Enhanced Skin Permeation of Diclofenac by Ion-Pair Formation and Further Enhancement by Microemulsion

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Enhancement of skin permeability of anionic diclofenac from non-aqueous vehicle isopropyl myristate (IPM) by ion-pair formation with either alkylamines or benzylamine as model cationic ions was examined in guinea pig dorsal skin. Diclofenac ion flux increased in the presence of these amines due to an increase in solubility. Maximum flux was observed in the presence of *n*-hexylamine, which induced 7.3-fold increase accompanied by a 45-fold increase in solubility. Permeability coefficients of the ionic form of diclofenac in the presence of benzylamine, *n*-hexylamine and *iso*-octylamine as counter ions in IPM were larger than those of the non-ionic form of diclofenac. Since the solubility of diclofenac was still limited, to obtain further enhancement of skin permeation, the effects of microemulsions as a vehicle consisting of phosphate buffered saline (PBS), isopropyl myristate (IPM), polyoxyethylene sorbitan monooleate (Tween 80) and ethanol were examined for transport of diclofenac-benzylamine ion-pairs. All microemulsion formulations tested increased diclofenac flux 4.9-fold to 10.7-fold over the value without a microemulsion accompanied by a 217-fold to 302-fold improvement in the solubility of diclofenac-benzylamine ion-pairs, but permeability coefficients were decreased 28—44 fold. Maximum enhancement was observed for a microemulsion with a ratio of PBS, IPM, ethanol and Tween 80 of 25 : 8 : 47 : 20 (w/w). The present findings suggest the usefulness of combined use of ion-pairs with microemulsions for enhancement of skin permeation of ionic drugs.

Key words diclofenac; skin permeation; ion-pair; microemulsion

The lipid lamella of the skin's stratum corneum acts as a barrier to the permeation of most drugs, and especially as a hydrophobic barrier against hydrophilic drugs and ionized drugs. One possible means of facilitating the transdermal delivery of ionic drugs is through ion-pair formation. Oppositely charged ions can interact to form new species known as ion-pairs. This association reduces or neutralizes electrostatic charges and consequently reduces the electrical conductivity in a non-polar milieu.[1)] In our previous study, an increase in skin permeation of salicylate in isopropyl myristate (IPM) was found in the presence of counter ions (alkylamines and benzylamine)[2)]; the increased permeation was attributed to the lipophilization of salicylate *via* an ion-pair.

Diclofenac, which is a therapeutically important non-steroidal anti-inflammatory drug, is extensively metabolized in the liver. Because of its short biological half-life, the drug needs to be administered quite frequently.[3)] Transdermal delivery of diclofenac may provide better patient compliance over oral administration. However, diclofenac is not easily absorbed on transdermal application.[4)] In this study we first examined the effects of amines, model cationic counter ions, on the *in vitro* percutaneous penetration of diclofenac through excised guinea pig dorsal skin. Guinea pig dorsal skin has been reported to be a good model for human skin.[5)]

We furthermore examined the effects of combined use of ion-pair formation and microemulsions. Microemulsions, characterized as thermodynamically stable and clear isotropic systems with droplet sizes in the sub-micron range, have also been studied in pharmaceutical applications.[6,7)] They typically consist of an aqueous phase, an organic phase, and a surfactant/cosurfactant component. They may act as penetration enhancers, depending on the oil/surfactant constituents.[8)] Currently they are recognized as good vehicles for the percutaneous absorption of drugs such as tyrosine, β-blockers, sucrose, ketoprofen and estradiol.[9—13)] Formulations based on microemulsions have several advantages over conventional formulations, namely: thermodynamic stability, enhanced drug solubilization and ease of manufacturing.[14)]

Although ion-pair formation is a promising means of facilitating the transdermal delivery of ionic drugs, the solubility of ion-pairs in both aqueous and non-aqueous vehicles is still limited to obtain sufficient absorption rates. Therefore, in this study, microemulsions containing diclofenac ion-pairs were also used to further improve the solubility and skin permeability of the ion-pairs. We used microemulsions consisting of PBS, IPM, polyoxyethylene sorbitan monooleate (Tween 80) and ethanol, which are the aqueous phase, oil phase, surfactant and cosurfactant, respectively, as model microemulsions. The microemulsions of these constituents have been revealed to be useful as a permeation enhancing system for hydrophobic drugs such as free-base lidocaine and estradiol, as well as hydrophilic drugs such as lidocaine hydrochloride salts and diltiazem.[15)]

