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Effect of penetration enhancers (pyrrolidone derivatives) on multilamellar liposomes of stratum corneum lipid: a study by UV spectroscopy and differential scanning calorimetry

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Summary

In order to elucidate the mechanisms of action of transdermal absorption-enhancing compounds, i.e., pyrrolidone derivatives (1-methyl-2-pyrrolidone, 1-ethyl-2-pyrrolidone, 1,5-dimethyl-2-pyrrolidone, 5-methyl-2-pyrrolidone), multilamellar liposome was prepared from the simulated stratum corneum lipids and employed as a model system for the barrier function of the stratum corneum. The liposomal membrane of the stratum corneum lipid liposome (SCLL) behaves as an osmometer and has an excellent barrier function. In addition, its phase transition temperatures are similar to those of human stratum corneum intercellular lipid region. Therefore, SCLL seems to be a useful skin model. The effect of the pyrrolidone derivatives on the osmotic behavior of SCLL was examined. As the result of the osmotic behaviors, pyrrolidone derivatives perturb the barrier function of the liposome. The more hydrophobic enhancers penetrate more easily into the lipid bilayer and reduce the barrier function of the membrane more effectively. The results of differential scanning thermograms of the SCLL suggest that the pyrrolidone derivatives had incorporated into the lipid layer in the liposome and increased the fluidity of the lipid layer in the liposome and such activity might have some correlation with the transdermal absorption-enhancing activity of these compounds.

Introduction

There has been a dramatic surge in interest in drug delivery through the skin to produce systemic effects by topical application. It has some merits of reducing hepatic first-pass elimination of drugs. It can also optimize therapeutic efficacies of drugs with a short half-life, and reduce problems of patient compliance (Chrisipher, 1971; Elias, 1983; Kydonieus, 1987; Sharade, 1988). The initial commercial success of nitroglycerine and scopolamine patches was a further impetus to invest in this approach. It also evoked a number of predictions regarding the future of this delivery route. However, so far there have been a very limited number of drugs available in transdermal patch systems and the future of drug delivery through this route has been criticized (Gardner,

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1987). The main problems of transdermal drug delivery are the poor permeability of most drugs through intact skin and the skin irritation or allergic responses that are induced by the drugs or vehicles (Chein, 1987; Gardner, 1987).

To improve the permeability of drugs through the skin, enhancers have been developed which would reversibly reduce the barrier resistance of the skin and thus allow the drug to penetrate to the viable tissues and enter the systemic circulation. They include lipophilic solvents such as dimethylsulfoxide (Kuriharabergstrom et al., 1987), *N,N'*-dimethylformamide (Barry, 1983) and 2-pyrrolidone derivatives (Akhter and Barry, 1985; Aungst et al., 1986; Sasaki et al., 1988, 1990), surfactants of various structure (Scheuplein and Ross, 1970; Dugard and Scheuplein, 1973; Shen et al., 1976) and fatty acids (Aungst et al., 1986; Chein, 1987; Yamada et al., 1987). Recently, azone (Beastall et al., 1988; Mahjour et al., 1989), decylmethylsulfoxide (Goodman and Barry, 1986) and terpenes (Williams and Barry, 1989, 1991) have been of great interest. The mechanism of transdermal absorption enhancers is not clear as yet. It has been suggested that they may interact with the stratum corneum, solubilize the lipids and thus enhance the transdermal transport of drugs (Hadgraft, 1989). Unsaturated fatty acids and azone have a property in common; they have a bulky moiety in their chemical structure. When these compounds are incorporated into the stratum corneum, they result in a less compact organization of the tissue and increase its fluidity. It is believed that this increment in fluidity reduces the barrier properties of the layer and enhances percutaneous drug absorption (Chein, 1987).

