# Enhancement Effects of Double-Chained Cationic Surfactants of *n*-Dimethyldialkylammoniums on Permeability of Salicylate through Guinea Pig Dorsal Skin

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**We examined the enhancement effects of the double-chained cationic surfactants of *n*-dimethyldialkylammoniums $(CH_3)_2N^+(C_nH_{2n+1})_2$ on the permeation of anionic salicylate through excised guinea pig dorsal skin at pH 7.4. Among them, *n*-dimethyldidecylammonium (2C10), which seemed to form micelles, had dose-dependent enhancement effects at concentrations of more than 0.1 mM, and about a ninety-fold increase in the permeability coefficient of salicylate was observed at 2 mM. The enhancement effect of 2C10 was larger than those of single-chained cationic surfactants of *n*-alkyltrimethylammoniums. *n*-Dimethyldilaurylammonium (2C12), which seemed to form bilayer vesicles, induced about a twenty five-fold increase in the permeability coefficient. The enhancement effects of *n*-dimethyldialkylammoniums decreased with the increase in their alkyl chain lengths. In contrast, only slight stimulation by these cationic surfactants was observed for silicon rubber membrane permeation of salicylate. Analysis of the retention of the salicylate in the skin suggested that the double-chained cationic surfactants-induced increase in the transfer of salicylate to the skin is the main reason for the marked stimulation of the skin permeation.**

**Key words** cationic surfactant; *n*-dimethyldialkylammonium; skin permeation; permeation enhancer; salicylate

It has been demonstrated that various kinds of surfactants disrupt the barrier function of skin and increase transdermal drug permeability.[1] Among them, long-chain cationic surfactants such as *n*-dodecyltrimethylammonium (1C12) are known to have marked enhancement effects on the transdermal permeation of hydrophilic drugs.[2,3] These cationic surfactants seem to interact with proteins in the stratum corneum as well as the lipid lamella, improve the hydrophilic property of the skin and enhance the skin permeation of relatively hydrophilic drugs.[3]

Different from the single-chained cationic surfactants, some of the double-chained cationic surfactants form bilayer vesicles just like phospholipids.[4—6] We recently examined the effects of double-chained cationic surfactants of *n*-dimethyldialkylammoniums $(CH_3)_2N^+(C_nH_{2n+1})_2$ on skin permeation of undissociated form of benzoic acid through excised guinea pig dorsal skin at acidic donor pH conditions.[6] *n*-Dimethyldialkylammoniums with relatively shorter alkyl chains such as dimethyldidecylammonium (2C10) seem to form micelles instead of vesicles. The findings on the five double-chained cationic surfactants tested ($n=10$—18) revealed the marked enhancement effects of the surfactants with relatively shorter alkyl chains, whose enhancement mechanism is possibly similar to that of single-chained cationic surfactants.

The enhancement effects of the cationic surfactants may be different depending on the electric charge of the drugs. Therefore, in this study we examined the effects of five *n*-dimethyldialkylammoniums; *n*-dimethyldidecylammonium (2C10), *n*-dimethyldilaurylammonium (2C12), *n*-dimethyldimyristylammonium (2C14), *n*-dimethyldipalmitylammonium (2C16) and *n*-dimethyldistearylammonium (2C18) on *in vitro* skin permeation of anionic drugs using salicylate, whose p$K_a$ value is 3.0,[7] as a model drug. We compared their enhancement effects with those on undissociated forms of drugs we previously reported.[6] We also compared their enhancement effects on the permeation of salicylate with those of single-chained cationic surfactants of *n*-alkyltrimethylammoniums and those of the vesicles consisting of *n*-dimethyldialkylammoniums and egg yolk phosphatidylcholine (egg yolk PC). In order to clarify the mechanism of the enhancement, we furthermore examined the effects of *n*-dimethyldialkylammoniums on the permeation of salicylate though silicon rubber membrane. We also examined their effects on the skin content of salicylate.

## Experimental

**Materials** Bromide salts of *n*-dimethyldialkylammoniums and *n*-alkyltrimethylammoniums were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Sodium salicylate, egg yolk phosphatidylcholine (egg yolk PC) and all other reagents were from Wako Pure Chemical Industries. Silicon rubber sheet was purchased from GE Toshiba Silicones Co., Ltd. (Ota, Japan).