Experimental

Materials Sodium diclofenac, ketoprofen, ethanol and polyoxyethylene sorbitan monooleate (Tween 80) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Hydrochloride salts of *n*-hexylamine, *iso*-octylamine and benzylamine were purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Isopropyl myristate (IPM) was obtained from Nacalai Tesque (Kyoto, Japan). A diclofenac-benzylamine ion-pair complex was prepared by addition of benzylamine hydrochloride salt (pK_a=9.4), in excess concentration, to sodium diclofenac (pK_a=4.7) at pH 7.4 and cooling at 0 °C. Recrystalization was performed by dissolving the crystal in ethanol and cooling at 0 °C. The acidic form of diclofenac was prepared by titrating sodium diclofenac with hydrochloric acid and cooling at 0 °C.

Microemulsion Preparation Microemulsions were prepared by weight using appropriate amounts of pH 7.1 phosphate buffered saline (PBS), IPM, Tween 80 and ethanol at 37 °C. Microemulsions were obtained by vortex

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Table 1. Composition (%, w/w) of Microemulsions

Vehicle	PBS	IPM	Ethanol	Tween 80
A	25	8	47	20
B	29	11	30	30
C	3	47	25	25
D	7	33	30	30

mixing for a few minutes. Drug-loaded microemulsions were prepared by saturating each formulation with an excess of diclofenac-benzylamine ion-pairs. The compositions of the microemulsions are shown in Table 1.

Measurement of *in Vitro* Skin Permeation *In vitro* skin permeation of diclofenac was performed as described.[16] Full thickness dorsal skin was excised from male guinea pigs and subcutaneous fat and other extraneous tissues were trimmed. The skin was then mounted in a Franz type diffusion cell with a water jacket (37 °C). The available diffusion area was approximately 0.71 cm^2 and the receiver cell had a capacity of about 4.45 ml. Then, 1.0 ml IPM was added to the donor cells while PBS adjusted to pH 7.4 was introduced into the receptor cells. Pretreatment was carried out for 12 h with stirring at 450 rpm by a magnetic stirrer. When a microemulsion was used, the microemulsion without any drugs was added to the donor cell for pretreatment. After washing both cells, sodium diclofenac and hydrochloride salts of either *n*-hexylamine or *iso*-octylamine, which were suspended in 1.0 ml IPM at final concentrations of diclofenac and alkylamines of 20 mM, were added to the donor cells and the permeation experiment was started. When benzylamine was used as the counter ion, a recrystalized diclofenac-benzylamine ion-pair complex was used at a final suspension of 20 mM. For microemulsions, recrystalized diclofenac-benzylamine saturated in 1.0 ml microemulsion was added to the donor cells. One hundred and fifty microliters of sample was taken from the receiver cells periodically over a period of 30 h.

The concentration of diclofenac was determined by HPLC (L-6000; Hitachi, Tokyo, Japan) with an L-4000 detector (Hitachi) at 276 nm. Separation was achieved on a reversed-phase column (Mightysil RP-18 GP, 4.6 mm i.d., 150 mm) using a mobile phase consisting of methanol, water and phosphoric acid (1200 : 800 : 1) for the analysis of diclofenac at a flow rate of 0.7 ml/min. Ketprofen was used as an internal standard for the analysis.

Apparent permeability coefficients (K_p) of diclofenac were obtained according to Eq. 1 from the initial straight portion of the permeation curve dM_R/dt.

$$K_p = \frac{dM_R}{dt} \cdot \frac{1}{A \cdot C_D} \quad (1)$$

where M_R is the amount of diclofenac permeated into the receiver compartment. A is the diffusion area and C_D is the concentration of diclofenac in the donor compartment.

Solubility Measurement The solubilities of diclofenac-amine ion-pairs were measured after their incubation in an excess amount of either IPM or microemulsion at 37 °C for 24 h. After quick centrifugation at 1000×***g*** for 2 min, the concentration of the supernatant was determined by HPLC as described above.

Particle Size Measurement The particle size of each microemulsion was determined with quasi-elastic light scattering using an FPAR-1000 fiber-optics particle analyzer (Otsuka electronics, Japan) at a scattering angle of 90°.