The stratum corneum is a compact and highly keratinized tissue with its lipids and proteins contributing to a complex structure which is relatively impermeable to water and other substances. Since the main role of stratum corneum is to act as a barrier, the majority of drugs will not penetrate at rates sufficiently high for any therapeutic effect. Enhancers thus must interact with and alter the proteins and/or the lipids to make the lipid moiety of the layer easier for molecules to diffuse through since it is thought to contribute much to the skin being an impermeable barrier (Swartzendruber et al., 1987). Stratum corneum is composed of ceramides, cholesterol, fatty acids and cholesterol ester (Wertz and Downing, 1987). The acyl chains in the ceramides are almost entirely straight and saturated, ideal for forming highly ordered impermeable membranes. It has been reported that removal of the lipids from the layer by an organic solvent leads to a 1000-fold increase in water permeability (Sweeney et al., 1966).

In this study, multilamellar liposomes were prepared from the simulated stratum corneum lipids, hence being referred to as stratum corneum lipid liposomes (SCLL). SCLL were employed as a model membrane of the stratum corneum lipid bilayer to investigate the effects of transdermal absorption enhancers on the barrier function of the stratum corneum. To estimate the barrier function of SCLL, the osmotic behavior of SCLL was measured in the presence of pyrrolidone derivatives such as 1-methyl-2-pyrrolidone, 1-ethyl-2-pyrrolidone, 1,5-dimethyl-2-pyrrolidone and 5-methyl-2-pyrrolidone. The effect of pyrrolidone derivatives on the phase transition temperature of SCLL was also investigated using differential scanning calorimetry (DSC), since the thermodynamic parameters for the gel-to-crystalline phase transition of liposomes are best obtained by DSC (Rolland et al., 1991).

Material and Methods

Materials

Ceramides, palmitic acid and cholesterol were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Palmitic acid and cholesterol were recrystallized several times in absolute ethanol. Cholesterol sulfate was prepared by the reaction of cholesterol with excess chlorosulfonic acid in pyridine, purified by silica gel column chromatography and recrystallized in *n*-propanol. 1-Methyl-2-pyrrolidone (MP), 1,5-dimethyl-2-pyrrolidone (DMP), 1-ethyl-2-pyrrolidone (EP), and 5-methyl-2-pyrrolidone (5-MP) were purchased from Aldrich Chemical Co. (St. Louis, MO, U.S.A.).

All other chemicals were of reagent grade and used without further purification.

Preparation of stratum corneum lipid liposomes (SCLL)

The composition of the stratum corneum lipids was simulated by dissolving ceramides (40%), cholesterol (25%), palmitic acid (25%) and cholesterol sulfate (10%) in a mixture of chloroform and methanol (9:1) (5 mg total lipids/ml solvent). The solution was evaporated to dryness in a round bottomed flask on a rotary evaporator. The lipid film was further dried in vacuum. This dried thin film was warmed at 75°C for 5 min. Then, the lipid film was suspended in an aqueous solution of 50 mM glucose (2 mM EDTA, 100 mM Tris-HCl buffer, pH 7.5) by vortexing and multilamellar liposomes were prepared. This preparation was then incubated at 37°C under nitrogen gas. Final lipid concentration of this dispersion was 5 mg of total lipid in 1 ml of 50 mM glucose solution.

Osmotic behavior of the SCLL

From the stock SCLL suspension, 0.2 ml of the SCLL suspension was taken and diluted with 3.0 ml of glucose solution of various concentrations to obtain the desired concentration gradients. C_{in}/C_{out}, the ratio of the glucose concentration in the inner parts of SCLL to that in the dilution medium, was varied from 0.2 to 2.0, keeping the interval approx. 0.2. In order to observe the effect of penetration enhancers on the barrier function of SCLL, various amounts of the penetration enhancers (MP, DMP, EP, 5-MP) were added to the glucose solution beforehand. The turbidity, apparent absorbance at 450 nm, was measured on a Pye Unicam 1750 UV/Vis Spectrophotometer after 4 h incubation of the mixed sample at 37°C.

