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Mechanism for enhancement effect of lipid disperse system on percutaneous absorption Part II

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Abstract

To further clarify the mechanism involved in the enhancement effect of lipid disperse systems (LDS) on percutaneous absorption, the effect of particle size of LDSs on percutaneous absorption of betahistine (BH), the comparison of the enhancement effect of LDS with the lipid mixtures or the plain LDS, the effect of pretreatment of skin with gel formulation on penetration of LDS-BH and the fluidising effect of LDSs on the stratum corneum (SC) lipids were estimated using Wistar and hairless rats. No major differences in BH absorption were seen between the gel formulations containing LDS with three different particle size (128 ± 4, 336 ± 15, 596 ± 37 nm), prepared using egg phosphatidylcholine (EPC), cholesterol and dicetylphosphate. The percutaneous absorbability of BH from the formulations containing the lipid mixtures or plain LDS did not reach to the extent from EPC–LDS formulation. Following pretreatment with gel formulation containing enhancer (D-limonene or *n*-octyl-β-D-thioglucoside), BH absorption significantly decreased at the initial stage after application compared with that from LDS formulation, suggesting the additive enhancement effect of LDS and enhancer on the absorption. The treatment of the SC of hairless rat with LDSs significantly decreased the rotational correlation time (τ_c) and shifted downwards the slope of curves (τ_c versus temperature) at temperatures ranging from 25 to 60°C, compared with that of untreated SC. However, the significant differences in the fluidising effect between LDSs with different particle size were not observed

Keywords: Percutaneous absorption; Lipid disperse system; Particle size; Fluidising effect; Stratum corneum lipid; Enhancement mechanism

1. Introduction

There is increasing evidence that liposomes (LDS) have a great potential as drug delivery

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systems, not only for intravenous delivery, but particularly for topical applications. It is generally accepted that liposomal drugs can be effectively delivered into and through the skin (Kimura et al., 1989; Michel et al., 1992). Mezei and Gulasekharam, (Mezei and Gulasekharam, 1980) and Singh and Mezei (Singh and Mezei, 1983) were the first to report the potential use of liposomes in topical applications for skin and eyes.

In spite of the well documented enhanced delivery of liposomes entrapped molecules, there was no detailed explanation about the mechanism by which such large objects as liposomes can cross the skin layers more efficiently than smaller molecules. There has been indication that the follicular pathway contributes to the liposomal delivery of drugs into the deeper skin strata (Du Plessis et al., 1992, 1994). In the previous study we have clarified that the LDS lipids were extensively incorporated into the intercellular domains of the stratum corneum (SC) and follicles, and that the fluidity of the SC lipid was significantly increased as a result of incorporation of fluid LDS lipids, e.g. egg phosphatidylcholine (phase transition temperature (τ_m), -15– -17°C), thus ensuring the rapid permeation of fluid LDS into the SC and the viable epidermis (Ogiso et al., 1996a).

The present study was undertaken in order to further clarify the mechanism involved in the enhancement of percutaneous absorption resulting from LDSs. The effect of particle size of LDS on percutaneous absorption, the comparison of the enhancement effect of LDS with the lipid mixtures consisting of the same composition as vesicles and the plain LDS, the effect of pretreatment of skin with gel formulation on the penetration of LDS-BH and the fluidising effect of LDS on the SC lipids were evaluated using rats.

2. Materials and methods

2.1. Materials

Betahistine (BH), 2-(2-methylaminoethyl)-pyridine, was obtained from Aldrich (Milwaukee, WI). Hiviswako 104 (carboxyvinyl polymer), a gel base, and diisopropanolamine were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Nacalai Tesque (Kyoto, Japan), respectively. D-Limonene and *n*-octyl-β-D-thioglucoside (OTG) were obtained from Nacalai Tesque and Wako Pure Chemical Industries, respectively. Egg yolk lecithin (EPC, 96.6%), cholesterol (CH) and dicetylphosphate were purchased from Nichiyu Liposome (Tokyo, Japan), Wako Pure Chemical and Nacalai Tesque, respectively. 16-Doxyl-stearic acid, a spin label, was obtained from Aldrich. All other chemicals and solvents used were of reagent grade or HPLC quality. Tri-*n*-caprylin (tricaprylin) and lauric acid were obtained from Tokyo Chemical Industries (Tokyo, Japan) and Nacalai Tesque, respectively.

