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A Novel Phytosphingosine Based 1-O-Acylceramide: Synthesis, Physicochemical Characterization, and Role in The Lipid Lamellar Organization

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Introduction:

The intercellular lipid matrix in the human stratum corneum (SC) is a unique lamellar arrangement in which ceramide, free fatty acid, and cholesterol are organized as the main lipids [1]. Lipid composition and organization in the SC play an essential role in the characteristic properties of the SC lipid matrix correlate to a skin barrier function [2]. There are at least 14 classes of ceramide in the human stratum corneum depending on sphingoids and fatty acids binding to the amino group of sphingoids to form an appropriate lipid lamella organization [3,4]. The numbers of ceramides identified in the mammalian stratum corneum have been increasing with the advent of analytical technology for lipids, largely thanks to the LC/MS/MS skills [2]. Given that there are hundreds of ceramide species in human skin, it is challenging to functional characterization and mass production of all the ceramide species for their application in clinics and cosmetics. Numerous studies on physicochemical properties of ceramides having different sphingoid types and alkyl chain lengths have been conducted. Molecular dynamics (MD) simulation is a powerful tool to investigate the structures and interactions among lipid components at the atomistic level and has been used in numerous studies of the SC [5,6]. 1-O-acylceramides as a new class of epidermal ceramide were first identified in humans and mice [7]. 1-O-acylceramides contain a different acyl chain at the 1-O-position. Based on their chemical structure and hydrophobicity, 1-O-acylceramides have been speculated to play an essential role in the stabilizing lipid lamellar structure and maintaining permeability barrier despite their relatively low abundance (2-3% of all ceramides) in the skin barrier. However, the role of this new ceramide class in skin barrier function has not been fully elucidated. No reports on phytosphingosine-based 1-O-acylceramide and functional characterization of this new class of ceramide have been known. In this study, we synthesized phytosphingosine-based 1-O-acylceramides, 1-O-stearoyl ceramide NP (CerENP), and proposed a 'bidirectional anchoring model' for the mode of action of 1-O-acylceramide. We performed physicochemical analyses and molecular dynamics (MD) simulations to verify the proposed model's possible role of 1-O-acylceramides in SC lipid lamellar organization. Finally, a vehicle-controlled human study was conducted to confirm this notion.

Materials & Methods:

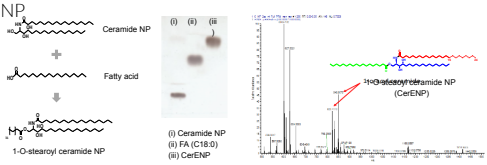
Synthesis and identification
Optimization of the 1-O-acylation process was done to obtain phytosphingosine-based 1-O-stearoyl ceramide NP. LC/MS and ^{1}H NMR analyses were conducted to identify the structure and conformation of the CerENP.

Physicochemical characterization and MD simulation
Multilayered lamellae structures were fabricated through the equimolar molecular assembly of ceramides (CerNP and CerENP), cholesterol, and stearic acid. The lamellar structure and phase properties of the fabricated multilayered lamellae were investigated by using DSC, XRD, and TEM analyses. MD simulation was performed to investigate the effect of CerENP on the LPP structure and skin barrier characteristics. Two LPP models were constructed, 1) Model A has an equimolar Cer:Chol:FFA ratio with a Cer mixture, CerEOS:CerNS:CerNP ratio of 46:41:13, 2) Model B contains additional CerENP (10% of CerEOS) from Model A. For hydrations, 5 waters per lipid were placed. All simulations were performed using GROMACS4 with CHARMM36 force field [8]. Steepest-descent energy minimization was performed first to relax the constructed system, which was further equilibrated by performing NVT (constant number of particles, volume, and 305 K
temperature) and NPT (constant number of particles, 1 bar pressure, and 305 K temperature) simulations with positional restraints on the heavy atoms. Productions runs were performed in NPT ensemble without any restraints to fully relax the system.