Determination of partition coefficients

Partition coefficients of the skin penetration enhancers (MP, DMP, EP, 5-MP) between distilled water and octanol were determined at 37°C according to the method of Kakemi et al. (1967). 1 ml of each enhancer was dissolved in 5 ml of distilled water saturated with octanol. The solution was then mixed with an equal volume of octanol and shake for 1 h at 37°C. The concentration of enhancers in the aqueous phase was measured using the UV/Vis Spectrophotometer after the partitioning equilibrated.

Differential scanning calorimetry of the SCLL

SCLL was frozen and lyophilized by a freeze dryer. The lyophilized SCLL was rehydrated with distilled water or water containing the enhancers. The thermograms were recorded from 20 to 120°C by DSC. Heating rate was 5°C/min.

Results and Discussion

Osmotic behavior of the stratum corneum lipid liposome

When multilamellar liposomes act as a perfect osmometer, they respond to an osmotic gradient and the total average volume of liposomes changes according to the osmotic gradient across the liposomal membrane as follows (Bangham et al., 1967; Bittman et al., 1981):

$$V_{total} = V_{act}(C_{in}/C_{out}) + V_{dead} \quad (1)$$

where V_{total} is the total average volume of liposomes, and V_{act} and V_{dead} denote the osmotically active and inactive volume of liposomes, respectively. C_{in}/C_{out} is the ratio of solute concentrations in the inner to those in the outer parts of liposome.

The volume change of liposomes can readily be observed by optical measurement according to the following relationship (Yoshikawa et al., 1983):

$$V = k(1/A)^{3/2} \quad (2)$$

where A is the absorbance at a given wavelength and k represents a constant. By combination of Eqns 1 and 2, an equation describing the linear relationship between $(1/A)^{3/2}$ and C_{in}/C_{out} can be derived as follows:

$$(1/A)^{3/2} = 1/k[V_{act}(C_{in}/C_{out}) + V_{dead}] \quad (3)$$

Fig. 1 shows the close linear relationship between C_{in}/C_{out} and $(1/A)^{3/2}$ in the SCLL. This

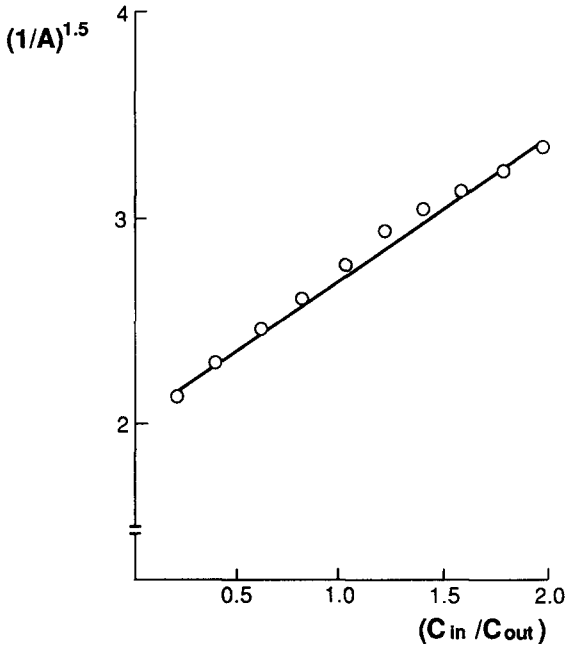

Fig. 1. Osmotic behavior of stratum corneum lipid liposome.

result indicates that the liposomal membrane of the SCLL behaves as an osmometer and has an excellent barrier function. The linear relationship between C_{in}/C_{out} and $(1/A)^{3/2}$ can be seen under hypertonic ($C_{in}/C_{out} = 0.2$) and hypotonic conditions up to $C_{in}/C_{out} = 2.0$, but under more hypotonic conditions than this, the liposomes cannot act as a perfect osmometer, and the plots gradually deviate from the previous linear relationship due to the release of glucose solutes from the liposomes. The equilibration times of SCLL and phospholipid vehicles were measured. The equilibration time of SCLL took 4 h which is 4-times longer than that of phospholipid liposomes (1 h). Water flux rate is less than phospholipid vehicles. SCLL composition is thought to play the role of a barrier function similar to the stratum corneum. Therefore, it is believed that SCLL can be used as a simple model of stratum corneum for the intercellular route.

