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Morphological effect of lipid carriers on permeation of lidocaine hydrochloride through lipid membranes

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ABSTRACT

We have studied how the transdermal delivery of lidocaine hydrochloride (LHC) is affected by the morphology of lipid carriers, liposomes and micelles, having the same lipid composition of 1-stearoyl-sn-glycero-3-phosphocholine (LPC) and cholesteryl hemisuccinate (CHEMS). *In vitro* drug permeation study, carried out on guinea pig skin, has revealed that the liposomes made of LPC and CHEMS significantly enhance the permeation rate of entrapped LHC; by contrast, the mixed micelles with the same composition decrease the degree of delivering co-existing LHC. Basically, we have also investigated the release kinetics of LHC through the cellulose membrane and found that both liposomes and micelles have a similar releasing profile. To experimentally demonstrate this unique behavior, we have observed the fluidity of stratum corneum liposomal membranes in the presence of either our liposomes or micelles. From this study, we have found that LPC/CHEMS liposomes fluidize the lipid membrane of stratum corneum lipids; however, lipid micelles rather make the membrane rigid. These findings highlight that controlling the morphology of drug carriers provides us with a means to modulate the permeability of encapsulated drug molecules.

1. Introduction

It has been known that when drugs are either encapsulated in liposomes or co-administered with liposomes, their permeation through the skin is enhanced (Du Plessis et al., 1994). Although attempts have been made to interpret this phenomenon by using the permeation enhancer mechanisms (Honeywell-Nguyen et al., 2003), currently, it is a common principle that traditional intact liposomes are not able to penetrate the skin without destroying themselves; it is an exceptional case that the vesicles that have an ability to deform their shape can penetrate the skin (Cevc et al., 2002; Kirjavainen et al., 1999b). Depending on the shape and structure of lipids as well as chemical composition, liposomes can fuse to the skin surface, which eventually disturbs the aligned structure of skin lipids. For example, mixing dioleoylphosphatidylcholine (DOPE) and lysophosphatidylcholine (LPC) that have a truncated cone and an inverted cone shapes, respectively, allows us to obtain more promoted permeation of fluorescent probe molecules into the skin, compared with the case of using liposomes made of rod-like lipids. This is due to lipid molecules with a fusogenic cone and an inverted cone type are a permeation enhancer (Kirjavainen et al., 1996; Bergstrand et al., 2003). Furthermore, lysophospholipids have an ability to lower the barrier function of skin layers, thus also enhancing their permeability (Davidsen et al., 2002).

By topically applying fusogenic lipids to the skin, we can achieve more improved permeation of drugs through the skin lipid membrane. Then, it is indeed interesting to consider that if we make a carrier system with the fusogenic lipids, we may obtain different permeation behaviors, especially depending on their morphology. In principle, the carriers with different morphologies can change their interfacial property to the penetrating media, thereby inducing a different type of molecular interactions with skin layer. Understanding how lipid carriers interact with the skin layer is truly important, since we can not only determine the degree of penetration of encapsulated drugs, but also explore flexible pathways that the drug molecules go through. However, the applicability of the concept of using carrier systems with different morphologies has never been introduced. To demonstrate this, we should fabricate the carriers having the same lipid composition as well as particle size. Using them, we can understand how their morphology has an influence on the penetration behaviors of encapsulated drugs.

In this study, we fabricate two types of drug carriers; micelles and vesicles, whose lipid membranes consist of an inverted

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cone-type lipid, LPC, and cholesterylhemisuccinate (CHEMS). They basically have the same lipid composition. Here we use lidocaine hydrochloride (LHC) as a model drug. Using these carriers encapsulating LHC, we observe *in vitro* skin penetration on guinea pig skin and also determine drug release kinetics through the cellulose membrane. Finally, we carry out a steady-state anisotropy analysis on stratum corneum lipid liposomes (SCLL) that have a similar lipid composition to the skin, which allows us to correlate the skin permeation with the morphological changes of SCLL membranes (El Maghraby et al., 2005).