Male Wistar rats and male hairless rats (WBN/Ila-Ht), weighing 230–260 g (Japan SLC, Shizuoka, Japan), were used throughout this experiment.

2.2. Preparation of gel formulations and their transdermal systems

2.2.1. Gel formulations

Gel formulations were prepared by the same method as described previously (Ogiso et al., 1996a).

2.2.2. Gel formulation containing LDS or lipid mixtures

BH (1 wt.) was mixed with a mixture (2.5 wt.) of EPC–CH–dicetylphosphate or lauric acid (10:1:1 mol/mol) dissolved in $CHCl_3$, followed by the evaporation of solvent, addition of 5% mannitol solution (3 wt.), and sonication (at 80 W, 25°C) for 25 min with chilling under nitrogen gas in a bath type sonicator. By the treatment large multilamellar vesicles (MLVs) were prepared. In some experiments, the MLV (EPC–LDS) prepared from EPC–CH–dicetylphosphate was further sonicated with a probe-type sonicator (Branson Sonifier, Model 250) at 80 W for 10 min under chilling and nitrogen gas. The resulting LDSs were extruded three times through two stacked Nucleopore polycarbonate filters (0.6, 0.2 and 0.1 μm in the series) at room temperature. The mean diameter of –LDS extruded through 0.6-, 0.2- and 0.1 μm filters, respectively,

Table 1
Composition of betahistine (BH) gel formulations

Composition (g)	Preparation 1	Preparation 2	Preparation 3	I[a]	II[a]
Hiviswako 104	1.0	1.0	1.0	1.0	1.0
Propylene glycol	20.0	20.0	20.0	20.0	20.0
Ethanol	15.0	15.0	15.0	15.0	15.0
Diisopropanol amine	0.5	0.5	0.5	0.5	0.5
BH	3.0	3.0[b]	3.0[b]	—	—
D-Limonene	4.0	4.0	—	4.0	—
OTG	—	—	—	—	1.5
Gentamicin sol.	1.0	1.0	1.0	1.0	1.0

The gel formulations were prepared by adding purified water to give a total weight of 100g.
[a] Gel formulations were prepared for pretreatment.
[b] (b–i) LDS or lipid mixtures containing BH (15.4%, w/w) (refer to Table 2) was mixed with the gel base.

was 596 ± 37 (LDS-600), 336 ± 15 (LDS-300) and 128 ± 4 (LDS-100) by dynamic laser light-scattering measurements using a submicron particle analyzer (Coulter N4, Counter Electron). From the microscopic observation, it was evident that large and small multilameller vesicles were formed, respectively. The plain EPC–LDS (empty bags), without addition of BH, was prepared by the same procedure and then the corresponding weight of BH was mixed gently. Each LDS with different particle sizes was mixed with the gel base containing solvents and enhancer lastly (Ogiso et al., 1996a).

Lipid mixtures were prepared by mixing BH (1 wt.) with a mixture (2.5 wt.) of EPC–CH–dicetylphosphate (10:1:1 mol/mol) or EPC–tricaprylin (1:1.5 w/w), or with EPC (2.5 wt.) alone after evaporation of $CHCl_3$, followed by mixing with the gel base. Details of the composition of gel formulations are listed in Table 1. The lipid constituents and particle size of LDSs and lipid mixtures are shown in Table 2.

The transdermal systems, containing 0.4 g (BH, 12 mg) of formulations and having an absorption area of 3.14 cm^2, were prepared using a corresponding gel formulation described in Table 1 as reported previously (Ogiso et al., 1991).