Effect of pyrrolidone derivatives on the osmotic behavior of the SCLL

The effects of pyrrolidone derivatives on the osmotic behavior of SCLL were examined. As shown in Figs 2–5, the enhancers increased the slopes of the graphs of liposome turbidity vs osmotic gradient in proportion to their concentration. These results mean that enhancers decrease the turbidity by solubilizing and interacting with the lipids. Above a certain concentration of the enhancer, the linear relationship was perturbed. This indicated that the liposomes had lysed and the liposomal membrane lost its barrier function, and hence the solute could move freely through the membrane. It was reported that pyrrolidone derivatives interacted with keratin in the stratum corneum (Barry, 1987a). This result also expressed the interaction of pyrrolidone derivatives with lipids. The concentration necessary to perturb the barrier function of the membrane was termed the minimum lytic activity concentration (MLAC). MLACs of the enhancers were 65, 20, 20 and 30% for MP, DMP, EP and 5-MP, respectively. MLAC corresponds to the

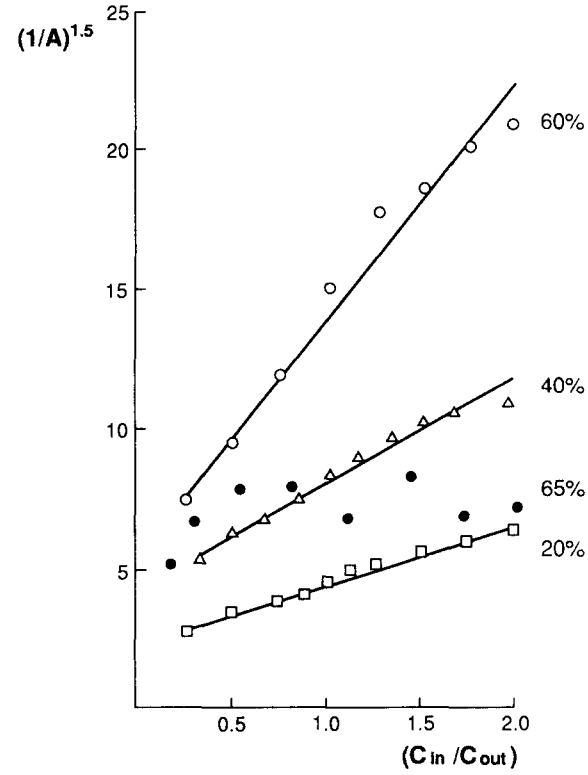

Fig. 2. Effect of MP on osmotic behavior of SCLL. The solid circles show that the linear relationship was perturbed.

concentration that dramatically increases the permeability of chemicals through the skin. These values were relative high. These results suggested that the percutaneous absorption-enhancing activity of pyrrolidone derivatives was simply a solvent effect and nonspecific; they might solubilize lipids and interact with lipid layer and swell the stratum corneum.

Relation between the partition coefficient and MLACs

The partition coefficients (P_c) of the pyrrolidone derivatives between *n*-octanol and water were measured. The P_c and MLAC values are listed in Table 1. The partition coefficients of the enhancers were 0.39, 0.86, 0.91, 0.79 for MP, DMP, EP and 5-MP, respectively. These results suggested that MLACs were related to the partition coefficients; the greater the partition coefficient, the smaller the MLAC. It was clearly shown that the more hydrophobic compounds penetrated more readily into the liposomal membrane, and reduced the barrier function of the membrane more effectively. From these results, it was clear that the most important factor in the percutaneous absorption-enhancing activity of the pyrrolidone derivatives was the partitioning of the enhancers into the lipid layer.