2. Materials and methods

2.1. Materials

1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) was purchased from Avanti Polar Lipids, Inc. (USA). 1-Stearoyl-sn-glycero-3-phosphocholine (LPC), ceramide III (CER III) and ceramide IIIB (CER IIIB) were kindly donated by Doosan Biotech (Korea). Palmitic acid (PA), cholesterol, cholesterol sulfate (CS) and cholesterylhemisuccinate (CHEMS) were purchased from Sigma–Aldrich (USA). Albino Hartley guinea pigs for *in vitro* skin permeation experiments were purchased from Charles River Laboratories (USA). The fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene (DPH), was purchased from Invitrogen (USA). Regenerated cellulose membrane for drug releasing test was purchased from Stectrumlabs® (Spectra/Por 6, MWCO 10 kDa). Lidocaine hydrochloride was purchased from Sigma–Aldrich (USA). All other chemicals used were of analytical grade.

2.2. Fabrication of LPC/CHEMS liposomes and micelles

To fabricate a liposome suspension, equimolar LPC and CHEMS were dissolved in ethanol (10%, w/w) at 70 °C and then mixed with double distilled water containing 0.01 vol% triethanolamine. This mixture was then homogenized by using a high-pressure homogenizer (Microfluidizer® M-110EH, Microfluidics™, USA, 1000 bar, 1 cycle), yielding a liposomal suspension. Micelles were prepared using the same procedure except that additional triethanolamine which can make phase transition of vesicle structure to micelle structure by changing pH of suspension was added just before finalize homogenization, and stirred for 6 h until the opaque suspension turns into translucent solution. pH of all the suspension media was adjusted to 6.5 by adding a citric acid solution (1%, v/v). LHC-incorporated liposomes and micelles were also prepared by using the same procedure except that 0.5 wt% LHC dissolved with lipids in ethanol was carefully mixed with water. The compositions of all formulations used in this study are listed in Table 1. LPC/CHEMS liposomes and micelle particles were observed by using a cryo-transmission electron microscopy (cryo-TEM) and sized by using a photocorrelation spectroscopy (Zetasizer 3000HSA, Malvern). DOPE/CHEMS liposomes and LHC dissolved in an ethanol/water mixture (1/9, v/v) were used as controls.

2.3. Preparation of stratum corneum lipid liposomes

SCLL were prepared by using the dehydration and rehydration method (Wertz et al., 1986). First, a lipid mixture, whose composition is CER III/CER IIIB/cholesterol/CS = 0.22/0.2/0.25/0.25/0.1 by weight fraction, was dissolved in the mixture of chloroform and methanol (9/1, v/v). Then, the solvents were completely evaporated at 45 °C. The lipid film hydrated by swirling for 5 min was sonicated for 1 h at 80 °C, resulting in a fine dispersion of SCLL. A steady-state fluorescence analysis was performed at 0.2 wt% SCLL concentration containing a small amount of a fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene (DPH), dissolved in tetrahydrofuran (2 mM). The molar ratio of lipid-to-probe was set to 1200:1 by weight, in which the probe concentration was 15 μM. Finally, these SCLL were incubated in a thermostatically controlled shaking water bath at 80 °C for 2 h (Yoneto et al., 1996).

2.4. Entrapment efficiency

The entrapment efficiency (e.e.) for these two carriers, liposomes and micelles was evaluated as a following equation (Lopez-Pinto et al., 2005):

$$\text{e.e.} = \frac{\text{total mass used for formulation preparation} - \text{mass of free form}}{\text{total mass used for formulation preparation}} \times 100$$

Separation of loaded amount of lidocaine hydrochloride (LHC) from free form was performed by Amicon® ultra-filtration purification kit (Millipore™, USA) with regenerated cellulose membrane (MWCO 3000), which can prohibit passing through colloidal carriers in these formulations. The drug concentration from each formulation was measured by means of HPLC method as described in Section 2.7. The total amount of LHC in each formulation was assessed by the same HPLC analysis procedure and liposomes and micelles were disrupted by 15 min sonication after adding methyl alcohol (formulation/methanol, 1/1). All formulations kept the pH range from 5.5 to 6.0 for this analysis.