2.3. In vivo percutaneous absorption experiment

The in vivo percutaneous absorption experiment using Wistar rats was carried out by the same method as described in our previous paper (Ogiso et al., 1996a).

Table 2
Composition and particle size of LDS and lipid mixtures

	Lipid composition (mol/mol)	Particle size (nm)
Liposome		
b	EPC, cholesterol, dicetyl phosphate (10:1:1)	596 ± 36
c	EPC, cholesterol, dicetyl phosphate (10:1:1)	336 ± 15
d	EPC, cholesterol, dicetyl phosphate (10:1:1)	128 ± 4
e	EPC, cholesterol, lauric acid (10:1:1)	642 ± 40
f	EPC, cholesterol, dicetyl phosphate (10:1:1)	594 ± 36
Lipid mixture		
g	EPC	—
h	EPC, cholesterol, dicetyl phosphate (10:1:1)	—
i	EPC, tricapryrine (1:1.5)	—

'f' plain LDS.
Each value represents the mean ± S.D. ($n = 4$).

2.4. Pretreatment with gel formulation containing D-limonene or OTG

The abdominal skin of Wistar rat, removed the hair, was pretreated with the gel formulation containing D-limonene (I) or OTG (II) without BH for 12 h, and then the remaining formulation was wiped off with gauze immersed with warm water. Immediately after wiping, the LDS-formulation (preparation 3), in which D-limonene was removed from preparation 2, was applied to the skin for 8 h. The blood samples were collected periodically.

2.5. ESR measurements of SC lipids

The horny layer (315 mg) prepared from hairless rat skin by trypsin treatment (Kligman and Christopheres, 1963) was homogenized in 9 ml of 0.85% NaCl-40 mM Tris–HCl buffer (pH 7.4) by use of a microhomogenizer. The treatment of homegenate with LDS and the spin labeling of the SC lipids were carried out by the same method as reported previously (Ogiso et al., 1996a). The ESR spectra of the packed samples were recorded with a JES-RE2X ESR spectrometer equipped with a variable-temperature accessory at 26–85°C. The rotational correlation time, τ_c, was calculated by the following formula (Keith et al., 1970): $\tau_c = 6.5 \times 10^{-10} W_0(h_0/h_{-1} - 1)$, where W_0 and h_0 are the width and height, respectively, of the medium-field line of the spectrum and h_{-1} is the height of the high-field line.

2.6. Measurement of zeta potential

The zeta potential of EPC–LDS (mean diameter, 596 nm) and plain EPC–LDS (594 nm) mixed with BH, after dilution with 5% mannitol, was calculated from the mean electrophoretic mobility (Lazer Zee Model 500, Pen Kem, NY).

2.7. Determination of SC lipids remaining in skin after application of gel formulations

The preparation of SC from Wistar rat and application of gel formulation to the SC were carried out by the same method as reported previously (Ogiso et al., 1996a).

2.8. Determination of BH

BH in plasma and sample was determined by HPLC method as described in our previous paper (Ogiso et al., 1996a).

2.9. Data analysis

The area under plasma concentration-time curve (AUC) up to the last sampling point was calculated by the trapezoidal method, and the AUC beyond the last observed plasma concentration (C_t) was extrapolated according to C_t/k_e, where k_e is the terminal elimination rate constant. The area under first moment curve (AUMC) and the mean residence time (MRT) were calculated by means of the moment analysis (Gibaldi and Perrier, 1982).

The means of all data are presented with their standard deviations. Statistical analysis was performed by using the nonpaired t-test, and $p \leq 0.05$ was considered significant.

