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Transdermal Delivery of Ceramide Using Sodium Deoxycholate-based Deformable Liposomes

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ABSTRACT – For transdermal delivery of ceramides, various liposomes formulations were studied and evaluated. Sodium deoxycholate (SDC), Tween 20 and Span 85 were used as edge activators. The skin permeation of ceramides was performed using a Franz cell apparatus with hairless mouse skin. Among edge activators, SDC showed the higher values of deformability index and skin permeation than did others. For optimization of formulations, we varied the ratios of lipids to edge activators and the compositions between phosphatidylcholine (PC) and ceramides. The optimal ratio of lipid to SDC was observed to be 6 : 1 (w : w) and that of PC and ceramide was 1 : 1. Our results suggest that the skin permeation of ceramides could be enhanced by optimized deformable formulations of liposomes containing SDC as a major edge activator.

Key words – Ceramide, Deformable liposomes, Sodium deoxycholate, Transdermal drug delivery

Main role of skin was to protect our body from the environment.[1)] Stratum corneum works as a barrier of skin permeability, but plays an important role in antimicrobial function, hydration function and protection of the skin from the UV light.[2)] Defects in the barrier functions of the skin may cause secondary infections such as atopic dermatitis.[3)] The lipid matrix in the stratum corneum consists of equimolar mixture of ceramide, cholesterol and free fatty acid. Among the components, ceramide is regarded as an essential and important component.[4)]

Topical application of ceramides has been effective therapeutic approach in skin disorder such as atopic dermatitis and psoriasis.[5)] Various ceramide derivatives have been applied onto the skin and shown to be effective against atopic dermatitis.[6)] Topical application of ceramide suppressed inflammatory reaction, and the production of cytokines such as interleukin-4 (IL-4) and tumor necrosis factor (TNF)-alpha expression.[7)] Numerous studies about atopic dermatitis using ceramide reported its therapeutic effect. Despite the significance of transdermal delivery of ceramides, little investigations have been done for enhancing the permeation of ceramide into the skin.

In this study, we formulated lipid-based skin permeation vehicles for delivery of ceramide. Although liposomes have been used for sustained drug delivery systems, conventional liposomes for transdermal drug delivery have suffered from the limited skin penetration because the rigidity of liposomal membranes may hamper the efficient penetration deep into the skin.[8)] Instead of conventional liposomes, deformable liposomes consisted of phospholipids and edge activators have drawn attention as transdermal delivery vehicles. Edge activators are usually single chain surfactants or detergents that confer the deformability of lipid bilayer for improved delivery of drugs into skin.[9)] However, most of the previous studies using deformable liposomes focused on the delivery of chemical drugs and genes. In this study, we aimed to deliver ceramide as a component of deformable liposomes for enhanced skin permeation of ceramide. We screened the optimal formulations of ceramide-containing deformable liposomes, and report here the optimal formulations for the delivery of ceramide.

Materials and Methods

Materials

Egg phosphatidyl choline (PC) was purchased from Avati Polar Lipid, Inc. (Birmingham, AL, USA). C-2 Ceramide was from Cayman chemical (Ann Arbor, MI, USA). Sodium deoxycholate (SDC) was from Fisher Scientific (Fair Lawn, NJ, USA). Span 85 was from Sigma Chemical Co (St Louis, MO, USA). Tween 20 was from USB (Cleveland, OH, USA). The Nuclepore polycarbonate membranes (pore size: 0.1, 0.2 μm)

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were from Whatman (Clifton, NJ, USA).

Liposomes preparation

PC and ceramide were dissolved in chloroform. To the mixture of various molar ratios of lipids, fluorescein isothiocyanate (FITC)-labeled oleylamine were added for tracing the liposomal penetration using a spectrofluorophotometer. The lipid mixtures were evaporated for reducing chloroform on a rotary evaporator, resulting in a thin film of lipids. The lipid thin films were hydrated in phosphate buffered saline (PBS) buffer (PH 7.4) with vigorous vortexing. The liposomes were extruded through 0.2 μm polycarbonate membrane filter using an extruder (Northern Lipids, British Columbia, Canada). The deformable liposomes with edge activators were prepared after getting thin lipid films. The ratio of lipids to edge activator was 6:1 (w:w) and edge activator in PBS buffer were used for hydration.