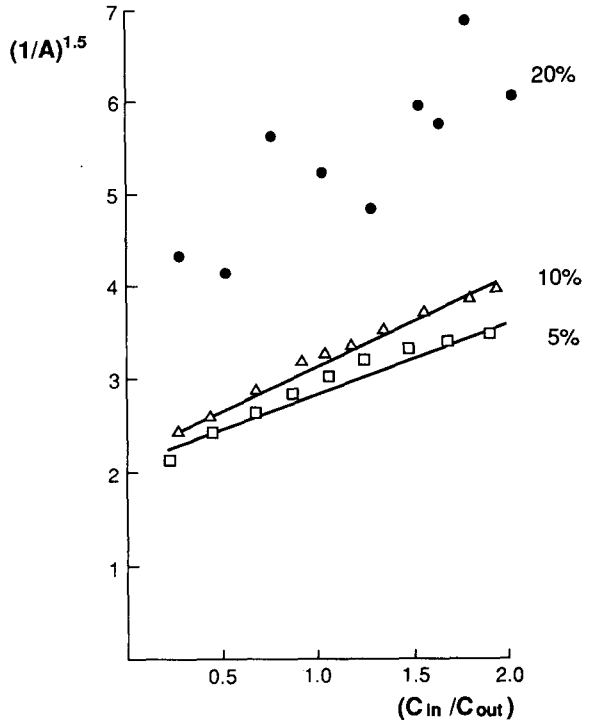

Fig. 3. Effect of DMP on osmotic behavior of SCLL. The solid circles show that the linear relationship was perturbed.

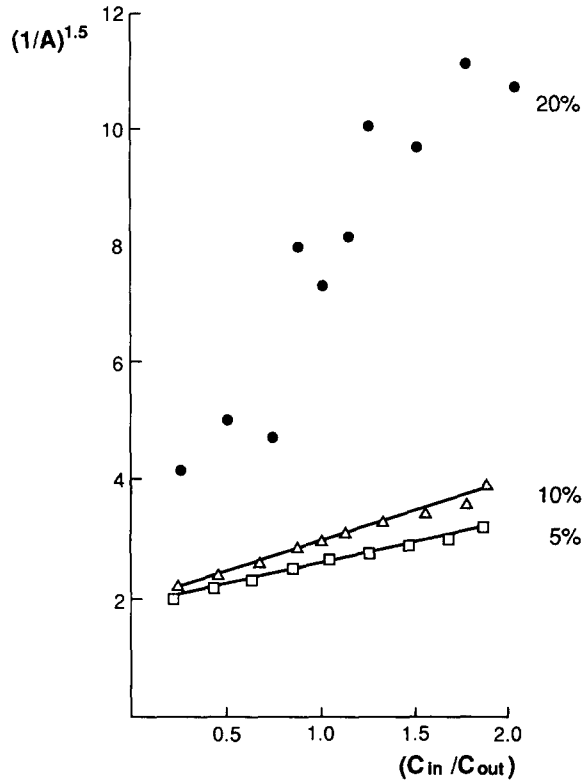

Fig. 4. Effect of EP on osmotic behavior of SCLL. The solid circles show that the linear relationship was perturbed.

The differential scanning thermograms of the SCLL

The differential scanning thermograms of the SCLL in the absence or presence of the pyrrolidone derivatives were measured and the results are shown in Figs 6–8. It was observed that the SCLL had three distinct endothermic transition points at 45°C (T_1), 67°C (T_2) and 98°C (T_3). Some researchers investigated thermal phase transitions in human stratum corneum using DSC (Goodman and Barry, 1986; Golden et al., 1986). Most of them observed two or three thermal phase transitions, near 40 and 70°C, or 40, 70 and

