Meeting of
the Pharmaceutical Society of Japan, Nagoya, April 1989.
3) D. Southwell, B. W. Barry, R. Evans and F. J. T. Fidler, J. Pharm.