3. Result and discussion

3.1. Effect of particle size of LDS on percutaneous absorption

The gel formulations containing EPC–LDS with three different particle sizes (128, 336 and 596 nm) were applied to the abdominal skin of rat. The plasma levels and the concentration profiles were approximately similar in the three formulations. As a result, no significant difference in BH absorption between the large MLVs (596 nm) and small vesicles (128 nm) was detectable, indicating that size of particles seems not to correlate with the penetration of this drug.

This is probably due to the fact that liposomal lipids interact with the intercellular and follicular lipids, after reaching to the deeper layer of skin, and liposomal lipids fuse or mix with the skin lipids (details will discuss later), thus resulting in the similar penetration behaviour of from fused lipids.

Consequently, no significant difference between the AUC and MRT for the three LDS formula-

tions was seen (Table 3). In other words, the concept that an optimum particle size for optimal drug delivery exists was neglected in this experiment. These results were keeping with the data of Michel et al. (Michel et al., 1992) that only a slight dependence of the extent of drug permeation into the SC on liposome diameter was observed.

3.2. Comparison of LDS with lipid mixtures or plain LDS on percutaneous absorption

To compare the absorption characteristics between LDS (preparation 2b and 2e) and the lipid mixtures (preparation 2g, 2h and 2i), LDS (EPC, CH, lauric acid), three kinds of lipid mixtures and the plain LDS (particle size, 594 ± 36 nm) were prepared (Table 2).

The gel formulations were prepared as mentioned above and the percutaneous absorption of BH from these formulations was estimated. The percutaneous absorbability of BH from the formulations containing the lipid mixtures (preparation 2g, 2h, 2i) and plain LDS (preparation 2f) did not reach to the extent from EPC–LDS formulation (preparation 2b) (Fig. 1; Table 3). The absorption of BH from preparation 2e was also less than that from preparation 2b (Fig. 1A; Table 3), probably due to the decreased flexibility of liposomal membrane by the incorporation of lauric acid (m.p. 44°C). However, the mixture (preparation 2i) consisting of EPC, tricaprilin and BH gave considerably high plasma concentrations during 1–5 h after application and a high AUC value out of these three formulations, although the peak plasma concentration (0.60 ± 0.06 μg/ml) was low. For the plain LDS added BH, the plasma concentrations of the drug were generally lower than those after dosing of EPC–LDS (preparation 2b). Of particular notice was that the lipid mixture (preparation 2h) of EPC, CH and dicetylphosphate, which are the same composition as the EPC–LDS, resulted in the low plasma concentrations compared with those after EPC–LDS (preparation 2b).

These results demonstrated the higher permeability of BH from the EPC–LDS compared with