**Preparation of Surfactant Solutions and Sonicated Dispersions** *n*-Dimethyldialkylammoniums were used after preparing the sonicated dispersions as described previously.[6] Bromide salts of *n*-dimethyldialkylammoniums were dissolved in chloroform, and the solvent was evaporated. Dried surfactant films were prepared by removing the solvent under vacuum evaporation. The cationic surfactants were hydrated and suspended by vortex mixing in phosphate-buffered saline (PBS). Then, the suspension was sonicated with a probe-type sonicator for 5 min at an output power of 80 W at 50 °C. When egg yolk PC was added as vesicle constituent, the suspension was sonicated at 30 °C under a stream of nitrogen.[8] Bromide salts of *n*-alkyltrimethylammoniums were dissolved in PBS and used for the permeation experiments.

**Measurement of *in Vitro* Skin Permeation** *In vitro* skin permeation of the drugs was examined as described previously.[9] Full thickness dorsal skin was excised from male guinea pigs and mounted in two-chamber diffusion cells with a water jacket (37 °C). The available diffusion area was 0.65 $cm^2$, and the mean half-cell volume was 5.4 ml. The donor cells were filled with PBS (pH 7.4) in the presence or absence of surfactants and the receiver cells were filled with PBS. Both donor and receiver cells were stirred at 450 rpm with a magnetic stirrer during 12-h pretreatment of the skin. After washing both donor and receiver cells, 20 mM sodium salicylate in PBS either in the presence or absence of the surfactants was added to the donor compartments, and the permeation experiment was started. One hundred fifty microliters of sample was taken from the receiver cells periodically over a maxi-

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mum period of 30 h. The concentration of salicylate was determined by HPLC (L-6000; Hitachi, Tokyo, Japan) with an L-4000 UV detector (Hitachi) at 303 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 (750 : 1250 : 1) at a flow rate of 0.68 ml/min. Ketotifen fumarate was used as an internal standard.

Apparent permeability coefficients $K_p$ of salicylate was obtained according to Eq. 1 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 salicylate in the receiver compartment and the compartment volume, respectively, $A$ is the diffusion area and $C_D$ is the concentration of salicylate in the donor compartment.

**Measurement of Silicon Rubber Membrane Permeation** Silicon rubber membrane (TSE221-5U, 0.5 mm thickness) was cut from the sheet and placed in the two-chamber diffusion cells. Permeation of salicylate through the silicon rubber membrane was examined in the same ways as described above at 37 °C.

**Measurement of Skin Content of Salicylate** Skin content of salicylate was measured as described previously.[10,11] After 6 h permeation of salicylate, the skins were removed from the cells and washed three times with ice-cold methanol. Following room temperature drying, each skin sample was weighed, cut up and placed in 10 ml of methanol, and then homogenized using a tissue homogenizer Polytron (Kinematica AG, Switzerland). The samples were then centrifuged and the supernatant layer was removed and the concentration of salicylate was determined by HPLC as described above.

**Statistical Analysis** Bonferroni's *t*-test for multiple comparisons or the Student's *t*-test was used to analyze the difference between the sets of data. A *p*-value less than 0.05 was considered significant.

## Results and Discussion

We examinied the effects of *n*-dimethyldialkylammoniums $(CH_3)_2N^+(C_nH_{2n+1})_2$ on the skin permeation of salicylate. Among them, 2C12, 2C14 and 2C16 seem to form bilayer vesicles, but 2C10 forms micelles and 2C18 is present as solid aggregates.[6] Since skin works as a barrier to ionic drugs, salicylate permeated through the skin very slowly as shown in Fig. 1. Double-chained cationic surfactant 2C10 dose-dependently increased the permeation in the concentration range of more than 0.1 mM, as shown in Figs. 1 and 2. The lag time decreased while the concentration of 2C10 increased, and almost disappeared at 2 mM. The cationic surfactant increased the permeability coefficient about ninety-fold at 2 mM. The enhancement ratios to the permeability coefficients of salicylate were much larger than those to the permeability coefficients of the undissociated form of benzoic acid by the same cationic surfactant on which we previously reported.[6] The enhancement effects of 2C10 were also larger than those of single-chained cationic surfactants of *n*-alkyltrimethylammoniums as shown in Table 1 for the effects of 2 mM *n*-decyltrimethylammonium (1C10), *n*-dodecyltrimethylammonium (1C12), *n*-tetradecyltrimethylammonium (1C14) and cetyltrimethylammonium (1C16).