Human study
Skin hydration and SC cohesion were measured by using a Corneometer and Tewameter TM300 (Courage & Khazaka, Cologne, Germany). SC cohesion was expressed by calculating ΔTEWL (ΔTEWL = TEWL immediately after tape stripping – basal TEWL). TEWL was measured after 15 tape-strippings on the forearm of volunteers. Permeation of CerENP into the deep layers of SC was analyzed by TLC, LC/MS from the SC sample collected by tape-stripping.

Results & Discussion:

Synthesis and identification of 1-O-stearoyl ceramide NP

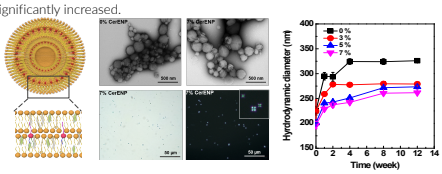


Optimization of the 1-O-acylation process was successfully established to obtain phytosphingosine-based 1-O-stearoyl ceramide NP, CerENP. LC/MS and 1H NMR analyses were conducted to confirm the structure and conformation of the CerENP.

Results & Discussion:

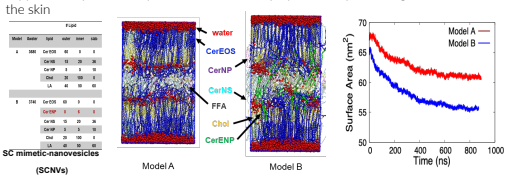
Improved stability of SCNVs by incorporated CerENP

SC mimetic-nanovesicle (SCNVs) using CerNP/cholesterol/fatty acid in combination with CerENP was prepared. The average particle size of the nanovesicles was 200–300 nm. With increasing amount of CerENP, the long-term stability was significantly increased.



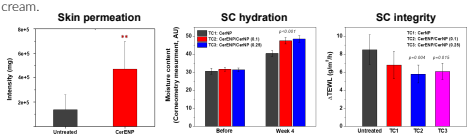
Effect of CerENP on the LPP structure (MD simulation)

The result of molecular dynamic (MD) simulations shows that the inserted CerENP molecules cause a significant change in the surface area (SA) by ~10% and may suppress the permeability of the SC, and thus play a role in preventing water loss from the skin



Skin permeation and human study on skin barrier functions

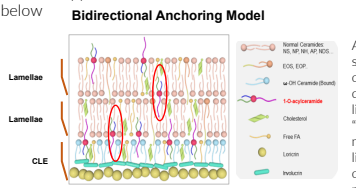
A significant improvement of skin barrier functions was observed from the skin site applied with a very low relative concentration of CerENP compared to CerNP. The ratio of CerNP to CerENP of TC2 was 10 to 1, yet more than a 17% increase in hydration was observed compared to TC1, which contains only 0.2% of CerNP in the cream.



Skin permeation after treatment with creams containing 0.5% CerENP. Data represent mean ± SEM (n= 5)

Skin barrier functions after 4-weeks of application of test cream compared to vehicle cream. Data represent mean ± SEM (n= 18)

Results support CerENP's mode of action as shown below



All the results strongly suggest that the mode of action of this new ceramide is very much likely exerted via a "bidirectional anchoring model" to stabilize the lipid lamellar organization as proposed.

Conclusions:

1-O-stearoyl ceramide NP, a novel phytosphingosine-based 1-O-acylceramide has been demonstrated to play an essential role in human SC. All the results strongly suggest that the mode of action of this new ceramide is very much likely exerted via a "bidirectional anchoring model" to stabilize the lipid lamellar organization as proposed. It is noteworthy that a synergistic effect was clearly demonstrated from the human skin test when it was formulated in combination with ceramide NP. We have proved that the effect of CerNP improving the skin barrier along with cholesterol and fatty acid was additionally boosted even if only a tiny amount of CerENP was added, which is as low as 1/10 of CerNP. To our knowledge, this is the first report on the investigation of CerENP on the boosting effect of CerENP together with CerNP. Combinatorial use of ENP and NP at an appropriate ratio could be a new normal in developing an ideal moisturizer for dry and atopic skin.

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