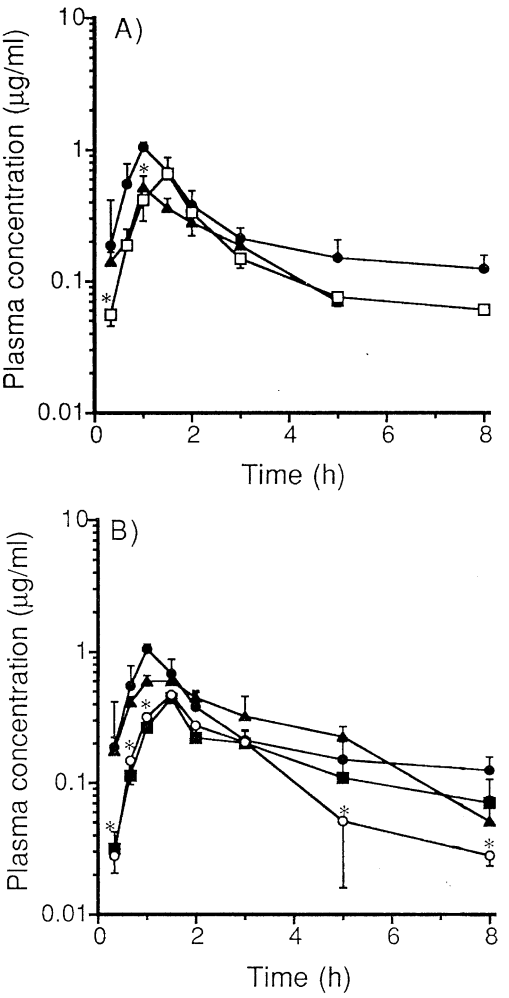

Fig. 1. Plasma concentration-time curves for BH after application of percutaneous absorption systems containing LDS, plain LDS or lipid mixtures. (A) LDS (●) preparation 2b (EPC–LDS); (▲) preparation 2e (EPC–CH-lauric acid); (□) preparation 2f (plain LDS). (B) Lipid mixtures (●) preparation 2b; (■) preparation 2g (EPC); (○) preparation 2h (EPC–CH-dicetylphosphate); (▲) preparation 2i (EPC–tricaprylin). Each point represents the mean ± S.D. ($n = 4$–5). * $P < 0.05$ vs. preparation 2b.

that from the lipid mixtures and plain LDS. The enhancement of percutaneous absorption resulting from EPC–LDS is probably explained as follows: the intercellular volume may be a factor of 3–7 times greater than was previously appreciated and is believed to be between 5 and 30% of the total tissue volume (Elias and Friend, 1975;

Table 3
Pharmacokinetic parameters following applicaion of percutaneous absorption systems containing LDS, plain LDS or lipid mixtures

	Preparation 2b	Preparation 2c	Preparation 2d	Preparation 2e	Preparation 2f	Preparation 2g	Preparation 2h	Preparation 2i
AUC (μg/h per ml)	3.61 ± 0.57	3.20 ± 0.78	2.92 ± 0.48	1.23 ± 0.12*	1.26 ± 0.11*	1.58 ± 0.39*	1.09 ± 0.17*	2.16 ± 0.47
MRT (h)	6.74 ± 0.89	5.33 ± 1.02	4.10 ± 0.63	2.76 ± 0.39*	2.49 ± 0.18*	5.42 ± 2.38	2.56 ± 0.47*	2.92 ± 0.22*
BA (%)[a]	4.51 ± 0.78	3.85 ± 1.07	3.98 ± 0.67	1.70 ± 0.16*	1.74 ± 0.15*	2.18 ± 0.53*	1.51 ± 0.23*	2.98 ± 0.64
Cmax (μg/ml)	1.13 ± 0.19	0.84 ± 0.08	1.20 ± 0.15	0.45 ± 0.04*	0.66 ± 0.07*	0.45 ± 0.04*	0.47 ± 0.04*	0.60 ± 0.06*

Each value represents the mean $\pm$ S.D. ($n = 4$).
[a] Bioavailability.
*$p < 0.05$ compared with preparation 2b.

Elias, 1981). On permeating of LDSs through the intercellular spaces and follicles, flexible LDSs such as EPC–LDS can permeate through the narrow maze-like passage between corneocytes filled partly with lamellar lipid structures and the inner root sheath of hair follicles, which creates a lipid-enriched environment with sebum, without significant interaction (electrostatic interactions) with the structured lipids, although there is an aid of the lipid environment fluidized by D-limonene to facilitate the permeation of LDS (Ogiso et al., 1995). A considerable part of LDSs would decay in the intercellular and follicular domains because of the fusion of vesicles with the lipid layers in these regions. The LDS-mixtures reached to the deeper lipid layers and viable epidermis, by the driving force arisen from transdermal concentration differential (LDS-lipids at high concentration on the stratum corneum to the viable epidermis containing lipids at low concentration), interact with surrounding lipids and cell membranes, and fuse or mix with these lipids, releasing BH from the mixtures. As a result of fusion, the lipid barrier of skin would be altered to more loose and permeable properties. It is shown that mixing of liposomes with the lipids in the intercellular layers could be one mechanism contributing to the enhancement of permeability of the skin to lipid vesicles (Blume et al., 1993). The rapid penetration of LDS-BH through the intercellular domains and follicles is demonstrated in our previous study (Ogiso et al., 1996a).