2C12, which seems to form bilayer vesicles, also stimulated the permeation of salicylate and increased the permeability coefficient about twenty five-fold at 2 mM. The decrease in the lag time was also observed (data not shown). The enhancement effects of *n*-dimethyldialkylammoniums decreased with the increase of the alkyl chain length, as also shown in Table 1 for their effects at 2 mM. The cationic surfactants with long alkyl chains did not have marked enhancement effects even at higher concentrations. As mentioned above, 2C10 seems to form micelles. In contrast, 2C12, 2C14 and 2C16 seem to form bilayer vesicles.[6] As previously reported for the ESR analysis on the order parameters of 5-doxylstearate in sonicated *n*-dimethyldialkylammoniums, the molecular packing of these cationic surfactants becomes

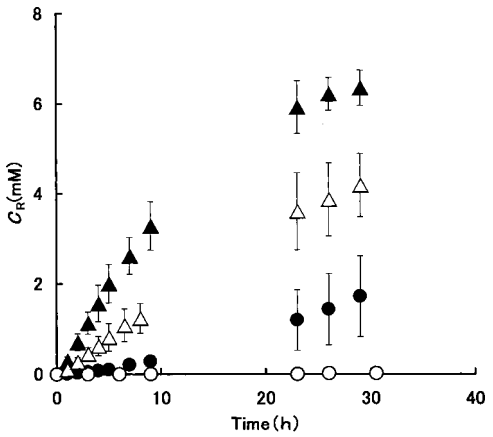


Fig. 1. Increase in Concentration of Salicylate in Receiver Component, $C_R$, due to Transfer through Guinea Pig Dorsal Skin in the Presence or Absence of 2C10

○, control; ●, with 0.5 mM 2C10; △, with 1 mM 2C10; ▲, with 2 mM 2C10. Data are means±S.D. of four experiments.

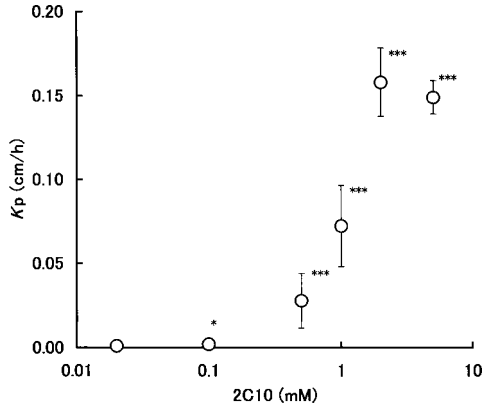


Fig. 2. Dose-Dependent Effects of 2C10 on the Apparent Permeability Coefficient ($K_p$) of Salicylate

Data are means±S.D. of four experiments. $*p<0.05$, $***p<0.001$ compared with the control value.

Table 1. Effects of 2 mM *n*-Dimethyldialkylammoniums and *n*-Alkyltrimethylammoniums on Permeability Coefficients ($K_p$) of Salicylate in the Presence or Absence of 2 mM Egg Yolk PC

| Surfactant | $K_p$ ($\times 10^{-2}$ cm h$^{-1}$) |
|---|---|
| None | 0.17±0.04 |
| *n*-Dimethyldidecylammonium (2C10) | 15.8±2.0*** |
| *n*-Dimethyldilaurylammonium (2C12) | 4.24±1.80** |
| *n*-Dimethyldimyristylammonium (2C14) | 1.67±0.83* |
| *n*-Dimethyldipalmitylammonium (2C16) | 0.36±0.07** |
| *n*-Dimethyldistearylammonium (2C18) | 0.21±0.07 |
| *n*-Decyltrimethylammonium (1C10) | 0.64±0.02*** |
| *n*-Dodecyltrimethylammonium (1C12) | 9.80±1.81*** |
| *n*-Tetradecyltrimethylammonium (1C14) | 8.81±1.84*** |
| *n*-Cetyltrimethylammonium (1C16) | 1.80±0.10*** |
| 2C10+egg yolk PC | 5.30±0.32***,*a)* |
| 2C12+egg yolk PC | 0.92±0.14***,*a)* |

Data are means±S.D. of four experiments. $*p<0.05$; $**p<0.01$; $***p<0.001$ compared with control value. *a)* $p<0.001$ compared with the values in the absence of egg yolk PC.

