Effect of Polyoil Fatty Acid Esters on Diclofenac Permeation through Rat Skin

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The effects of a series of polyoil fatty acid esters (sefsols) on diclofenac permeation through rat skin were investigated using in vitro and in vivo methods. Four monooesters and one diester of sefsol were selected as a vehicle components. The effects of each sefsol on in vitro diclofenac permeation were compared at a concentration of 5% sefsol in water. Monooesters of sefsol, except the glyceryl monoester, enhanced the percutaneous permeation of diclofenac. The highest enhancement was observed in propylene glycol mononcaprylate. The plasma concentration of diclofenac was increased dramatically by the addition of 10% propylene glycol monocaprylate when applying the diclofenac sodium suspension to abdominal rat skin in vivo. These results suggest that the monooesters of polyoil fatty acid are potential candidates to enhance the transdermal absorption of diclofenac sodium.

Key words: diclofenac sodium; sefsol; polyoil fatty acid ester; permeation enhancer; skin permeation

Percutaneous administration of nonsteroidal anti-inflammatory drugs has been extensively studied as a drug delivery system promising systemic efficacy. Diclofenac sodium, which is a strong anti-inflammatory agent, has been reported to be poorly absorbed by transdermal application. It is well known that the selection of a vehicle is a very important factor in the percutaneous absorption of a drug. In our previous study, a we investigated the effect of several oleaginous vehicles on the transdermal permeation of diclofenac sodium, and found that several vehicles improved the permeation of this drug.

Fatty acid-alcohol esters are commonly used as adjuvants for cosmetics and pharmaceuticals. Ozawa et al. 2 a reported the effect of various fatty acid-alcohol monoesters on the permeation of hydrocortisone butyrate propionate. Inagi et al. 3 b and Okumura et al. 4 a also reported the effect of fatty acid-alcohol mono-, di- or triesters on the drug permeation. As such esters are not miscible with water, they are used as an emulsion or a solution solubilized by adding ethanol.

Polyoil fatty acid esters (sefsols) are immiscible with water. However, in preliminary studies we found that the monooesters of sefsols were solubilized at a concentration of 5% and more by the presence of diclofenac sodium. Therefore, in the present study, the effects of several sefsols on the transdermal permeation of diclofenac were studied by applying a diclofenac sodium suspension containing one of the sefsols on rat skin in vitro and in vivo.

MATERIALS AND METHODS

Materials Diclofenac sodium was kindly supplied by Ciba Geigy, Japan (Takarazuka, Japan). Sefsols and hydrogenated soya phospholipids (lecithin) were supplied by Nikko Chemicals Co., Ltd. (Tokyo, Japan). Azone was also supplied by Nelson Research and Development Co. (Irvine, CA). The structures and abbreviations of sefsols used in this study are summarized in Chart 1. Other reagents used were of analytical grade and were used without further purification.

Preparation of Test Suspension A slightly excess amount of diclofenac sodium was suspended in a mixture of sefsol and water. The mixture was allowed to stand at 32°C under agitation for 12 h. The resultant suspension was applied to rat skin in vitro and in vivo. To investigate the effect of other additives, a 5% compound was added to the mixture of 10% sefsol in water.

In Vitro Permeation Study Abdominal hair of male Wistar rats (250 to 300 g) was removed with an electric clipper one day before the experiment. Skin excised immediately before the experiment was mounted on a Franz-type diffusion cell. Ten ml of 0.1 m sodium phosphate buffer (pH 7.2) was used as the receptor medium, and 1 g of a test suspension was placed on the donor side with a parafilm occlusion. The surface area of the skin exposed to the applied preparation was 0.785 cm² (diameter = 1.0 cm). The receptor medium was kept at 32°C while being stirred with a magnetic stirrer at 600 rpm.

Aliquots (0.1 ml) of the receptor medium were withdrawn periodically for 24 h and replaced with an equal volume of fresh buffer. The concentration of diclofenac in the sample was determined by high performance liquid chromatography (HPLC).

The steady state flux, J, was obtained from the initial linear portion of the penetration curve which was obtained by plotting an accumulated amount of diclofenac

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Chart 1. Structures and Abbreviations
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against time. The permeability coefficient was calculated by Eq. 1.

\[ J = P \cdot A \cdot C_a \]  

(1)

where \( P \) = the permeability coefficient, \( A \) = the surface area (0.785 cm\(^2\)) for the diffusion cells used in the present study), and \( C_a \) = the solubility of diclofenac sodium in the applied suspension.