Size measurement of deformable liposomes

The size of deformable liposomes was determined using ELS-8000 dynamic light scattering instrument (ELS-8000, Photal, Osaka, Japan). The samples were diluted with PBS buffer and then moved into a four side cuvette in an ELS-8000 dynamic light scattering instrument. Next, the hydrodynamic diameters of the particles were measured via dynamic He-Ne laser (10 mW) light scattering at an angle of 90° at 24.1°C. A software package (ELS-8000 software) supplied by the manufacturer was used to analyze the data.

Measurement of liposome deformability

The deformability of liposomes was determined by measuring the size of liposomes and passed volume ratio after extruding liposomes. The pressure (0.5 MPa) was used to drive the diluted liposomes through 100 nm polycarbonate membrane filter for 5 minutes. The extruded volume was measured by syringe. The deformability of liposomes was calculated by using the following equation: $D=J(Rv/Rp)^2$. J was the amount of suspension after extrusion. Rv was the size of liposomes. Rp was the pore of the polycarbonate membrane.[10]

***In vitro* skin permeation test**

A Franz cell apparatus were used to test the skin permeability of various deformable liposomes formulations. The diameter of Franz cell apparatus was 15 mm and a diffusion area was 1.76 cm^2. Moreover, the acceptor volume was 10 mL. Receptor chambers were filled with 10 mL PBS, and maintained at 37°C. Coated magnetic bar was stirred at 600 rpm in the receptor medium during experiments. Freshly excised hairless mouse skin was mounted Franz cell apparatus and deformable liposomes were loaded on the skin. An aliquot (100 μL) sample was collected from the receptor phase at 1, 3, 6, 12 and 24 hours after the application of deformable liposomes and replaced with the same volume of PBS immediately. The cumulative amounts of deformable liposomes penetrating skin were measured by spectrofluorophotometer with an excitation wavelength of 485 nm and an emission wavelength of 535 nm.

Results and Discussions

Deformability of liposomes

It has been known that edge activators give deformability to liposomes and the conventional rigid liposomes without edge activator have limitation in effectively penetrating the skin.[11] We calculated the deformability index of various liposomes that we prepared.[12] First, we monitored the size distribution of deformable liposomes with Tween 20, SDC and Span 85 (Table I). The particle size of Tween 20-based deformable liposomes was the smallest, 162.0±25.5 nm. Although the mean diameters of SDC-based deformable liposomes were 195.2±9.9 nm, extruded volume was larger than Tween 20-based deformable liposomes. It has been indicated that the deformability of SDC or sodium cholate-based liposomes were higher than Tween 80-based deformable liposomes.[13] Size of liposomes may be important for skin penetration, but it may be not the most crucial factor governing the skin penetration. The size of Span 85-based deformable liposomes was the largest and deformability index was the lowest (Table II). The average size of SDC-based deformable liposomes was 30 nm larger than Tween 20-based liposomes, however, SDC provided higher deformability than did Tween 20. Among the edge activators tested, SDC provided the highest deformability due to the highest extruded volume. This result indicates that both the particle size and extruded volume might be important factors

Table I–*The Size Distribution of Deformable Liposome*

Edge activators	Size of liposome (nm)
SDC	195.2±9.9
Tween 20	162.0±25.5
Span 85	233.0±3.1

Table II–*Deformability Index Value of Deformable Liposome*

Edge activators	Deformability index
SDC	2.97±0.07
Tween 20	2.88±0.01
Span 85	2.33±0.00

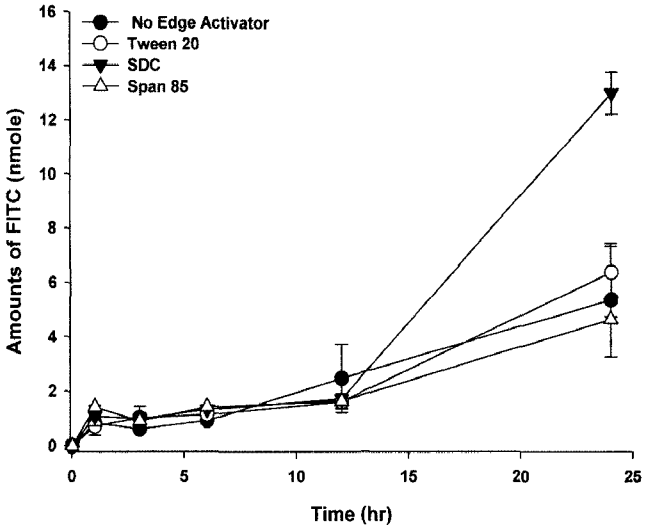

Figure 1—Effect of edge activators on the skin permeation of deformable liposomes. Sampling was done at time intervals using a Franz cell apparatus. The cumulative amounts of deformable liposomes permeated through the skin were measured by a spectrofluorophotometer.