Statistical Analysis One way analysis of variance and Bonferroni's *post-hoc* test were used to analyze differences between the sets of data. A *p*-value less than 0.05 was considered significant.

Results

Effects of Ion-Pair Formation with Counter Ions on Skin Permeation of Diclofenac We first examined the effects of amines (*n*-hexylamine, *iso*-octylamine and benzylamine) as counter ions on the permeation of diclofenac through guinea pig dorsal skin by suspension in IPM. Figure 1 shows that these amines enhanced the penetration of diclofenac. As shown in Table 2, a 2.9-fold to 7.3-fold increase in diclofenac flux was observed by ion-pair formation with

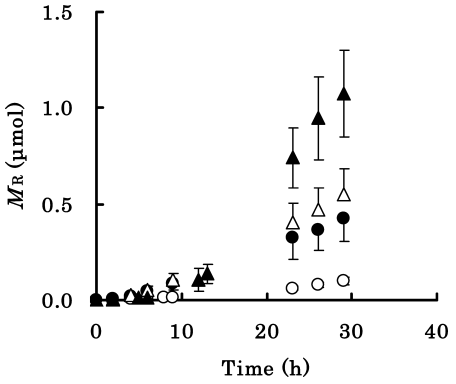

Fig. 1. Amount of Diclofenac Permeated into the Receiver Compartment, M_R, through Excised Guinea Pig Dorsal Skin in the Presence of Alkylamines and Benzylamine as Counter Ions

○, control (sodium diclofenac); ●, benzylamine; △, *iso*-octylamine; ▲, *n*-hexylamine. Data are means±S.D. of four experiments.

Table 2. Effects of Alkylamines and Benzylamine as Counter Ions on the Solubility in IPM, Flux and Permeability Coefficients (K_p) of Diclofenac through Excised Guinea Pig Dorsal Skin

Amine	Solubility (mM)	Flux ($\times 10^{-2}$ μmol·cm^{-2}·h^{-1})	K_p ($\times 10^{-2}$ cm·h^{-1})
None[a]	0.071	0.75±0.16	10.5±2.2
Benzylamine	1.32	2.15±0.60	1.63±0.45***
n-Hexylamine	3.18	5.44±1.17***	1.71±0.37***
iso-Octylamine	4.54	2.81±0.64*	0.62±0.14***
Diclofenac (nonionic)	20[b]	5.00±0.47***	0.25±0.02***

a) Sodium diclofenac was used. *b*) 20 mM acidic form of diclofenac was used without addition of amines. Data are means±S.D. of four experiments. * $p<0.05$, *** $p<0.001$, compared with the value of diclofenac ions in the absence of amines.

the amines. In the presence of *n*-hexylamine, the flux was the highest. This finding was consistent with our previous finding for salicylate-amine ion-pairs, in which flux of salicylate was increased significantly by amines and maximum flux was found when *n*-hexylamine was present as a counter ion.[2]

The permeation data of diclofenac-amine ion-pairs were also compared with that of the 20 mM non-ionic form of diclofenac in IPM. As shown in Table 2, the permeability coefficients of sodium diclofenac in the presence of benzylamine, *n*-hexylamine and *iso*-octylamine were larger than those of the non-ionic form of diclofenac, whose solubility in IPM was much larger than 20 mM. The permeability coefficients of sodium diclofenac present as an ion-pair with amines were 2.5-times to 6.8-times larger than that of diclofenac. This finding was also consistent with our previous finding, especially for the improvement in solubility for salicylate-amine ion-pairs, in which the permeability coefficients of salicylate present as ion-pairs with amines were larger than that of salicylic acid.[2] However, the solubility of the ion-pairs in IPM was still limited.

Effects of Microemulsions on Skin Permeation of Diclofenac To further improve skin permeation, we next examined the effects of microemulsions on the permeation of diclofenac through guinea pig dorsal skin by using a saturated solution of diclofenac-benzylamine ion-pairs in a microemulsion consisting of PBS as the aqueous phase, IPM as the oil phase, Tween 80 as surfactant and ethanol as a cosurfactant. The pH of the aqueous phase was adjusted to 7.1,

