Effect of Vehicles on Diclofenac Permeation across Excised Rat Skin

Koichi TAKAHASHI,*^a Takashi SUZUKI,^b Hitomi SAKANO,^a and Nobuyasu MIZUNO^a

Faculty of Pharmaceutical Sciences, Mukogawa Women's University,^a 11–68 Koshien Kyuban-cho, Nishinomiya, Hyogo 663, Japan and Shiseido Research Center (2) Pharmaceutical Research Center,^b 2–12–1 Fukuura, Kanazawa-ku, Yokohama 236, Japan. Received July 18, 1994; accepted December 8, 1994

The *in vitro* percutaneous permeation of diclofenac from various vehicles was examined using rat abdominal skin as a model membrane. The oleaginous vehicles used in this study consisted of three components: *i.e.* fatty acid, fatty acid ester and nonpolar oil. The solubilities of sodium diclofenac in formulated vehicles were above 0.2 M. The effect of each oleaginous component in the vehicle on the permeation of diclofenac across the skin was in the following order: oleic acid > isostearic acid, diisopropyl adipate = diethyl sebacate > Panasate 875 and squalane > liquid paraffin.

To clarify the reason for the differences in permeation among the fatty acid esters, the release of diclofenac through either porous or lipoidal membranes from these vehicles *in vitro* and the solubility of sodium diclofenac in the vehicles were studied. However, no relationship was observed between the release rate or solubility and skin permeability. The skin permeation of diclofenac increased following pretreatment with diisopropyl adipate or diethyl sebacate, but not with middle chain triglyceride (Panasate 875). These results suggested that the main reason may be the enhancement effect of fatty acid esters.

Emulsions and creams containing 3% sodium diclofenac were prepared using the above oleaginous vehicles. A large flux and short lag time were observed in these preparations compared with an aqueous suspension of sodium diclofenac. The incorporation of urea significantly enhanced the permeation of diclofenac from these preparations. These results suggest that the emulsion and cream prepared in this study are useful for development for practical purposes.

Key words sodium diclofenac; percutaneous permeation; oleaginous vehicle; enhancement; emulsion; cream

The therapeutic efficacy of a topically applied drug depends on its ability to penetrate the skin and accumulate in the deeper skin layers. The extent of this absorption varies depending on both the physicochemical properties of the penetrant and its formulation. However, skin forms an effective barrier against the permeation of foreign materials. To overcome this, many investigators have studied the effects of penetration enhancers^{1–3)} and vehicles^{4,5)} on drug permeation. The vehicle composition can affect both drug release and skin permeability properties. Important physicochemical factors which can improve the vehicle include the solubility of the drug in the vehicle and the transfer of drug from the vehicle to the skin.

Sodium diclofenac, which is a strong anti-inflammatory agent, is a water soluble drug. Previously, we reported the effect of several oleaginous vehicles containing octanol on the permeation of diclofenac and suggested that the selection of vehicle and the solubility of sodium diclofenac in the vehicle were the important factors.⁶⁾ However, it is difficult to increase the solubility of this drug in an oleaginous vehicle without incorporating alcohol. In the present study, we selected several oleaginous components in which to dissolve sodium diclofenac at a high concentration and sought the optimal vehicle for the skin permeation of sodium diclofenac. Next, we prepared an emulsion and cream using the same oleaginous vehicle and studied the permeation of diclofenac from the emulsion and the cream. We also studied the effect of urea on permeation from these formulations.

MATERIALS AND METHODS

Materials Sodium diclofenac was kindly supplied by Ciba Geigy Japan (Takarazuka, Japan). Crotamiton,

isostearic acid, oleic acid (Extraolein 90), middle chain triglyceride (Panasate 875), diisopropyl adipate, diethyl sebacate, squalane and liquid paraffin (Cresutol 172) were kindly supplied by Shiseido (Yokohama, Japan). An ethylene-vinyl acetate copolymer (EVA) membrane (composed with ethylene-vinyl and acetate at 90:10; thickness of 40 μ m) was obtained from Tamapoly Co., Ltd. (Tokyo, Japan). A membrane filter (Fluorinert, pore size 0.2 μ m) was obtained from Nihon Millipore, Ltd. (Tokyo, Japan). Other reagents used were of analytical grade and were used without further purification.

Preparation of Formulated Vehicles Codes for and constituents of the formulated vehicles are listed in Tables 1 and 3.

***In Vitro* Permeation Study** After the removal of abdominal hair from male Wistar rats (250 to 300 g) with electric clippers, the abdominal skin was removed and 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 0.5 g of the test vehicle was placed on the donor side. The surface area exposed for diffusion was 0.785 cm² (diameter = 1.0 cm). The receptor medium was kept at 32 °C and stirred with a magnetic stirrer. 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 fresh buffer was added. The concentration of diclofenac in the sample was determined by a high-performance liquid chromatography (HPLC).