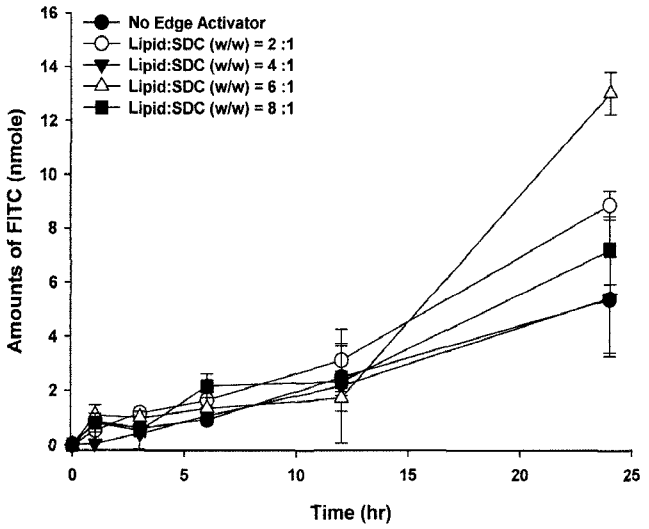

Figure 2—Formulation-dependent skin permeation of SDC-based deformable liposomes. Sampling was done at time intervals using a Franz cell apparatus. The cumulative amounts of deformable liposomes permeated through the skin were measured by a spectrofluorophotometer.

for deformability.

Effects of edge activator in the skin permeation of deformable liposomes

As a key component in the deformable liposomes, the edge activator could affect the extent of skin permeation.[14] In this study, we tested Tween 20, Span 85, and SDC as edge activators for skin permeation. We conjugated FITC to oleylamine for fluorescence detection using the methods described previously.[15] FITC-oleylamine was used as a lipid-membrane component of deformable liposomes as a marker.

Up to 12 hr, the penetration of deformable liposomes was minimal, indicating the relatively long lag time for deformable liposomes (Fig. 1). After 24 hours, SDC-based deformable liposomes showed the highest skin permeation. Deformable liposomes with SDC provided about two-fold higher level of skin penetration than did Tween 20- and Span 85-based deformable liposomes. Even though Span 85 was used as an edge activator in liposomes, the permeability of liposomes was the lowest, almost similar to conventional liposomes. Previous study reported that Span family was a good enhancer for transdermal drug delivery vehicle with nitrendipine.[16] For delivery of ceramide lipid, SDC seems to be a suitable edge activators for the formulation of deformable liposomes. Deformable liposomes seem to have a relatively long lag time. The penetration of deformable liposomes across stratum corneum might have contributed to the lag time. The deformation of spherical structure and the restructuring of edge activator-containing liposomes may take time to pass through the epidermis layer. Given the lag time, the use of additional enhancer which can shorten the lag time may be formulated in the deformable liposomes in the future.

Optimization of SDC composition

Since SDC-based deformable liposomes provided the highest skin permeation, we fixed SDC as an edge activator. It has been reported that the compositions of deformable liposomes, especially the ratio between the lipid and the edge activator may affect the transdermal delivery efficiency.[17] We tested five different ratios of lipids to SDC to find out the optimal composition of deformable ceramide liposomes (Fig. 2). Among the various ratios between the lipids to SDC, the ratio of 6:1 presented the highest permeation than did the other ratios of liposomes. The second efficient formulation was observed at the ratio of 2:1, followed by 8:1. This observation indicates that the too high content of SDC may reduce the functionality of deformable liposomes.