2.5. In vitro permeation test

In vitro permeation of LHC through abdominal skins of Albino Hartley guinea pig (Charles River Laboratories, USA) was evaluated by using a Franz-type vertical diffusion cell system (Microette Plus™ Auto-sampling system, Hanson Research, USA). After scarifying these rodents, abdominal skin was carefully excised. The volume of the receptor compartment was 7 ml and the effective diffusion area was 1.76 cm^2. All experiments were carried out for 24 h at 32 °C (±0.5 °C). One milliliter of each receptor solution was automatically withdrawn with the time interval of 0.5, 1, 3, 6, 12, 18, and 24 h, right after each formulation was applied to the upper reservoir compartment. Each time of sampling the receptor compartment, we completely exchanged it with a fresh phosphate buffered saline, thereby maintaining a sink condition. We analyzed LHC concentration by using HPLC JASCO, HSC-3000, Japan.

Table 1
Composition of liposomes, micelles, and control formulations.

Ingredient (wt%)	Liposome	Micelle	Control 1 (liposome)	Control 2
LPC	0.5	0.5	–	–
DOPE	–	–	0.5	–
CHEMS	0.3	0.3	0.3	–
Lidocaine hydrochloride	0.5	0.5	0.5	0.5
EtOH	1.0	1.0	1.0	1.0
Water	88.7	78.6	88.7	89.5
Triethanolamine	0.01	0.1	0.01	0.01
Citric acid (1 wt%)	–	1.0	–	–

2.6. Diffusion study through synthetic membranes

In vitro diffusion study of LHC trapped in liposomes and micelles was performed through a regenerated cellulose membrane (Spectra Por 6, MWCO 10,000). For this, we also used the vertical type Franz diffusion cell system. Receptor compartment filled with phosphate buffer solution was constantly stirred at 200 rpm and maintained a thermostatic condition at 32 °C (±0.5 °C) throughout the experiments. Applying each test formulation (6 ml) onto the cellulose membrane, we characterized the permeability of LHC by taking 1 ml sample from the receptor compartment every 2 h for 24 h (Fang et al., 2006).

2.7. HPLC analysis

Samples collected from the receptor compartment were filtered through a 0.45 μm PVDF (polyvinylidene fluoride) membrane syringe filter and analyzed by using HPLC (Jasco, Japan) that uses a pre-packed reverse phase column (Waters™, 4.6 mm × 250 mm × 5 μm, CNRP). A 0.02 M phosphate buffer (pH 6.0)–acetonitrile (6/4, v/v) solution was used as an isocratic mobile phase at a flow rate of 1 ml min^{-1} with detection at 210 nm (Carafa et al., 2002). Reference curves were linear over the range of 12.5–100 μg ml^{-1}. The steady-state flux is given as follows: $J_{ss} = 1/A(dQ/dt)$, where J_{ss} is the flux of LHC (μg cm^{-2} h^{-1}) at a steady-state, Q is the accumulated amount of LHC in the receptor solution over test time, A refers to the contact area of the donor compartment, 1.76 cm^2. Data analysis was carried out by using ANOVA, in which p-value of <0.05 was considered statistically significant.

2.8. Fluorescent anisotropy measurements

DPH-labeled SCLL were mixed with an equal volume of either LPC liposomes or micellar solutions and incubated for 2 h at 32 °C. Then, we measured the steady-state polarization (Høyrup et al., 2001). The emission spectra and polarization data were obtained at 37 °C by using a fluorescence spectrophotometer (F-4500 Hitachi, Japan). The steady-state was conditioned by doing the experiment in a water circulating bath. The excitation wavelength was 360 nm for DPH, and emission was measured by equipping a 400 nm long-pass filter. SCLL in water without DPH were used as a blank. The anisotropy, r was measured as follows: $r = (I_P - I_V)/(I_p + 2I_V)$, where I_P is the fluorescence intensity of the emitted light that is polarized parallel to the excited light, and I_V is the fluorescence intensity of the emitted light that is polarized perpendicular to the excited light.