For the pretreatment study, skin was pretreated with 20 μ l of vehicle or a 40% aqueous solution of ethanol as the control. After 2 h, an aqueous suspension (0.5 ml) of sodium diclofenac was placed on the donor side.

Solubility Study An excess amount of sodium diclofenac was added to the test vehicle. The mixture was

* To whom correspondence should be addressed.

then stirred at 32 °C for 24 h. The solution was centrifuged at 100000 rpm (32 °C) for 30 min. The drug concentration of the supernatant was measured by HPLC.

In Vitro Release Study The release of 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. A lipoidal membrane (EVA membrane) or porous membrane (Fluorinert) was used as the separation membrane.

Analysis The HPLC system consisted of a pump (880-PU, Jasco, Tokyo, Japan), and a detector (875-UV, Jasco), a 4.6 mm × 250 mm column packed with Nucleosil 100-5C₁₈ (Macherey-Nagel, Germany), and an integrator (C-R3A, Shimadzu, Kyoto, Japan). The flow rate was 1.0 ml/min and the separation was performed at ambient temperature. The mobile phase composition was 18.7 mM H₃PO₄:methanol=20:80 and the UV detection was performed at 282 nm.

Statistical Analysis Data were represented as the mean with standard deviation (mean ± S.D.). Statistical evaluation of the data was carried out using the Student's *t*-test.

RESULTS AND DISCUSSION

Effect of Oleaginous Components on the Permeation of Sodium Diclofenac The solubility of a drug in a vehicle is an important factor in selecting the vehicle, because the permeation rate depends on the concentration of the drug in the vehicle. It is difficult to increase the solubility of sodium diclofenac in an oleaginous vehicle without using alcohol (e.g. ethanol), because sodium diclofenac is a water soluble drug. To make a preparation (emulsion or cream) containing 3% sodium diclofenac, we selected several oleaginous components (Table 1). The formulations consisted of three different components: *i.e.* fatty acid, fatty acid ester and a nonpolar oil. Crotonamiton was also selected to increase the the solubility of sodium dichlofenac and was added to all vehicles. All of these components are commonly used as cosmetic and drug additives. The solubilities of sodium diclofenac in the formulated vehicles were above 0.2 M, and the highest solubility was observed in code 5 among the vehicles used in this study (Table 2).

In comparing the fatty acid components used in this study, a larger flux was observed in oleic acid (code 1) than in isostearic acid (code 2) (Fig. 1 and Table 2). It is well known that oleic acid enhances the skin permeation of many drugs.⁷⁻¹⁰ This large flux may be the effect of oleic acid as a penetration enhancer. But, for our preliminary study, it was difficult to prepare a stable emulsion or cream using the same content of oleic acid. We selected isostearic acid as the fatty acid component to study the effects of other components on the permeation.

Middle chain triglyceride (Panasate 875), diisopropyl adipate and diethyl sebacate were used as fatty acid ester components. The flux was significantly increased with diisopropyl adipate (code 3) and diethyl sebacate (code 4) in comparison to Panasate 875 (code 1) (Fig. 1 and Table 2). For the comparison of a nonpolar oily component, the flux of diclofenac from squalane (code 4) was higher than that of liquid paraffin (code 5) (Fig. 1 and Table 2). The same results were obtained in our previous reports using

Table 1. Codes for and Constituents of the Oleaginous Vehicles

Constituents	Code				
	1	2	3	4	5
Sodium diclofenac	3.0	3.0	3.0	3.0	3.0
Crotonamiton	2.5	2.5	2.5	2.5	2.5
Isostearic acid	6.0	—	6.0	6.0	6.0
Oleic acid	—	6.0	—	—	—
MCT ^{a)}	17.0	17.0	—	—	—
Diisopropyl adipate	—	—	17.0	—	—
Diethyl sebacate	—	—	—	17.0	17.0
Squalane	4.5	4.5	4.5	4.5	—
Liquid paraffin	—	—	—	—	4.5

a) Middle chain triglyceride (Panasate 875). All values in gram.

Table 2. Permeation Parameters, Release Control Factor and Solubilities of Sodium Diclofenac