On the other hand, lipid mixtures, such as preparation 2g and 2h, would not permeate through the narrow passages rapidly as shown in Fig. 1, because the lipid mixtures would facilely mix with the intercellular lipids and sebum on the outer layers of skin and so diffuse slowly into deeper lipid layers.

The zeta potential of EPC–LDS ((b) in Table 2) and plain EPC–LDS (f) added BH was −45.6 and −5.4 mV, respectively. Judging from the result, it is considered that the plain LDS would be changed the surface electrostatic properties by the attachment of cationic BH, consequently the attachment increases the interaction of vesicles with the intercellular and sebaceous lipids by the electrostatic force and thus leads the comparatively late permeation of vesicles, in contrast with the EPC–LDS, of which surface is charged in negative. It is reported that counterions such as fixed, negative charge bearing end groups, (e.g. carboxylic and phosphate groups) contained in intercellular lipids (Elias et al., 1977; Elias, 1981) would potentiate cationic drug binding by electrostatic interactions (Kitagawa et al., 1990). Additionally, in the relative paucity of positively charged end groups in human skin, the diffusion of salicylate anion is not intensively interfered (Pardo et al., 1992).

As a result, EPC–LDSs would suffer the weak electrostatic repulsion in the intercellular domains and follicles. Since the electrostatic interactions results in a net slow down of the overall diffusion rate as mentioned above, the repulsion would also ensure the rapid penetration of EPC–LDS in these domains based on the concentration differential.

3.3. Effect of pretreatment with gel formulation containing D-Limonene or OTG on percutaneous absorption of LDS-BH

The plasma concentration-time curves for BH after pretreatment are depicted in Fig. 2, with the concentrations after application of preparation 1 and 2b. The absorption of BH comparatively delayed during the early time periods compared with that following LDS formulation (preparation 2b), and the plasma concentrations were much less than the levels after application of preparation 2b (Table 4). These results suggest that LDS and enhancers, especially D-limonene, acted on the enhancement of BH absorption additively, probably due to the partial extraction of the intercellular and follicular lipids by the enhancer and the rapid penetration of LDS into the domains of which were enlarged by the extraction, as shown in our previous paper (Ogiso et al., 1992; 1996a).

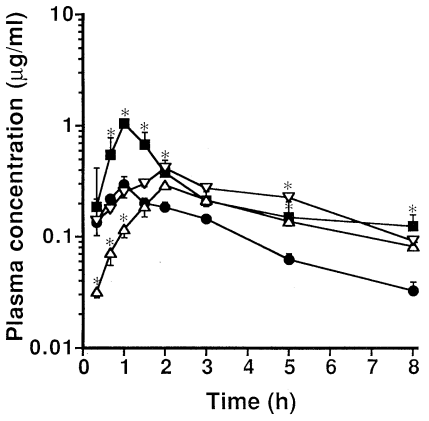

Fig. 2. Plasma concentration-time curves for BH after application of percutaneous absorption systems: (●) preparation 1; (■) preparation 2b; (△) preparation 3 after pretreatment with I; (▽) preparation 3 after pretreatment with II. Each point represents the mean ± S.D. ($n = 4–5$). * $p < 0.05$ vs. preparation 2b.