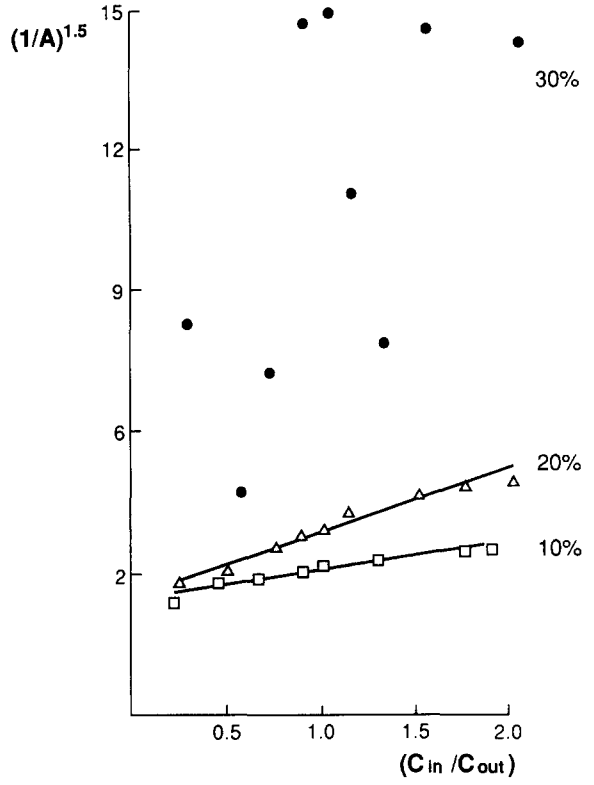

Fig. 5. Effect of 5-MP on osmotic behavior of SCLL. The solid circles show that the linear relationship was perturbed.

80°C arising from the phase transitions of the stratum corneum lipids. It was suggested that the transition seen near 40°C should be allocated to lipid melting, possibly arising from sebaceous lipids or cholesterol side-chain motion, the transition at 70°C is due to the melting of the lipid chain portion buried in the barrier structure and the endotherm near 80°C is due to the break-up of the association between lipid polar head groups

TABLE 1

Partition coefficient (P_c) and minimum lytic activity concentration (MLAC) of enhancer

Enhancer	P_c	MLAC (%)
MP	0.39	65
DMP	0.86	20
EP	0.91	20
5-MP	0.79	30

P_c = [octanol]/[water].

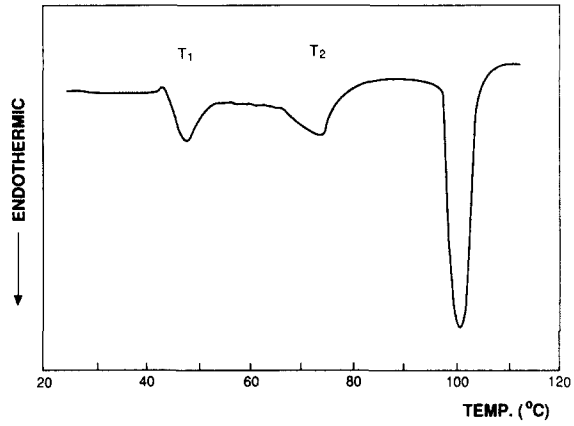

Fig. 6. The DSC profile of stratum corneum lipid liposome.

together with disruption of cholesterol-stiffened regions (Barry, 1987b). Thus, the phase transition at T_1 was ascribed to the phase transition resulting from the cholesterol side chain and that at T_2 resulting from the phase change of gel to lipid crystal of the hydrocarbon chains of palmitic acid and ceramide in the bilayer structure and break-up of the association between lipid polar head groups. The transition near 100°C might be due to vaporization of bound water.