Code	$J^a)$ ($\mu\text{mol}/\text{cm}^2/\text{h}$)	$L_t^a)$ (h)	$k^b)$	$J'^c)$ ($\text{nmol}/\text{cm}^2/\text{h}$)	$S^d)$ (M)
1	0.45 ± 0.05	6.44 ± 0.24	1.29 ± 0.11	3.71 ± 0.25	0.26
2	$0.94 \pm 0.15^*$	6.65 ± 0.34	1.26 ± 0.16	4.05 ± 0.18	0.24
3	$1.53 \pm 0.28^*$	6.29 ± 0.39	1.49 ± 0.15	3.92 ± 0.11	0.28
4	$1.62 \pm 0.21^{***}$	6.61 ± 0.46	1.42 ± 0.13	$5.38 \pm 0.25^*$	0.29
5	$0.78 \pm 0.15^*$	6.32 ± 0.24	1.39 ± 0.13	4.50 ± 0.57	0.34

a) The values of mean flux (J) and lag time (L_t) were calculated from the data of 8, 10 and 12 h in Fig. 1 using a least squares method. b) The values of release control factor (k) were calculated from the straight line in Fig. 2 using a least squares method. c) The values of steady state flux (J') were calculated from the straight line in Fig. 3 using a least squares method. d) The solubility of sodium diclofenac was measured at 32 °C. * $p < 0.05$ versus code 1. ** $p < 0.05$ versus code 5. Each value represents the mean ± S.D. ($n = 3-5$).

Fig. 1. Permeation Profiles of Diclofenac from the Oleaginous Vehicles through Rat Skin

Key: ○, code 1; ●, code 2; □, code 3; ■, code 4; △, code 5. Each value represents the mean ± S.D. ($n = 3-4$). * $p < 0.05$ versus code 1.

simple oleaginous vehicles.⁶⁾

Release of Diclofenac from Oleaginous Vehicles With respect to drug permeation through the skin from vehicles, a drug should first diffuse out from the vehicle to the skin. To clarify the reason for the differences in permeation among the oleaginous vehicles, especially among codes 1, 3, 4 and 5, the release of diclofenac from the vehicles *in vitro* was studied. A porous membrane was used as the

Fig. 2. Release Profiles of Diclofenac from the Oleaginous Vehicles through a Porous Membrane

Key: ○, code 1; ●, code 2; □, code 3; ■, code 4; △, code 5. Each value represents the mean ($n=3-5$).

separation membrane.

The release profiles of diclofenac from the oleaginous vehicles through the porous membrane are presented in Fig. 2. 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, a leaching-type drug release process, as proposed by Higuchi,¹¹⁾ may be applied for the release of diclofenac from the vehicles. The slope (k) of the straight line in Fig. 2 was calculated and listed in Table 2. Significant differences were not observed among the vehicles. These results suggested that the process of diffusion through the vehicle is not the main reason for the differences in the skin permeation of diclofenac from the vehicles.

It has been well established that the stratum corneum is a heterogenous membrane consisting of a mosaic of cornified cells containing cross-linked keratin filaments and intercellular lipid-containing regions.¹²⁾ This suggests that there are distinct lipophilic and hydrophilic pathways in the stratum corneum.^{13,14)} Hatanaka *et al.*¹⁵⁾ reported the possibility of predicting the skin permeability of drugs with an artificial membrane. In their reports, lipoidal and porous membranes were used as models of lipophilic and hydrophilic pathways, respectively. The permeation of diclofenac from the vehicles through an EVA membrane was investigated in the present study. As the EVA membrane is a lipoidal membrane, the amount of a water soluble compound, such as sodium diclofenac, which permeates through the EVA membrane may be little or none. Hatanaka *et al.*¹⁵⁾ also suggested the low permeability of diclofenac from the aqueous suspension of sodium diclofenac through a silicone membrane. In this study, diclofenac was not detected in the receptor medium during the experimental period of 8 h when the aqueous suspension of sodium diclofenac was used. However, diclofenac permeated through the membrane from the oleaginous vehicle (Fig. 3). The values of steady state flux (J') are listed in Table 2. For the difference in permeation between an aqueous suspension and an oleaginous vehicle, two possibilities may be considered: one is a transfer of sodium ions from sodium diclofenac to fatty

Fig. 3. Release Profiles of Diclofenac from the Oleaginous Vehicles through a Lipoidal Membrane

Key: ○, code 1; ●, code 2; □, code 3; ■, code 4; △, code 5. Each value represent the mean ($n=3-4$).

acid. It is difficult to demonstrate whether diclofenac is a sodium salt or free acid in the vehicles. But the solubility of sodium diclofenac in the vehicle, except in fatty acids, decreased extremely (0.36 mM in the vehicle except oleic acid in code 2). This result suggests strongly the possibility mentioned above. The other is a cosolvent effect of the vehicle components. It is reported that some cosolvents enhance drug permeation.^{16,17)} In this study, some components in the vehicle also changed the permeation of diclofenac (mentioned below). The values of the flux (J') were significantly increased in code 4 compared with code 1. And slightly large values in code 4, compared with code 1, and in code 4 compared with code 5, were observed, but they were not significant. Thus, it may be considered that the partitions of diclofenac between the vehicles and lipoidal membrane differ between code 1 and code 4 or 5, and between code 4 and code 5.

