Enhanced Skin Permeation of Cationic Drug Ketotifen through Excised Guinea Pig Dorsal Skin by Surfactants with Different Electric Charges

Shuji KITAGAWA* and Ai IKARASHI

Niigata University of Pharmacy and Applied Life Sciences, Faculty of Pharmaceutical Sciences; 5–13–2 Kamishin'ei-cho, Niigata 950–2081, Japan. Received April 28, 2003; accepted July 30, 2003

Using excised guinea pig dorsal skin, we examined the effects of three surfactants, anionic sodium dodecylsulfate (SDS), cationic *n*-dodecyltrimethylammonium bromide and non-ionic *n*-dodecyl-β-D-maltoside, all of which commonly have an *n*-dodecyl group, on *in vitro* skin permeation of the cationic drug ketotifen. All these three surfactants increased the skin permeation of ketotifen. Among the surfactants tested, anionic SDS had the largest enhancement effects, and significantly increased the permeation at concentrations over 1 mM. The enhancement effect of the same anionic surfactant on the permeation of anionic salicylate was smaller and similar to that of cationic *n*-dodecyltrimethylammonium. The enhancement effects of SDS on ketotifen permeation were more marked than those of the cationic surfactant but differed from previous findings of their effects on other drugs permeation. Analysis of the retention of ketotifen in the skin suggested that SDS-induced increase in the transfer of hydrophilic ketotifen to the skin is the main reason for the marked increase in skin permeation.

Key words ketotifen; surfactant; skin permeation; absorption enhancer; sodium dodecylsulfate

Ketotifen is one of the candidate drugs for developing a transdermal therapeutic system (TTS), because of its superior pharmacological action against asthma at low plasma concentrations.[1] However, since the drug is present as cations at skin pH (pK_a value of ketotifen is 8.5[2]), it exhibits low skin permeability. Therefore, application of a chemical or physical permeation enhancing system is necessary to develop its TTS potential. Thus the effects of chemical enhancers such as L-lactic acid–ethanol–isopropyl myristate have been examined.[1,3]

Alkyl surfactants have been revealed to work as a chemical enhancer on skin permeation of various drugs.[4,5] Among them, alkylammoniums such as *n*-dodecyltrimethylammonium are known to show marked enhancement effects on skin permeation of drugs such as methyl nicotinamide[6] and nonionized form of benzoic acid.[7] Chemical enhancers have been demonstrated to work after penetration into the stratum corneum by increasing either the partition of the drugs to the skin or their diffusion rates in the stratum corneum.[8] Since ketotifen is a cationic drug, electric interaction with enhancers may also affect their enhancement effects. Therefore, to clarify whether such an interaction is involved or not, in this study we examined the effects of alkyl surfactants with different electric charges. We examined the effects of anionic sodium dodecylsulfate (SDS), cationic *n*-dodecyltrimethylammonium bromide and non-ionic *n*-dodecyl-β-D-maltoside, all of which commonly have *n*-dodecyl group as their alkyl chains, on *in vitro* skin permeation of ketotifen. We compared the effects of these surfactants on ketotifen permeation with their effects on the anionic salicylate permeation. Furthermore, we examined the effects of these surfactants on the intradermal concentration of ketotifen.

Experimental

Materials Bromide salt of *n*-dodecyltrimethylammonium and 3-(lauryldimethylammonio)-1-propanesulfonate were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Ketotifen fumarate was from Sigma Chemical Co. (St. Louis, MO, U.S.A.). *n*-Dodecyl-β-D-maltoside was from Dojindo Laboratories (Kumamoto, Japan). Sodium dodecylsulfate (SDS) and all other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Skin Preparations Full thickness dorsal skin was excised from male guinea pigs and subcutaneous fat and other extraneous tissues were trimmed. Delipidized skin was prepared as described previously.[9] Stratum corneum lipids were extracted by incubating the excised skin with a chloroform–methanol mixture (2 : 1 vol) for 12 h and washing it extensively with phosphate buffered saline (PBS).

Measurement of *in Vitro* Skin Permeation *In vitro* skin permeation of ketotifen was examined as described previously.[10] The skin was mounted in a Franz cell with water jackets (37 °C). The available diffusion area was about 0.64 cm^2, and the lower cell volume was about 4.5 ml. The upper cells were filled with 1 ml saline either in the presence or absence of surfactants, and the receiver cells with PBS (pH 7.4). The lower cells were stirred at 450 rpm by a magnetic stirrer during 12 h pretreatment of the skin. After washing both cells, 20 mM ketotifen maleate dissolved in saline either in the presence or absence of surfactants was added to the upper compartments, and the permeation experiment was started. The pH of the ketotifen solution was 3.4. One hundred fifty microliters of sample was taken from the lower cells periodically over a maximal period of 28 h. The concentration of ketotifen was determined by HPLC (L-6000; Hitachi, Tokyo, Japan) with an L-4000 UV detector (Hitachi) at 300 nm. Separation was achieved on a reversed-phase column (Mightysil RP-18, 4.6 mm i.d., 150 mm) using a mobile phase consisting of methanol, water and phosphoric acid (750 : 1250 : 1, v/v) at a flow rate of 0.68 ml/min. Sodium salicylate was used as an internal standard.