3. Results and discussion

3.1. Vesicle-to-micelle phase transition

Our liposomes consisting of an equimolar ratio of LPC and CHEMS have a similar morphology to liposomes of LPC/cholesterol (Davidsen et al., 2002). They form a milky suspension with the mixture of uni- and multi-lamellar vesicles, as shown in Fig. 1a. Their mean particle size, measured by using the photocorrelation spectroscopy, is approximately 160 nm (Fig. 1c). A big feature of our fabrication method is to co-assemble CHEMS to the lipid bilayers. CHEMS plays an essential role in triggering the phase transition of lipid layers. As known, CHEMS is a mildly acidic amphiphilic molecule. Therefore, its solubility increases in basic aqueous condition, which makes it act as a bile salt, thereby reorganizing the liposomal layer to a mixed-micellar structure. Thus detergent-like transformation of vesicles usually occurs (Michel Ollivon et al., 2000; Annegret Hildebrand et al., 2004). By using this unique approach, we are able to produce a fine dispersion of micelles; it is almost colorless and translucent. The micelle size is in the nanometer length scale, as can be seen in the cryo-TEM micrograph in Fig. 1b; micelles are much smaller than 10 nm. This process is not reversible even after we recover the pH of the suspension. The technique we use in this approach is indeed advantageous in that we are able to induce the phase transition from vesicles to micelles of lipid particles not by changing their chemical composition, but by temporal shifting pH of the dispersion medium, excluding any factors that can affect the skin permeation of drug. Consequently, the entrapment of LHC within liposomes was reached at 12% and that of micelles was approximately 10%.

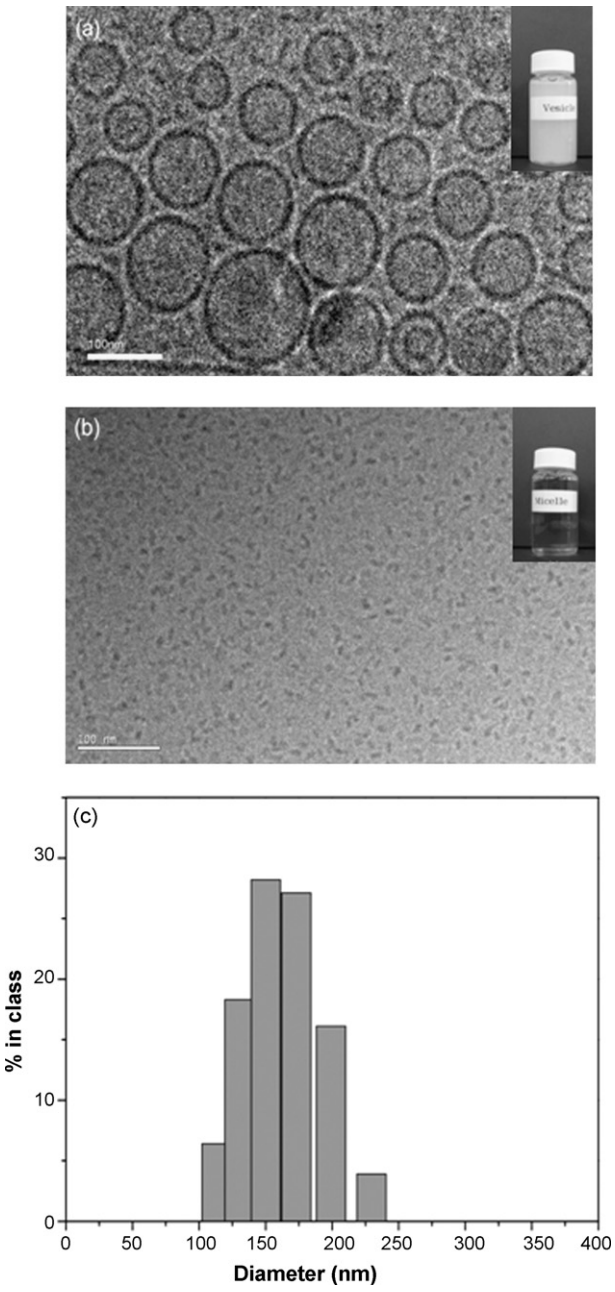

Fig. 1. Cryo-transmission electron spectroscopy images of LPC/CHEMS liposomes (a) and micelles (b) after the phase transition of liposomes by temporary escalating pH of liposome suspension. The size range of liposomes was measured by dynamic light scattering (c). Average diameter, Z_{ave}, reaches ~160 nm (±12.5).