In Vivo Percutaneous Absorption Study Abdominal hair of male Wistar rats weighing 250—300 g was shaved with an electric clipper one day before the experiment. After anaesthetizing the rats with urethane solution (20%, 0.6 ml/100 g B.W., i.p.), a columnar cylinder (15 mm inner diameter) was fixed on the abdominal skin surface with glue (Aron Alpha, obtained from Toa Chemicals Co., Tokyo) at the edge. One g of a test suspension was placed into the cylinder and was applied to the skin with a paraffin occlusion. Two hundred \( \mu \)l of blood was collected from a jugular vein at designated time intervals and centrifuged to obtain plasma. The area under the curve (AUC) of diclofenac concentration in plasma vs. time was calculated by the trapezoidal rule.

Measurement of Solubility A suspension of diclofenac sodium, which was prepared as described in the preparation of test suspensions, was filtered through a membrane filter (pore size: 0.45 \( \mu \)m). The concentration of diclofenac in the filtrate was measured by HPLC. Since the diester of selsol was not miscible with water, even after the addition of diclofenac sodium, and the solubility of diclofenac sodium in the diester is very low, the solubility of diclofenac sodium in the mixture of 5\% diester and 95\% water was assumed to be the same as that in water.

Analytical Methods Assay of diclofenac was carried out by HPLC. One hundred \( \mu \)l of sample solution in the in vitro experiment was mixed with 100 \( \mu \)l of the mobile phase, and 20 \( \mu \)l of the mixed solution was injected onto a HPLC column. In the plasma sample, a 0.1 ml aliquot of plasma was deproteinized with 1 ml of acetonitrile. After centrifugation (10000 \( \times \) g, 20 min), the solvent of the supernatant (0.9 ml) was evaporated to dryness under reduced pressure. The residue was dissolved by adding 200 \( \mu \)l of the mobile phase, and 80 \( \mu \)l of the resultant solution was injected onto a HPLC column. A HPLC system equipped with a pump (880-PU, Jasco, Tokyo, Japan), a UV-detector (875-UV, Jasco), a column (4.6 \( \times \) 250 mm) packed with Nucleosil 100-5C(18) (Macherey-Nagel, Germany) and an integrator (C-R3A, Shimadzu, Kyoto, Japan) was used. The mobile phase composition was 8.7 mmol H(3)PO(4) \cdot CH(3)OH = 23:77. The flow rate was 1.0 ml/min and the separation was performed at ambient temperature. The limitation of assay was 50 ng/ml and the coefficient of variation was 2.7%.

Statistical Analysis Data are represented as the mean with standard deviation (mean ± S.D.). Statistical evaluation of the data was made by the Student’s t-test at a level of 5\%.

RESULTS AND DISCUSSION

Effect of Selsols on the in Vitro Penetration of Diclofenac The effect of five selsols on the permeation of diclofenac was studied by using diclofenac sodium suspension in 5\% selsol aqueous solution. Okumura et al.\(^4\) reported the effect of S-318, S-228 and S-810 (glyceryl tricaprylate) on the penetration of water soluble drugs. In this study, they used the emulsion as a vehicle because these selsols are not miscible with water. Also, all selsols used in the present study were not miscible with water. However, the 5\% monoesters of selsol became miscible with water after the addition of diclofenac sodium (the contents of diclofenac sodium are 48, 32, 35 and 50 mg/ml in 5\% aqueous solutions of S-118, S-218, S-318 and S-1126, respectively). The diester (S-228) did not dissolve in water, even after the addition of diclofenac sodium, and it resulted in an emulsion suspending diclofenac sodium. The effect of diclofenac sodium on the surface tension of water was investigated using a Surface Tensiometer ST-1 (Shimadzu, Kyoto, Japan). The surface tension of water decreased in relation to an increase in the concentration of diclofenac sodium in water (Fig. 1). From these results, diclofenac sodium may act as a surfactant in the vehicle and dissolve selsol in water. However, the reason why the diester of selsol was not miscible with water, even after the addition of diclofenac sodium, is not clear. Further physico-chemical investigations may be needed to make clear the mechanism.

Figure 2 shows the transport profiles of diclofenac through rat abdominal skin. The steady-state flux (J) and lag time (L) can be calculated from the straight line plotted in Fig. 2. The permeation parameters and solubilities of diclofenac sodium are summarized in Table 1. The solubilities of diclofenac sodium in monoesters of selsol were about 2—3.5 times higher than that in water. Selsols, except for S-318, significantly enhanced the permeation of diclofenac, and the enhancement effect was in the following order: S-218 > S-1126 > S-118 > S-228 > S-318 = water. The value of L increased significantly in S-218, S-318 and S-1126. Since we used the drug suspension as a donor solution, the permeation of drugs is proportional to the partition coefficient of drug between the vehicle and skin and the diffusivity of drugs in the skin barrier, and L is inversely proportional to the diffusivity of a drug in
the skin. S-118, S-218, S-318 and S-1126 show a similar
effect on the solubility of diclofenac sodium. However,
many differences in the permeation enhancement of
diclofenac were observed. On the other hand, S-218 shows
a greater lag time, although diclofenac showed the greatest
permeation coefficient. These results suggest that the
enhancement effect of ethylene glycol monoesters and
propylene glycol monoesters at a low concentration in
water depends mainly on the increase in diclofenac
partition from the vehicle to the skin.