Using Eq. 1, the apparent permeability coefficient K_p of ketotifen was obtained from the initial straight portion of the permeation curve dC_R/dt.

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

where C_R and V_R are the concentration of ketotifen in the lower cell and the lower cell volume, respectively, A is the diffusion area and C_D is the concentration of ketotifen in the upper compartment.

Measurement of the Intradermal Concentration of Ketotifen Intradermal concentration of ketotifen was measured as described previously.[11] After 24 h permeation of ketotifen, the skins were removed from the cells and washed three times with ice-cold methanol. Following room temperature drying, each skin was weighed, minced and placed in 10 ml of methanol, then homogenized using a tissue homogenizer Polytoron (Kinematica AG, Switzerland). The samples were then centrifuged and the supernatant layer was used to determine the concentration of ketotifen by HPLC as described above.

Statistical Analysis Mann–Whitney's *U*-test was used to analyze differences between sets of data. The level of significance was adjusted by Bonferroni's method. A p-value less than 0.05 was considered significant.

Results and Discussion

We first examined the effects of various surfactants on the permeation of ketotifen through guinea pig dorsal skin. As

* To whom correspondence should be addressed. e-mail: kitagawa@niigata-pharm.ac.jp

Table 1. Effects of 2 mM Surfactants on Flux (J) and Apparent Permeability Coefficients (K_p) of Ketotifen

Surfactants	J ($\times 10^{-3}$ μmol·cm^{-2}·h^{-1})	K_p ($\times 10^{-4}$ cm·h^{-1})
None	1.56±0.52	0.78±0.26
SDS	6.62±0.83***,*a)*	3.66±0.46***,*a)*
n-Dodecyltrimethylammonium bromide	3.78±0.78**	1.89±0.39**
n-Dodecyl-β-D-maltoside	3.26±0.16***	1.63±0.08***
3-(Lauryldimethylammonio)-1-propanesulfonate	2.54±0.54	1.27±0.27

Data are means±S.D. of four experiments. ** $p<0.01$, *** $p<0.001$, compared to control. *a)* $p<0.01$, compared to the values in the presence of *n*-dodecyltrimethylammonium or *n*-dodecyl-β-D-maltoside. Concentration in donor compartment in the presence of sodium dodecylsulfate was 18.1±0.7 mM, and that in the presence or absence of other surfactants was 20 mM.

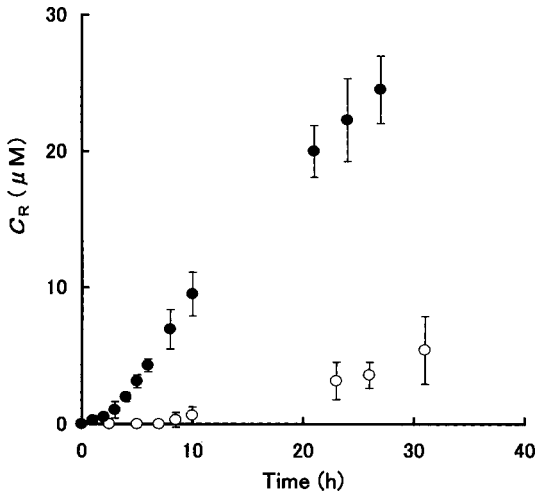

Fig. 1. Effect of SDS on Increase in Concentration of Ketotifen in Receiver Compartment, C_R, due to Transfer through Guinea Pig Dorsal Skin

○, control; ●, with 2 mM SDS. Data are means±S.D. of four experiments.

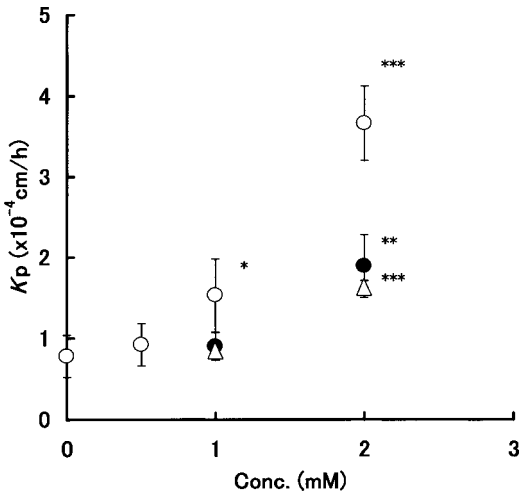

Fig. 2. Dose-dependent Effects of SDS, *n*-Dodecyltrimethylammonium and *n*-Dodecyl-β-D-maltoside on Permeability Coefficient of Ketotifen