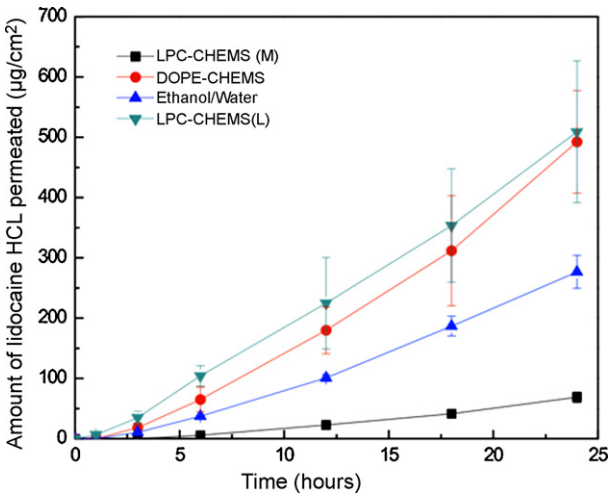

Fig. 2. Cumulative permeation amount of LHC for four different formulations by *in vitro* Franz cell diffusion system through abdominal guinea pig skin ($n = 6$, ±S.D). The formulations we used were LPC/CHEMS liposomes (L), LPC/CHEMS micelles (M), DOPE/CHEMS liposomes, and ethanol control solution.

3.2. In vitro drug permeation study

To observe the skin permeation behavior of LHC encapsulated in liposomes and micelles, we have performed *in vitro* guinea pig abdominal skin permeation tests by using Franz diffusion cells. The result is shown in Fig. 2. We have observed that LHC in LPC/CHEMS liposomes has a better ability to permeate the skin, compared with the case of using conventional DOPE/CHEMS liposomes, a positive control (Control 1). In our study, it is a surprise to notice that LHC in LPC/CHEMS micelles permeates the skin with a much lower concentration, which is less than the case of using liposomes by factor of approximately 5. These permeability values are even smaller (9.4 cm/h) than those of using an ethanol solution (3.32 cm/h, ethanol/water = 2/8, v/v), which is a negative control (Control 2) that has a chemical enhancing effect. This big difference in permeability of LHC reproducibly occurs; actually, we have achieved this permeability data after carrying out three independent experiments, of which p-value between samples is <0.05. One might point out that the length scales of drug-carrying materials could change the permeation behaviors. However, liposomes, having a bigger particle size, show better permeability than micelles, implying that the length scales of carriers have little effect on it. Our observations obviously demonstrate well that the morphology of drug carriers directly affects the permeation of encapsulated drug molecules therein (Guo et al., 2000), which is possible by fabricating the carriers with the same chemical composition.

3.3. Diffusion behaviors of LHC from different lipid carriers

To quantitatively characterize the permeation behaviors of LHC in the systems of LPC/CHEMS liposomes and micelles, skin permeability values were measured (Table 2).Using Fick's first law, we can obtain the amount of drug permeated, as a following equation:

$$\frac{Q}{A} = KLC_0\left(\frac{Dt}{L^2} - \frac{1}{6}\right) \quad (1)$$

where A (cm^2) is the effective skin diffusion area, C_0 is the initial concentration of LHC in the formulations ($\mu g\,ml^{-1}$), D is the diffusion coefficient and corresponds to the drug diffusion coefficient and corresponds to the drug diffusion capacity through the skin, L is the skin thickness and K is the partition coefficient between skin and vehicle (Fresno Contreras et al., 2005). Q is the total amount of drug permeated skin was calculated and partition and diffusion coefficients are obtained. The lag-time τ, which is obtained by back-extrapolation of the steady-state slope to its intersection with the time-axis. The diffusion coefficient D and partition coefficient K are represented as follows:

$$\tau = \frac{L^2}{6D} \quad (2)$$

At the steady-state, the flux J_s ($\mu g/cm^2$ h) was calculated using the linear portion of the correlation between the accumulated amount of LHC by unit area and time, where we could obtain partition coefficient K:

$$J_s = \frac{C_0 KD}{L} \quad (3)$$

As listed in Table 2, LHC in LPC/CHEMS and DOPE/CHEMS liposomes has quite close D values. These values are also similar to that of the ethanol solution. Although D value of LPC/CHEMS micelles is slightly different, their K value is remarkably lower than that of DOPE/CHEMS liposomes. This means that the structure of lipid carriers considerably changes the degree of partitioning LHC into the skin, which is likely due to their adsorption to the lipid phase of the skin.