Effect of Pretreatment of Fatty Acid Esters on Permeation of Diclofenac through Rat Skin From the above results, we could not explain the difference in skin permeation between diclofenac from code 1 and code 3. The enhancement effect of fatty acid esters on the permeation of drugs through skin has been reported previously.¹⁸⁻²⁰⁾ Diisopropyl adipate and diethyl sebacate significantly increased the permeation of diclofenac by the pretreatment, but Panasate 875 did not (Fig. 4). Ozawa *et al.*¹⁹⁾ suggested that isopropyl myristate has a direct effect on the barrier function. Inagi *et al.*²¹⁾ also reported that the association (hydrogen-bond) of indomethacin and diisopropyl adipate is important for inducing the percutaneous absorption of indomethacin. The skin permeation of diclofenac may be easily enhanced by diisopropyl adipate and diethyl sebacate contained in the vehicles (code 3 and code 4). The mechanism of these fatty acid esters as penetration enhancers were not studied in this investigation, and further study may be needed to clarify the mechanism.

Permeation of Sodium Diclofenac from Emulsion and Cream On the basis of the above results, we prepared

Fig. 4. Pretreatment Effect of Fatty Acid Esters on the Permeation of Diclofenac

Key: ○, control (40% ethanol solution); ●, MCT; □, diisopropyl adipate; ■, diethyl sebacate. Skin was pretreated with 20 μ l of the vehicle or a 40% aqueous solution of ethanol as a control before the 2 h permeation study. Each value represents the mean $\pm$ S.D. ($n=4$). * $p<0.05$ versus H_2O .

Table 3. Codes for and Constituents of Emulsion and Cream

Constituents	Code			
	6	7	8	9
Sodium diclofenac	3.0	3.0	3.0	3.0
Crotamiton	3.0	3.0	3.0	3.0
Isostearic acid	6.0	6.0	6.0	6.0
Diethyl sebacate	17.0	17.0	17.0	17.0
Squalane	2.0	2.0	2.0	2.0
Urea	—	3.0	—	3.0
Surfactant	1.5	1.5	1.5	1.5
Hiviswako-104	—	—	1.0	1.0
Water	To make 100% (w/w)			

All values in gram.

an emulsion and a cream containing the same oleaginous component of code 4. Code 4 and its constituents are listed in Table 3. These preparations containing 3% sodium diclofenac were stable at room temperature throughout the experiment period (6 months). The amount of diclofenac which permeated from the cream (code 8) was slightly larger than that from the emulsion (code 6), but the values were not significantly different (Fig. 5, Table 4). A large flux and short lag time were observed in these preparations compared with the aqueous suspension of sodium diclofenac (Table 4). However, the flux rates observed in these preparations were about 10 times lower than that calculated from the data in code 4, because the content of sodium diclofenac was 9.1% in code 4 and 3% in the other preparations. These preparations are heterogeneous systems, whereas the aqueous suspension and oleaginous vehicles are homogeneous. Also, the concentrations of sodium diclofenac in the formulations differ between the oleaginous component and the water component. These suggest that a simple comparison between the permeation parameters of these formulations and that of an aqueous suspension or oleaginous vehicles may lead to an erroneous discussion. Nishihata *et al.*²²⁾ suggested a combined effect of alcohol and urea on the skin permeation of indomethacin. To further improve the diclofenac permeation from these

Fig. 5. Permeation Profiles of Diclofenac from Emulsion or Cream through Rat Skin

Key: ○, code 6; ●, code 7; □, code 8; ■, code 9. Each value represents the mean $\pm$ S.D. ($n=3-4$). * $p<0.05$ versus without urea.

Table 4. Permeation Parameters of Diclofenac from Emulsion and Cream

	H_2O^b	Code			
		6	7	8	9
$J^a \times 10^2$	1.98 ± 0.41	$3.35 \pm 0.36^*$	$5.85 \pm 0.85^{***}$	$3.99 \pm 0.47^*$	$7.48 \pm 1.10^{***}$
($\mu\text{mol}/\text{cm}^2$)					
Lt^a (h)	9.8 ± 1.0	$3.0 \pm 0.1^*$	$2.8 \pm 0.2^*$	$2.8 \pm 0.3^*$	$2.8 \pm 0.6^*$

a) The values of steady state flux (J) and lag time (Lt) were calculated from the straight line in Fig. 5 using a least squares method. b) The values were calculated from the straight line in Fig. 4 using a least squares method. * $p<0.05$ versus H_2O . ** $p<0.05$ versus without urea. Each value represents the mean $\pm$ S.D. ($n=4$).

formulations, we investigated the effect of urea. The flux of diclofenac from the emulsion and the cream containing urea was larger than that from the formulation without urea (Fig. 5, Table 4). The enhancing effect of urea on the skin permeation of a drug is partially explained on the basis of the hydration enhancement of cornified skin, but is not completely explained at present.

It may be considered that the present study may contribute to the development of a clinically available topical preparation of sodium diclofenac.

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