Effect of the Concentration of S-218 on the Permeation
of Diclofenac Among the surfactants used in this study, the
highest enhancement effect was observed in S-218. Table
2 shows the effect of the concentration of S-218 on the
permeation parameters and solubilities of diclofenac
sodium. In all concentrations, S-218 became miscible
with water after the addition of diclofenac sodium. The
solubility increased with an increase in the concentration of S-218,
and was almost constant in the concentration range from
20 to 60%. These results suggest that S-218 at a low
concentration may affect the partition of diclofenac
from the vehicle to the skin, and S-218 at higher
concentrations may affect the diffusivity of diclofenac in the
skin.

Effects of the Other Additives on the Permeation of
Diclofenac It is well known that some compounds enhance
the permeation of drugs, and that further enhancement
was observed in the combination of the enhancers and
other compounds (or other enhancers). Okumura et al. suggested that the mechanisms of the action of
S-318 and Azone as permeation enhancers are similar.
This may suggest that S-218 affects the fluidity of the
stratum corneum. On the other hand, S-218 is a good
solvent for diclofenac sodium, because its solubility in
S-218 alone was about 9 times higher than that in water
(data not shown). From these results, ten compounds were
selected to investigate their combined effect with S-218.
The vehicle which contains 5% other additive and 10% S-218 in water was used, and diclofenac sodium was
also suspended in the vehicle. The results are summarized
in Table 3. The solubilities of diclofenac sodium were in-

<table>
<thead>
<tr>
<th>S-218 content (%)</th>
<th>5.0</th>
<th>10.0</th>
<th>20.0</th>
<th>40.0</th>
<th>60.0</th>
<th>100.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>$J^\alpha$ (µmol/cm²/h)</td>
<td>1.43 ± 0.14</td>
<td>2.07 ± 0.39</td>
<td>1.98 ± 0.14</td>
<td>1.87 ± 0.37</td>
<td>1.76 ± 0.17</td>
<td>1.99 ± 0.41</td>
</tr>
<tr>
<td>$L^\alpha$ (h)</td>
<td>10.34 ± 0.95</td>
<td>9.60 ± 0.67</td>
<td>2.68 ± 0.64</td>
<td>1.55 ± 1.35</td>
<td>1.56 ± 0.77</td>
<td>5.71 ± 1.10</td>
</tr>
<tr>
<td>$P^\alpha$ (× 10³ cm/h)</td>
<td>9.08 ± 0.90</td>
<td>9.82 ± 1.85</td>
<td>6.56 ± 0.46</td>
<td>4.34 ± 0.86</td>
<td>3.86 ± 0.37</td>
<td>5.46 ± 1.12</td>
</tr>
<tr>
<td>Solubility (mg/g)</td>
<td>50</td>
<td>67</td>
<td>96</td>
<td>137</td>
<td>145</td>
<td>116</td>
</tr>
</tbody>
</table>

[Table 2: Permeation Parameters and Solubilities of Diclofenac Sodium in Vehicles Containing Different Concentrations of S-218]

| a) The values were calculated from the straight lines of the permeated amount of diclofenac vs. time. b) Calculated from $J$ and the solubility of diclofenac sodium in the donor compartment. c) Measured at 32°C. Each value represents the mean ± S.D. (n = 3 to 4). |
Table 3. Effect of Other Additives on Permeation Parameters of Diclofenac Sodium