loose with the decrease in their alkyl chain lengths due to the repulsion of the cationic charges.[6] Thus, their interaction with skin components is likely to occur by penetration of their monomers into the stratum corneum,[6] as suggested for the enhancement effects of single-chained *n*-alkyltrimethylammoniums on skin permeation of benzoic acid[3] and as also suggested by the hemolytic effects of the same double-chained cationic surfactants.[8] Reduction of the enhancement effects of *n*-dimethyldialkylammoniums by the stable vesicle formation due to the addition of egg yolk PC,[8] which is shown in Table 1, also supports this. As suggested in the previous report on their interaction with the erythrocyte membranes,[8] the partition of the double-chained cationic surfactants into the skin also seems to have been decreased by the presence of PC vesicles. As well as the formation of the stable vesicles, this also seems to account for the reduction of the enhancement effects.

Ion-pair formation has been suggested to be involved in the enhancement mechanism of various alkylammonium compounds.[12] To examine the possibility of the involvement of the ion-pair formation between salicylate and the cationic surfactants such as 2C10, we next examined the effects of *n*-dimethyldialkylammoniums on the permeation of silicon rubber membrane; Since permeation of drugs through silicon rubber membrane follows the pH-partition hypothethis,[13] and the membrane has been used to study the possibility of the contribution of the ion-pair formation on the enhancement effects of various skin permeation enhancers.[14] Undissociated forms of salicylic acid rapidly permeated through the silicon rubber membrane, which was shown as a large permeability coefficient value at acidic pH (pH 2.6) in Table 2. In contrast, salicylate hardly permeated through the membrane. 2C10 only slightly induced the permeation of salicylate. Therefore, the contribution of ion-pair formation may be small on the skin permeation enhancement by the cationic surfactants.

To reveal the mechanism of the enhancement effects of the cationic surfactants, we next examined their effects on the skin content of salicylate. As shown in Table 3 on the effects of 2 mM *n*-dimethyldialkylammoniums on the skin content of salicytate after 6 h, 2C10 induced a sixty-fold increase. The increase in the skin content by the double-chained surfactants shown in Table 3 corresponded to the increase in the permeability coefficients of salicylate shown in Table 1. The present findings suggested that the increase in the transfer of salicylate to the skin is the main reason for the marked increase in skin permeation.

As suggested for the single-chained surfactants,[3] monomers of *n*-dimethyldialkylammoniums with relatively shorter alkyl chains may interact with proteins of the stratum corneum as well as with the lipid lamella, which will stimulate the permeation of ionic drugs such as salicylate used in this study. The cationic surfactants may interact with anionic components of the stratum corneum, change the electoric property there, and stimulate the transfer of the anionic drug into the skin. In that case high concentration of the cationic surfactants in the stratum corneum may induce the electric repulsion among them and cause the saturation phenomenon in their enhancement effects as observed for 2C10. Since the enhancement effect of 2C10 was larger than the single-chained *n*-alkyltrimethylammoniums, the transfer of 2C10 to the stratum corneum may be larger than that of single-chained surfactants.

Table 2. Permeability Coefficients ($K_p$) of Salicylate through Silicon Rubber Membrane in the Presence or Absence of 2 mM 2C10 and That of Salicylic Acid in Its Absence

| Surfactant | $K_p$ ($\times 10^{-4}$ cm h$^{-1}$) |
|---|---|
| None | 0.00±0.00 |
| 2C10 | 0.21±0.08 |
| None (pH 2.6) | 393±28 |

Data are means±S.D. of four experiments.

Table 3. Skin Content of Salicylate after 6 h in the Presence of 2 mM *n*-Dimethyldialkylammoniums

| *n*-Dimethyldialkylammonium | μg salicylate/mg dried skin |
|---|---|
| None | 0.18±0.05 |
| 2C10 | 10.3±3.6** |
| 2C12 | 2.90±0.84** |
| 2C14 | 0.86±0.10*** |

Data are means±S.D. of four experiments. ** $p<0.01$; *** $p<0.001$ compared with control value.

In addition to the increase in the transfer of salicylate to the skin induced by the cationic surfactants, other mechanisms may also contribute to their enhancement effects. The extraction of stratum corneum lipids by the micelles of 2C10 may also contribute to its marked enhancement effects. Furthermore, since the decrease in the lag time was observed, there is a possibility that the cationic surfactants induce the reduction of the available diffusion length in the stratum corneum as well as the increase in the diffusion rate of salicylate.

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