Previously, in the deformable liposomes carrying melatonin, the optimal formulation of melatonin-loaded liposomes for the highest skin permeation was reported to be 6:1 between the lipid and the edge activator.[18] Optimal ratio of tetanus toxoid-loaded deformable liposomes consisted of PC and SDC was 85:15 (w/w) and this formulation showed maximum deformability and entrapment efficiency.[19] The ratio of 6:1 as observed in melatonin-loaded deformable liposomes is consistent with our finding on the deformable ceramide liposomes. The mechanisms by which the ratio of 6:1 exerted the most effective skin penetration remain to be studied further. The

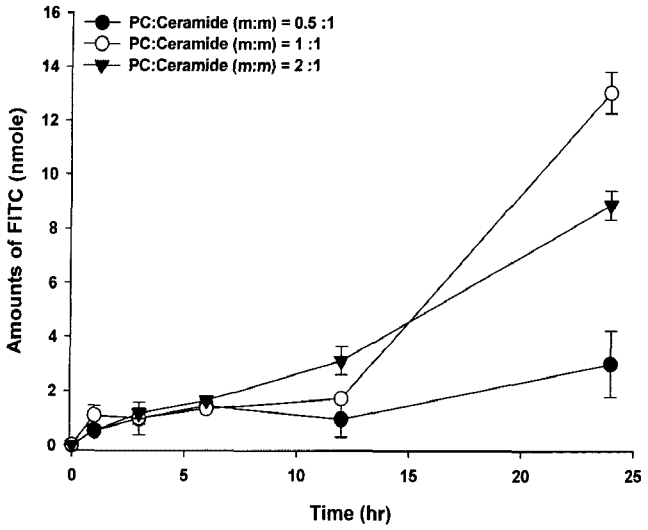

Figure 3–Influence of lipid compositions on skin permeation of SDC-based liposomes. Sampling was done at time intervals using a Franz cell apparatus. The cumulative amounts of deformable liposomes permeated through the skin were measured by a spectrofluorophotometer.

possibility exists that this ratio may enhance the easy perturbation of lipid packing, provide the high flexibility and promote the reconstitution of the liposome particle shape.

Optimization of lipid compositions of SDC-based deformable liposomes

Given the results of the ratio screening between lipid to SDC, we fixed the ratio of lipid to SDC as 6:1, and further optimized the ratio between PC and ceramide. The lipid portion of SDC-based deformable liposomes was composed of PC and ceramide. Most deformable liposomes have been formulated with only one lipid, PC, except gene delivery purposes in which cationic lipid components have been used.[20] Since we used ceramide as a functional component to alleviate atopic dermatitis, we fixed the amount of ceramide and changed lipid composition to test the best ratio of lipid to ceramide which may provide the highest skin penetration. The various SDC-based deformable liposomes showed different skin penetration capability (Fig. 3). As the ratio of PC to ceramide increased, the amount of FITC-oleylamine in acceptor solution increased up to 12 hours. However, at 24 hr after incubation, the deformable liposomes containing the same molar ratio of PC and ceramide showed the highest permeation than other deformable liposomes. The skin permeation of SDC-based liposomes was the lowest in case of the composition containing PC and ceramide as the ratio of 0.5:1. Such a low level of skin penetration might be due to the membrane rigidity conferred by the higher portion of ceramide. Many drug and natural product like a placenta extract have been studied for atopic dermatitis. Deformable liposomes might be considered for transdermal delivery systems of these natural products in the future.

Conclusion

In this study, deformable liposomes were prepared for delivery of ceramide into skin. Many cosmetic products contain ceramide for alleviation of atopic dermatitis. However, given the presence of immune cells in dermis layer, topic formulations may not deliver ceramide to the target sites in sufficient amounts. Since deformable liposomes have been used for enhanced transdermal delivery of several drugs and genes, we formulated ceramide-containing deformable liposomes for atopic dermatitis. We found that SDC might be a suitable edge activator for ceramide-containing liposomes. Moreover, using SDC as an edge activator, we optimized the ratio between lipid to SDC and the ratio of lipid compositions. At the ratio of lipid to edge activator was 6:1, the SDC-based liposomes provided the highest skin permeation. Moreover, we observed that the SDC-based deformable liposomes functions more effectively at the equal composition between PC and ceramide as lipid component. These results indicate that SDC-based deformable liposomes might be developed for enhanced delivery of ceramide liposomes.

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