3.4. Content of ceramides, phospholipid phosphorus and triglycerides following in vitro treatment with formulations

The lipid contents in the SC were determined following application of gel formulation containing LDS-100 (preparation 2d) or plain LDS (preparation 2f) in comparison with the data of LDS-600 (preparation 2b) obtained in our previous study (Ogiso et al., 1996a). As a result, the application of preparation 2d caused a pronounced decrease in the ceramide (about 45% decrease) and triglyceride content (42–48% decrease) compared with the control values, (8.61 ± 1.51 $\mu g/cm^2$ ceramides and 108.99 ± 5.68 $\mu g/cm^2$ triglycerides) (Ogiso et al., 1996a), 3 and 6 h after application. The decreased extent in both lipids agreed well with that obtained by application of preparation 2b. The phospholipid phosphorus content in the SC was increased by a factor of 2 at the same h after treatment with preparation 2d (phospholipid phosphorus at 3 h; control skin, 3.37 ± 0.35 $\mu g/cm^2$; treated skin, 7.21 ± 1.18 (2b), 6.53 ± 1.03 $\mu g/cm^2$ (2d)). On the other hand, when preparation 2f was applied, the decrease in ceramides and triglycerides was 24 and 20%, respectively, compared with those of control SC, and the increase in phospholipid phosphorus was only 18–20%, 3 and 6 h after application. These results indicate that the LDSs with different particle sizes partitioned into the SC to the same extent, and that the partition of the plain LDS added BH was less than that of LDS.

3.5. Thermal transition pattern of SC lipids following treatment with LDSs

In previous paper, we clarified that LDS-600 decreased the τ_c values and increased the fluidity of SC lipids (Ogiso et al., 1996a). To further confirm the fusion of LDS with the intercellular lipids and the fluidising effect of EPC–LDS (b, c, d and f in Table 2), the ESR spectra of the SC lipids using a deeper probe, 16-doxyl-stearic acid, were recorded after treatment with LDS with

Table 4
Pharmacokinetic parameters following applicaion of percutaneous absorption systems

	Preparation 1	Preparation 2b	Preparation 3-I[a]	Preparation 3-II[a]
AUC (μg/h per ml)	0.95 ± 0.04	3.61 ± 0.57*	1.55 ± 0.05*	2.07 ± 0.14*
MRT (h)	3.21 ± 0.06	6.74 ± 0.89*	5.68 ± 0.49*	4.46 ± 0.27*
BA (%)[b]	1.31 ± 0.05	4.51 ± 0.78*	2.14 ± 0.08*	2.86 ± 0.20*
C_{max} (μg/ml)	0.30 ± 0.02	1.13 ± 0.19*	0.29 ± 0.02	0.42 ± 0.06*

Each value represents the mean ± S.D. ($n = 4–5$).
[a] The values for preparation 3-I and 3-II are the parameters obtained by applying preparation 3 after pretreatment with I and II, respectively.
[b] Bioavailability.
*$p < 0.05$ compared with preparation 1.

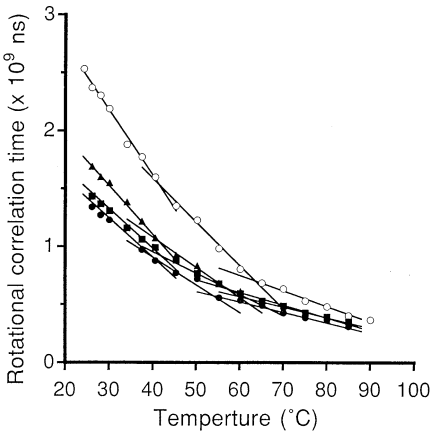

Fig. 3. Temperature dependence of the motion parameters of lipid spin label in SC: (○) untreated SC; (●) LDS (596 nm); (■) LDS (336 nm); (▲) LDS (128 nm). Point and line are the mean observed value and the regression line, respectively. The data of preparation 2f were not shown because of the accordance with the plots of preparation 2d.

different particle sizes. Fig. 3 denotes the plots of apparent rotational correlation time (τ_c) versus temperature for 16-doxyl-stearic acid spectra of the SC lipids. The curves represented the temperature dependence of τ_c values, indicating the increased fluidity, rotational disorder, of the lipids with increasing temperatures. The plots of τ_c values of the untreated SC lipids indicated an abrupt inflection at 40.1 ± 0.6 and 62.8 ± 1.9°C. Since values of τ_c at given temperature serve as an index of membrane lipid viscosity (Morse et al., 1975), the break temperature represents the phase transition of the lipids from gel to liquid-crystalline phase (White et al., 1988).