To further characterize the diffusion of LHC molecules into the skin, we have carried out LHC releasing tests by using both of LPC/CHEMS liposomes and micelles, in which the cellulose membrane was used, thus excluding any interactions of our carriers with the skin lipid membrane. It has been found that although our two lipid carriers appear to display a similar release profile, as shown in Fig. 3, they show a lower diffusion of LHC through the cellulose membrane, compared with the ethanol solution. This means that thermodynamic releasing of LHC encapsulated in these two carriers, liposomes and micelles may not be significant to make skin permeation different. Furthermore, in our additional experiment,

Table 2
Kinetic parameters of LHC skin permeation.

Formulation	J_{ss} ($\mu g\,cm^{-2}$)	P ($cm\,h^{-1}$)	D ($cm^2\,h^{-1}$)	K
DOPE/CHEMS liposomes	29.82	5.96×10^{-3}	3.93×10^{-5}	5.36
LPC/CHEMS liposomes	22.39	4.48×10^{-3}	3.90×10^{-5}	3.99
LPC/CHEMS micelles	4.67	9.3×10^{-4}	3.21×10^{-5}	1.02
EtOH/water (1/9)	16.6	3.32×10^{-3}	3.85×10^{-5}	3.02

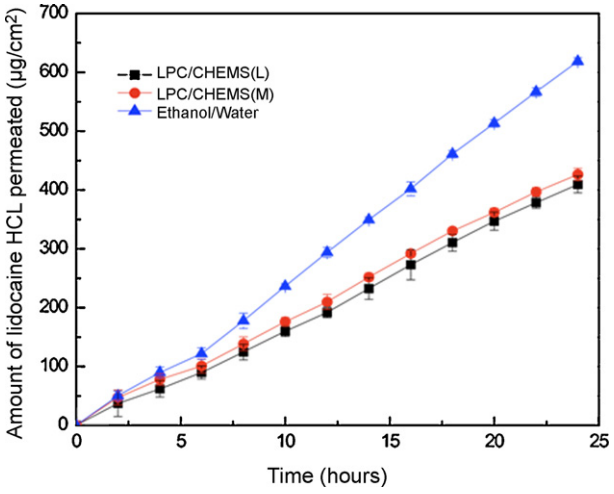

Fig. 3. *In vitro* releasing of LHC through the cellulose membrane ($n = 6$, ±S.D). The formulations are identical to the cases of *in vitro* skin permeation.