○, SDS; ●, *n*-dodecyltrimethylammonium; △, *n*-dodecyl-β-D-maltoside. Data are means±S.D. of four experiments. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, compared to control.

shown in Fig. 1, ketotifen permeated through the skin very slowly. Since rapid permeation of the drug was observed in delipidized skin (flux through it (0.968±0.112 μmol·cm^{-2}·h^{-1}) was about 600 times greater than that through intact skin), the stratum corneum lipid lamella was considered a barrier against the permeation of this cationic drug. Anionic SDS enhanced permeation as shown in Fig. 1 and Table 1 for its effect at 2 mM. To avoid the change in thermodynamic activity of ketotifen by the addition of surfactants, we limited the concentration of the surfactants to at most one tenth of that of ketotifen. SDS induced 4 to 5-fold increase in the flux and permeability coefficient at that concentration. Decrease in lag time was also observed. At 1 mM only SDS had enhancement effects as shown in Fig. 2 for the dose-dependent effects of these surfactants. Cationic *n*-dodecyltrimethylammonium and non-ionic *n*-dodecyl-β-D-maltoside also enhanced permeation as shown in Table 1 for their effects at 2 mM, but these effects were smaller than that of SDS. There was no significant effect observed for zwitter ionic 3-(lauryldimethylammonio)-1-propanesulfonate.

To examine whether the electric charge of the surfactants is an important factor influencing permeation enhancement, we next examined the effects of 2 mM of the same surfactants on the permeation of anionic salicylate. As shown in Table 2, SDS increased the permeability coefficient 2.3-fold. The enhancement effect was smaller than that of the same surfactant on ketotifen permeation shown above. It was similar to that of cationic *n*-dodecyltrimethylammonium on salicylate permeation, which induced about 2.4-fold increase in the permeability coefficient.

Table 2. Effects of 2 mM Surfactants on Apparent Permeability Coefficients (K_p) of Salicylate

Surfactants	K_p ($\times 10^{-3}$ cm·h^{-1})
None	1.04±0.18
SDS	2.40±0.47**
n-Dodecyltrimethylammonium bromide	2.52±0.16***
n-Dodecyl-β-D-maltoside	1.51±0.33

Data are means±S.D. of four experiments. ** $p<0.01$, *** $p<0.001$, compared to control.

Table 3. Intradermal Concentration of Ketotifen after 24 h in Presence and Absence of 2 mM Surfactants

Surfactants	μg ketotifen/mg dry skin
None	1.80±0.25
SDS	8.40±1.68***
n-Dodecyltrimethylammonium bromide	1.79±0.17
n-Dodecyl-β-D-maltoside	2.07±0.34

Data are means±S.D. of four experiments. *** $p<0.001$, compared to control.

To demonstrate the mechanism involved in the enhancement by the surfactants, especially that by SDS which showed the largest enhancement effects, we then examined the effects of the surfactants on the intradermal concentration of ketotifen. As shown in Table 3, 2 mM SDS increased the intradermal concentration 4.7-fold, which corresponded to

the increase in permeability coefficient. However, the other two surfactants did not significantly increase the intradermal drug concentration.

The present findings demonstrated that among the surfactants tested anionic SDS provided the most marked enhancement effect on the skin permeation of cationic ketotifen. This differed from the effect on anionic salicylate permeation as demonstrated in this work. The findings were also in contrast with previous findings on the effects of the surfactants.[6,7,12,13] It has been demonstrated that cationic surfactants are more destructive to skin tissues causing a greater increase in flux than anionic surfactants.[12,13] We also reported that the enhancement effects of cationic *n*-alkyltrimethylammoniums such as *n*-dodecyltrimethylammonium on permeation of the nonionized form of benzoic acid were larger than those of SDS in excised guinea pig skin.[7]

The mechanism of marked SDS-induced enhancement of skin permeation by ketotifen is not clear. It could not be simply explained by extraction of skin lipids by the surfactant. It has been suggested ion-pair formation and its transfer to the skin is involved between ionic drugs and enhancers with opposite electric charges.[14] Such an ion-pair formation may be involved in the anionic surfactant-induced enhancement process. If not, some kind of ionic interaction between cationic ketotifen and anionic SDS also seems to be involved in the permeation enhancement process in addition to the surfactant-induced perturbation of lipid lamella. Partition of anionic surfactants such as SDS to the lipid lamella of the stratum corneum may change the surface potential of the lamella and stimulate cationic ketotifen, which is a highly hydrophilic drug and has a low partition behavior to the skin surface, to transfer there.

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