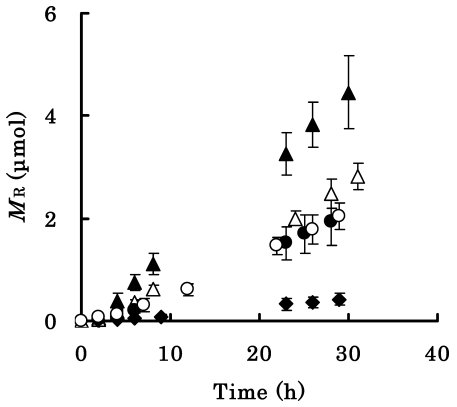

Fig. 2. Amount of Diclofenac Permeated into the Receiver Compartment, M_R, through Excised Guinea Pig Dorsal Skin after the Addition of Diclofenac-Benzylamine Ion-Pairs in Microemulsion Systems

◆, control (in IPM without microemulsion); ▲, microemulsion A; △, microemulsion B; ●, microemulsion C; ○, microemulsion D. Data are means±S.D. of four experiments.

Table 3. Effects of Microemulsions on the Solubility, Flux and Permeability Coefficients of Diclofenac Dissolved as Diclofenac-Benzylamine Ion-Pairs through Excised Guinea Pig Dorsal Skin

Microemulsion	Solubility (mM)	Flux ($\times10^{-2}$ μmol·cm^{-2}·h^{-1})	K_p ($\times10^{-2}$ cm·h^{-1})
None[a)]	1.32	2.15±0.60	1.63±0.45
A	398	23.1±0.6***	0.058±0.002***
B	324	13.6±1.4***	0.042±0.004***
C	290	11.0±2.5***	0.038±0.009***
D	286	10.6±0.6***	0.037±0.002***

a) Diclofenac-benzylamine ion-pairs dissolved in IPM were used in the absence of a microemulsion. Data are means±S.D. of four experiments. ***p<0.001, compared with the value in the absence of a microemulsion.

which was larger than the pK_a value of diclofenac (4.7). Diclofenac-benzylamine ion-pairs suspended in IPM were used as a control. As shown in Fig. 2 and Table 3, the microemulsion system provided significant enhancement of the transport across the skin for all the formulations tested. Steady state flux values of diclofenac from the microemulsions were 4.9-fold to 10.7-fold higher than from the value in the IPM vehicle without a microemulsion. Maximum flux was observed for microemulsion A with a ratio of PBS, IPM, ethanol and Tween 80 of 25 : 8 : 47 : 20. Enhancement effects on flux in the presence of the other three microemulsions (B, C and D) were similar to each other. On the other hand, the permeability coefficients for diclofenac from the microemulsions were 28—44 times lower than the control. Diclofenac solubility in the microemulsion was 217—302 times greater than that in IPM. The mean particle diameter of microemulsion A was 6.2±0.5 nm, and the others were similar (between 5 and 7 nm).

Discussion

The present findings revealed that ion-pair formation in a non-aqueous vehicle IPM improved the skin permeation of diclofenac Ion-pair formation increased solubility caused by reducing or neutralizing the electrostatic charge. Although permeability coefficients decreased with ion-pair formation, the decrease was less than the increase in solubility. Actually, permeability coefficients of diclofenac-amine ion-pairs were larger than that of non-ionic diclofenac. This is probably because relatively high partition coefficients between non-aqueous vehicles and the skin can be expected for ion-pairs which seem to be weakly associated, as reported for the lidocaine-alkanoate ion-pair.[17)]

The present findings also revealed that microemulsions further improved the skin permeation of diclofenac ion-pairs as well as their solubility. Although permeability coefficients decreased in the presence of microemulsions, the decreases were smaller than the increases in the solubility. The decreases in partition coefficients between vehicle and skin may be small. Although further study is necessary to demonstrate the mechanism, high solubility and sufficient partition coefficient may be the reason for enhanced permeation of ion-pairs by microemulsions.

Among microemulsions tested, maximum flux was observed for microemulsion A with a ratio of PBS, IPM, ethanol and Tween80 of 25 : 8 : 47 : 20. The permeability coefficient of diclofenac-benzylamine ion-pair in the same micriemulsion was larger than those in other microemulsions. Microemulsion A contained the highest amount of ethanol. Increasing the amount of ethanol in the microemulsion may have favorable effects on the skin permeation of diclofenac. Ethanol may change the interaction between the microemulsion and the skin. Moreover, ethanol may work as a penetration enhancer as ethanol alone has been used as a permeation enhancer for many drugs.[18)]

On the other hand, there was no significant difference in permeation when microemulsions B, C and D were used. Microemulsion C, which contained the highest concentration of IPM, did not result in a significant enhancement compared to B and D, although IPM has been shown to exhibit a high skin penetration-enhancing effect on its own.[19)] This may be due to the fact that IPM had difficulty penetrating the stratum corneum because of the encapsulation of the surfactant and cosurfactant.