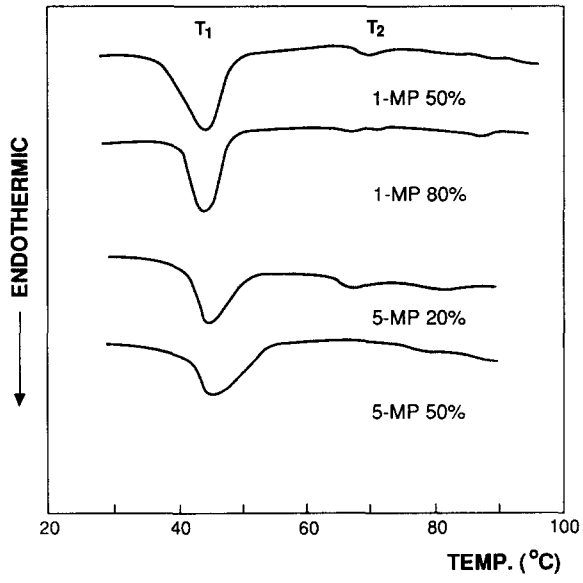

Fig. 7. The DSC of stratum corneum lipid liposome in the presence of MP and 5-MP.

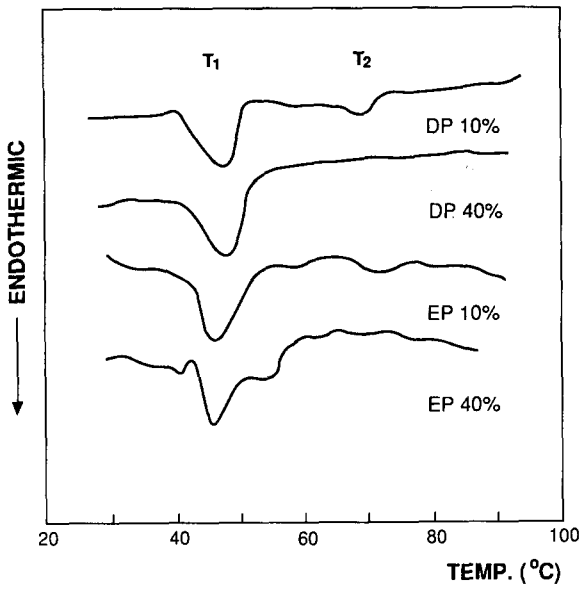

Fig. 8. The DSC of stratum corneum lipid liposome in the presence of DP and EP.

The hydrocarbon chains of lipid have played an important role in transporting drugs (Wertz and Downing, 1987). The interdigitation of chains in the middle of the hydrophobic region stabilized membrane and hydrophobic region acted as a diffusion resistance against drug. Therefore, T_2 lipid phase transition was likely to be important in drug penetration. When the SCLL were treated with penetration enhancers, the peak area at T_2 was reduced and the peak shifted progressively towards lower temperature with increased concentration of enhancers as shown in Figs 7 and 8. At high enhancer concentration, it nearly smeared out. However, the peaks at T_1 remained almost unchanged even in the presence of the enhancers.

These results imply that incorporation of the pyrrolidone derivatives into the lipid layer induces loosening of the lipid packing in the layer, increasing molecular motions in the lipid layer and decreasing intermolecular forces among the lipid molecules. This leads to a reduction in the phase transition temperature of lipids from the gel to liquid-crystalline state. This indicates that the incorporation of pyrrolidone derivatives into the lipid layer increases the fluidity of the lipid bilayer and reduces the resistance against the flow of substances through this layer.

Conclusions

The liposomal membrane of SCLL behaves as an osmometer and has an excellent barrier function. Additionally, phase transition temperatures of SCLL are similar to those of the human stratum corneum intercellular lipid region. Therefore, SCLL seems to be a useful model of skin. The effects of the pyrrolidone derivatives on the osmotic behavior of the SCLL suggest that they perturb the barrier function of the liposome. The MLACs and the partition coefficients of the pyrrolidone derivatives were related; the greater the partition coefficients, the smaller the MLAC. This suggests that the more hydrophobic enhancers penetrate into the lipid layer more easily and reduce the barrier function of the membrane more effectively. The effect of the pyrrolidone derivatives on the differential scanning thermograms of the SCLL suggests that the pyrrolidone derivatives have incorporated into the lipid layer in the liposome and significantly increased the fluidity of the lipid layer in the liposome. Such activity might have some correlation with the transdermal absorption-enhancing activity of these compounds.

Acknowledgement

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