<table>
<thead>
<tr>
<th></th>
<th>(J^a) ((\mu\text{mol/cm}^2\text{h}))</th>
<th>(L^b) (h)</th>
<th>Solubility-c (mg/g)</th>
<th>(P^d) ((\times 10^3) cm/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-218(^a)</td>
<td>2.07 ± 0.39</td>
<td>9.60 ± 0.67</td>
<td>67</td>
<td>9.83 ± 1.85</td>
</tr>
<tr>
<td>P.G.</td>
<td>1.65 ± 0.17</td>
<td>9.75 ± 1.08</td>
<td>86</td>
<td>6.10 ± 0.63</td>
</tr>
<tr>
<td>13-B.G.</td>
<td>0.20 ± 0.07</td>
<td>8.80 ± 0.61</td>
<td>84</td>
<td>0.76 ± 0.27</td>
</tr>
<tr>
<td>Crotamiton</td>
<td>0.11 ± 0.02</td>
<td>10.26 ± 0.39</td>
<td>165</td>
<td>0.33 ± 0.06</td>
</tr>
<tr>
<td>Azone</td>
<td>1.71 ± 0.21</td>
<td>8.05 ± 1.23</td>
<td>75</td>
<td>7.25 ± 0.89</td>
</tr>
<tr>
<td>Glycerin</td>
<td>0.28 ± 0.14</td>
<td>10.53 ± 0.50</td>
<td>73</td>
<td>1.22 ± 0.61</td>
</tr>
<tr>
<td>Urea</td>
<td>1.74 ± 0.19</td>
<td>11.73 ± 0.61</td>
<td>85</td>
<td>6.51 ± 0.71</td>
</tr>
<tr>
<td>Lecithin</td>
<td>0.087 ± 0.010</td>
<td>7.75 ± 0.73</td>
<td>95</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td>Decanol</td>
<td>1.34 ± 0.11</td>
<td>6.18 ± 2.32</td>
<td>98</td>
<td>4.35 ± 0.36</td>
</tr>
<tr>
<td>Caprylate sodium</td>
<td>0.84 ± 0.13</td>
<td>9.66 ± 1.41</td>
<td>65</td>
<td>4.11 ± 0.64</td>
</tr>
<tr>
<td>Laurate sodium</td>
<td>1.32 ± 0.20</td>
<td>7.73 ± 1.14</td>
<td>73</td>
<td>5.75 ± 0.87</td>
</tr>
</tbody>
</table>

\(^a\) 10% of S-218 in water was used. \(^b\) The values were calculated from the straight lines of the permeated amount of diclofenac vs. time. \(^c\) Calculated from \(J\) and the solubility of diclofenac sodium in the donor compartment. \(^d\) Measured at 32°C. Other compounds (5%) were added to the vehicle (10% S-218 in water). Each value represents the mean ± S.D. \((n = 3 \text{ to } 4)\).

Fig. 3. Plasma Diclofenac Concentration after Application of Diclofenac Sodium Solution to the Abdominal Skin of Rat

Key: ●, with S-218; ○, without S-218. Each value represents the mean ± S.D. \((n = 4)\).

creased with the addition of some compounds compared with 10% S-218 in water, especially with crotamiton. Although the values of \(P\) in these vehicles were higher than that in \(H_2O\), the combined effect was not observed in all additives. The enhancers may enact its enhancement effect in the skin. Therefore, the decrease may account for the decreased concentration of S-218 in the skin.

In Vivo Percutaneous Absorption Studies Figure 3 represents the in vivo absorption profiles of diclofenac through abdominal skin in rats, and the pharmacokinetic parameters are summarized in Table 4. Although the solubility of diclofenac sodium in 10% S-218 was about 4 times higher than that in water (Tables 1 and 3), the \(AUC_{0-\infty}\) and \(C_{\text{max}}\) were higher by 30 and 40 times, respectively, compared with water. The apparent skin permeation \(J\) of diclofenac in steady-state plasma concentration was calculated by the following equation.

\[ J = CL \cdot C_{\text{ss}} / A \]  

(2)

where \(CL\) is the total body clearance of diclofenac, \(C_{\text{ss}}\) is the steady-state plasma concentration of diclofenac and \(A\) is the surface area. The enhancement factor (EF) can be calculated from Eq. 3.

\[ EF_{\text{in vivo}} = J_{\text{S-218}} / J_{\text{water}} = C_{\text{ss}, \text{S-218}} / C_{\text{ss}, \text{water}} \]

(3)

because \(A\) is the same and \(CL\) may be the same between water and 10% S-218. The value of \(EF_{\text{in vivo}}\) calculated by \(C_{\text{max}}\) instead of \(C_{\text{ss}}\) was about 40, and the value of \(EF_{\text{in vitro}}\) was about 160 (Tables 1 and 2). The value of \(L\) in vitro was higher than that in vivo. Several reasons may be considered for the in vitro—in vivo difference. Hatanaka et al.\(^{12}\) reported differences between in vitro and in vivo permeation, and they discussed the reasons from the viewpoint of drug permeation. Sefsol partitions to skin from vehicle and its enhancing effect takes place in the skin. It is considered that the behavior of sefsol in the skin may differ between in vitro and in vivo. In this study, the permeation enhancing mechanism and the behavior in the skin of sefsol is not clear, but we are planning to investigate the effect of sefsol in the skin. In conclusion, monoesters of sefsol are potential candidates for enhancing the transdermal absorption of diclofenac sodium.

**REFERENCES**