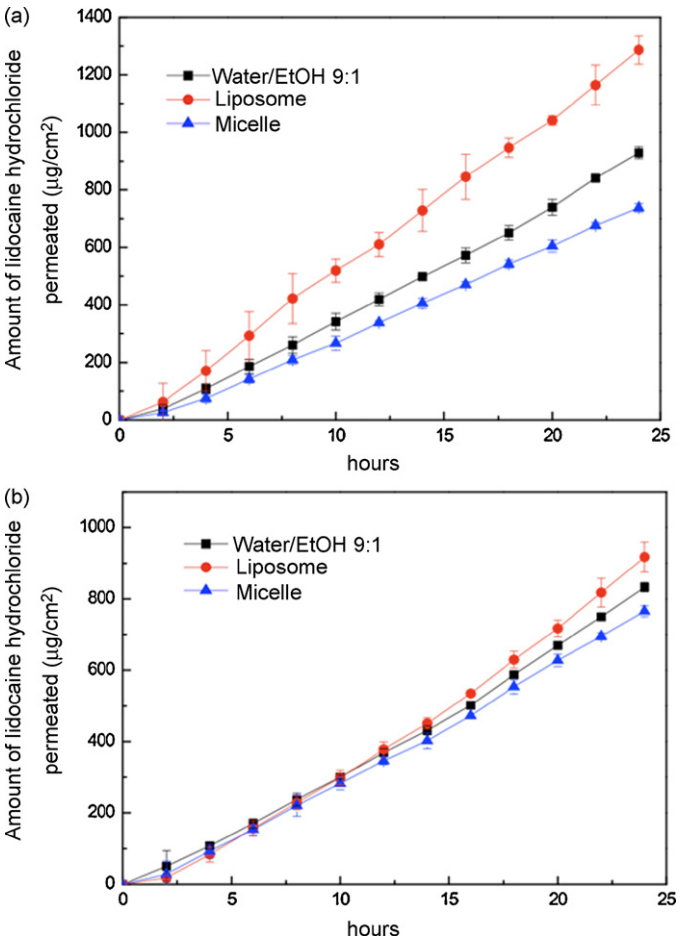

Fig. 4. Comparative study of *in vitro* LHC permeation. (a) Cumulative permeation amount of LHC encapsulated in LPC/CHEMS liposomes (L), LPC/CHEMS micelles (M). (b) Cumulative permeation amount of LHC exogenously added in LPC/CHEMS liposomes (L), LPC/CHEMS micelles (M), DOPE/CHEMS liposomes, and ethanol control solution. ($n = 6$, ±S.D).

simply mixed LHC with the dispersion of our two types of blank lipid carriers, no drug encapsulated, could not show significant effect in partitioning of LHC through the skin, which is comparable to the case of using lipids carriers, liposomes and micelles encapsulating LHC, as shown in Fig. 4. This explains that the lipid carriers could not give significant effect to the permeation of free-formed LHC. All these results show well that LPC/CHEMS carriers can regulate the permeability of LHC encapsulated due primarily to the drug partitioning property to the skin, which can be solely regulated by fabricating them with a different membrane structure.

3.4. Interaction of lipid particles with stratum corneum lipid liposomes

By exploiting the stratum corneum lipid liposomes (SCLL), which are known to have the same composition as the intercellular lipid phase of the skin, we are able to figure out how liposomes and micelles interact with the skin layer. By mixing LPC/CHEMS liposomes and micelles with SCLL, It has been tried to analyze any changes in physical property of SCLL membranes. For this, the steady-state fluorescence anisotropy and the membrane fluidity of SCLL in the presence of LPC/CHEMS carriers (see Table 3) were studied. Adding LPC/CHEMS liposomes to SCLL allows us to have lower γ value, meaning the fluidity of the SCLL membrane increases; by contrast, addition of LPC/CHEMS micelles leads to a decrease in membrane fluidity. This indicates that the permeability of drug

Table 3
Fluidity parameter, γ_{ss}, measured by using DPH immobilized in SCLL.

Formulation	γ_{ss}
SCLL	0.244 ± 0.005
SCLL + LPC/CHEMS liposomes	0.225 ± 0.003
SCLL + LPC/CHEMS micelles	0.260 ± 0.004

molecules through the skin, i.e., the partition coefficient of skin, is closely related with underlying changes in the membrane fluidity of the skin; more fluidity of the skin lipid membrane, more effective transdermal drug delivery (Kirjavainen et al., 1999a). In our study, it has been found that the interaction of LPC/CHEMS micelles with SCLL makes the SCLL membrane rigid, thus, hampering an effective transportation of drug molecules to the skin. These results seem to attribute to the fact that liposomes may fuse to the SCLL lipid membranes, eventually, disturbing the well-assembled structure of skin lipids; whereas, micelles may thickly adhere to the outermost membrane layer of the skin, thus, physically strengthening the lipid membrane.

4. Conclusion

In this study, we introduce a facile approach to regulate transporting drugs through the skin, which is possible by fabricating carrying materials with different morphologies. The key of our approach is to tune the interaction with the lipid phase of the skin by changing the geometry of the lipid molecules. Solely temporarily changing pH of the dispersion medium enables the carriers to phase change from liposomes to micelles. The carriers with different morphologies generate an opportunity to control over the degree of interaction with SCLL. It has been also confirmed that by directly measuring the membrane fluidity, which allows us to correlate the result with drug partitioning. More fusible carrying system provides more effective transdermal delivery. Since our understandings are very useful for overcoming the barrier function of the skin, we are expecting we can develop a practical means to non-invasively deliver drugs through the skin with controllable dosages and kinetics.

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