Treatment of the SC with LDSs with different particle sizes and with lauric acid (data of the latter not shown in Fig. 3) significantly decreased the τ_c value and shifted downwards the slope of curves at temperatures ranging from 26 to 60°C, suggesting the increased disorder of the intercellular lipids based on mixing with EPC. At the physiological temperatures between 32°C (the surface temperature) and 37°C, treatment with LDS dramatically lowered the τ_c values compared with those of the control skin (Fig. 3; Table 5). The dramatic decrease of τ_c value indicates that EPC–LDS incorporated into the intercellular domains of the SC and into the follicles (Ogiso et al., 1996a) changed the physical characteristics of these lipids and significantly fluidised these lipids in comparison with the lipids of untreated skin, consequently facilitating the drug diffusion in the domains at the physiological temperatures. However, fluidising effect was not significantly different between LDSs with different particle sizes, and both phase transition temperatures were little altered by the mixing of LDSs (Table 5). This result agreed well with the data obtained in the in vivo percutaneous absorption experiment (Fig. 1). This suggests that the approximately similar amounts of LDSs were incorporated into the SC. On the other hand, the break point at higher temperature shifted to left on this treatment. Thus, LDS treatment led to a reduction in the higher phase transition temperature of lipids, although the reduction based on LDS with lauric acid was comparatively less, this being probably due to increased loosening of lipid packing in the lipid layer mixed with EPC (Table 5). The similar concept is shown by the study on the differential scanning thermograms of the liposomes of SC lipid (Kim et al., 1993). We have already reported that the SC lipids of rat probably exist as the gel, crystalline state below 40°C, the fluid, liquid-crystalline state at the temperature higher than 64°C and the mesomorphic state (coexistence of gel and liquid-crystalline states) between 40 and 64°C (Ogiso et al., 1996b). The coexistence of crystalline and liquid alkylchains in untreated SC of murine at normal physiological temperature is shown by X-ray diffraction and FTIR experiments (Knuston et al., 1987; White et al., 1988).

Table 5
Apparent break temperatures in τ_c versus temperature plots

Treatment	Apparent break temperature (°C)[a]	
Untreated	40.09 ± 0.57	62.77 ± 1.88
LDS (596 nm)	39.04 ± 2.12	56.29 ± 1.17*
LDS (336 nm)	38.87 ± 1.01	59.84 ± 1.76
LDS (128 nm)	40.06 ± 1.85	57.53 ± 1.36*
LDS-lauric acid (605 nm)[b]	39.97 ± 1.78	60.38 ± 2.39

[a] Expressed as mean ± S.D. ($n = 3$–4).
[b] EPC, cholesterol, lauric acid (10:1:1).
*$p < 0.05$ compared with the untreated.

4. Conclusions

Percutaneous absorption of LDS-BH was not influenced by the particle size of vesicles in formulations; the EPC–LDS with three different particle sizes (128, 336 and 596 nm) gave the similar AUC and MRT value. With the absorption of BH, the gel formulation containing EPC–LDS showed the much high plasma levels of the drug compared with the lipid mixtures and the plain LDS with BH, probably due to the differences in the permeation rate of LDSs into the intercellular lipid domains and the inner root sheath of follicles. The τ_c values of SC lipids were decreased and the slope of the plots of τ_c versus temperature shifted downwards by the treatment with EPC–LDS.

As a result, the mechanism for enhancement effect of LDS on percutaneous absorption is probably due to that flexible LDS such as EPC–LDS can facilely permeate through the passage between corneocytes and follicles without the electrostatic interaction with the structured lipids and can reach to the deeper lipid layers in these regions, although the LDS is followed by partial fusion with the lipid layers in these regions. The LDS-mixtures interact with the surrounding lipids and cell membranes, thus releasing the drug from the mixtures and leading the absorption.

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