Microemulsions may also work as drug carriers to the skin. This may have also contributed to the increase in the flux of the diclofenac-benzylamine ion-pair. It is possible that a dermally applied microemulsion could penetrate the stratum corneum and exist intact in the whole horny layer.[20)]

According to the findings revealed in a previous study[2)] and the present study, ion-pair formation could be a useful tool to facilitate transdermal absorption of ionic drugs. Combined use of ion-pairs with microemulsions will be developed for future clinical transdermal therapeutic delivery systems for ionic drugs. In this study we used microemulsions consisting of PBS, IPM, ethanol and Tween80 as model microemulsions. Since there is a possibility that a high concentration of alcohols and surfactants induce skin irritation, further studies such as the selection of microemulsions with no skin irritation are necessary for future clinical use.

Acknowledgements This study was supported by a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan (18590041) and the Promotion and Mutual Aid for Private schools in Japan.

References

1) Megwa S. A., Cross S. E., Whitehouse M. W., Benson H. A. E., Roberts M. S., *J. Pharm. Pharmacol.*, **52**, 929—940 (2000).
2) Kamal M. A. H. M., Iimura N., Nabekura T., Kitagawa S., *Chem. Pharm. Bull.*, **54**, 481—484 (2006).

3) Menasse R., Hedwall P. R., Kraetz J., Pericin C., Riester L., Sallmann A., Ziel R., Jaque R., *Scand. J. Rheumatol.*, **22** (Suppl.), 5—16 (1978).
4) Nishihata T., Kotera K., Nakano Y., Yamazaki M., *Chem. Pharm. Bull.*, **35**, 3807—3812 (1987).
5) Kitagawa S., Hosokai A., Kaseda Y., Yamamoto N., Kaneko Y., Matsuoka E., *Int. J. Pharmaceut.*, **161**, 115—122 (1998).
6) Gallarate M., Gasco M. R., Trotta M., *Acta Pharm. Technol.*, **34**, 102—105 (1988).
7) Iwamoto K., Kato T., Kawahara M., Koyama N., Watanabe S., Miyake Y., Sunamoto J., *J. Pharm. Sci.*, **80**, 219—224 (1991).
8) Kreilgaard M., *Adv. Drug Deliv. Rev.*, **54** (Suppl.), S77—S98 (2002).
9) Fevrier F., Bobin M. F., Lafforgue C., Martini M. C., *S. T. P. Pharm. Sci.*, **1**, 60—63 (1991).
10) Kemken J., Ziegler A., Mueller B. W., *J. Pharm. Pharmacol.*, **43**, 679—684 (1991).
11) Begona D.-C. M., Iglesias-Vilas G., Blanco-Mendez J., Arturo L.-Q. M., Marty J. P., Guy R. H., *Eur. J. Pharm. Biopharm.*, **43**, 37—42 (1997).
12) Rhee Y.-S., Choi J.-G., Park E.-S., Chi S.-C., *Int. J. Pharmaceut.*, **228**, 161—170 (2001).
13) Peltola S., Saarinen-Savolainen P., Kiesvaara J., Suhonen T. M., Urtti A., *Int. J. Pharmaceut.*, **254**, 99—107 (2003).
14) Laurence M. Y., Rees G. D., *Adv. Drug Deliv. Rev.*, **45**, 89—121 (2000).
15) Lee P. J., Langer R., Shastri V. P., *Pharm. Res.*, **20**, 264—269 (2003).
16) Kitagawa S., Yokochi N., Murooka N., *Int. J. Pharmaceut.*, **126**, 49—56 (1995).
17) Nash R. A., Mehta D. B., Matias J. R., Orentreich N., *Skin Pharmacol.*, **5**, 160—170 (1992).
18) Gao S., Sing J., *Int. J. Pharmaceut.*, **165**, 45—55 (1998).
19) Aungst B. J., Rogers N. J., Shefter E., *Int. J. Pharmacet.*, **33**, 225—234 (1986).
20) Kweon J.-H., Chi S.-C., Park E.-S., *Arch. Pharm. Res.*, **27**, 351—356 (2004).