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Permeability and microstructure of model stratum corneum lipid membranes containing ceramides with long (C16) and very long (C24) acyl chains

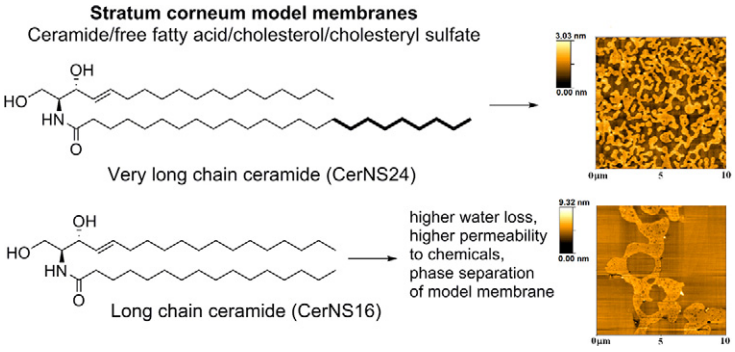
Petra Pullmannová [a,*], Ludmila Pavlíková [a], Andrej Kováčik [a], Michaela Sochorová [a], Barbora Školová [a,b], Petr Slepička [c], Jaroslav Maixner [c], Jarmila Zbytovská [b,c], Kateřina Vávrová [a]

[a] Skin Barrier Research Group, Charles University, Faculty of Pharmacy in Hradec Králové, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
[b] Charles University, Faculty of Pharmacy in Hradec Králové, Department of Pharmaceutical Technology, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
[c] University of Chemistry and Technology Prague, Technická 5, 166 28 Prague, Czech Republic

HIGHLIGHTS

- Shortening of ceramide chains from 24 to 16 carbons increased membrane permeability.
- C16 acyl ceramide does not mix well with other skin barrier lipids.
- A higher area per lipid in C16 ceramide monolayers than in C24 ceramide monolayers

GRAPHICAL ABSTRACT



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ABSTRACT

The Stratum corneum (SC) prevents water loss from the body and absorption of chemicals. SC intercellular spaces contain ceramides (Cer), free fatty acids (FFA), cholesterol (Chol) and cholesteryl sulfate (CholS). Cer with "very long" acyl chains (for example, *N*-lignoceroyl-sphingosine, CerNS24) are important for skin barrier function, whereas increased levels of "long" acyl Cer (for example, *N*-palmitoyl-sphingosine, CerNS16) occur in patients suffering from atopic eczema or psoriasis. We studied the impact of the replacement of CerNS24 by CerNS16 on the barrier properties and microstructure of model SC lipid membranes composed of Cer/FFA/Chol/CholS. Membranes containing the long CerNS16 were significantly more permeable to water (by 38–53%), theophylline (by 50–55%) and indomethacin (by 83–120%) than those containing the very long CerNS24 (either with lignoceric acid or a mixture of long to very long chain FFA). Langmuir monolayers with CerNS24 were more condensed than with CerNS16 and atomic force microscopy showed differences in domain formation. X-ray powder

Abbreviations: AFM, Atomic force microscopy; Cer, Ceramide/s; Chol, Cholesterol; FFA, Fatty acid/s; FFA(16-24), Mixture of free fatty acids with 16, 18, 20, 22 and 24 carbons; CerNS16, *N*-hexadecanoyl-D-*erythro*-sphingosine; IND, Indomethacin; LIG, Lignoceric acid; CholS, Sodium cholesteryl sulfate; RH, Relative humidity; SC, Stratum corneum; CerNS24, *N*-tetracosanoyl-D-*erythro*-sphingosine; TH, Theophylline; XRPD, X-ray powder diffraction.

* Corresponding author at: Skin Barrier Research Group, Department of Inorganic and Organic Chemistry, Charles University, Faculty of Pharmacy in Hradec Králové, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic.
E-mail address: pullmanp@faf.cuni.cz (P. Pullmannová).

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diffraction revealed that CerNS24-based membranes formed one lamellar phase and separated Chol, whereas the CerNS16-based membranes formed up to three phases and Chol. These results suggest that replacement of CerNS24 by CerNS16 has a direct negative impact on membrane structure and permeability.

1. Introduction

The outermost layer of the skin of terrestrial mammals, including humans – the stratum corneum (SC) – protects the body from both desiccation and the entry of substances from the environment. The SC consists of cornified cells, corneocytes, embedded in an extracellular matrix of highly ordered lipids. These lipids consist of ceramides (Cer), free fatty acids (FFA) and cholesterol (Chol) in an approximately equimolar ratio and a minor fraction of cholesteryl sulfate (CholS) [1]. Skin Cer (i.e., *N*-acylsphingosines) are a heterogeneous class of sphingolipids that are indispensable for the epidermal homeostasis. To date, 15 classes of free Cer have been identified in the human SC; these classes are based on five different sphingoid bases and three types of *N*-acyl chains, including the ultra-long acyl Cer (EO-class Cer), which contain 30-34C acyl chains with a linoleic acid ester linked to an ω-hydroxyl [2,3]. In addition to the common double chain Cer, two recently identified Cer classes contain a third chain at position 1 (1-*O*-acyl Cer) [4].

The human skin barrier Cer are characterized by having very long (20–26 carbons) or ultra-long (>28 carbons) acyl chains [2–4].[1] The biosynthesis of such very long or ultra-long acyl chains requires the elongation of palmitoyl coenzyme A by a series of reactions catalyzed by a family of elongases (ELOVL) (Fig. 1).

The acyl chains are then attached to dihydrosphingosine by one of six Cer synthases, which have distinct preferences in terms of acyl structure, leading to the formation of various Cer classes (Fig. 1). The Cer synthase 2 generates ceramides with the "very long" acyl chain length of 22–24 carbons. Intracellular Cer are further processed to their more polar precursors, i.e., sphingomyelin and glucosylceramide, and stored in lamellar bodies. At the stratum granulosum/SC interface, these fluid-phase precursors are enzymatically processed to yield Cer and other barrier lipids. The transition from fluid to crystalline lipids is likely further aided by the corneocyte lipid envelope (Cer covalently bonded to the cell surface proteins) that is supposed to have a templating effect on the orientation of the free lipids (for a review, see Ref. [4]).

Pewzner-Jung et al. demonstrated that the lipid profile and biophysical properties of liver lipid extract were altered in Cer synthase 2 null mouse, which lost the activity of this enzyme. Cer in Cer synthase 2 null mouse were devoid of very long acyl chains (C22 – C24), but the total Cer level was unaltered due to an increased amount of "long" acyl chains Cer with 16 carbons [5]. The length of the Cer acyl chain appears to be important for numerous physiological and pathophysiological properties of Cer [4–6] and also for the effects of Cer on the lateral organization of lipid membranes [7–9].

Several chronic skin diseases, such as psoriasis and atopic eczema, are accompanied by an altered skin lipid profile [10–12]. Recent works showed that atopic dermatitis patients had decreased levels of sphingosine-based C24 Cer with "very long" acyl chains (for example *N*-lignoceroyl-sphingosine, CerNS24) and an increased proportion of C16 Cer with "long" acyl chains (for example *N*-palmitoyl-sphingosine, CerNS16) [11,13]. This acyl chain length shift correlated with the aberrant lipid organization and increased transepidermal water loss observed in these patients [13]. Similar "shortening" of Cer acyl chains was also found in psoriasis, cultured human keratinocytes treated with interferon gamma [14], and a murine atopic dermatitis model [15].

Thus, it appears that the biosynthesis of the very long Cer (in addition to the ultra-long acyl Cer) is an important step in skin barrier development and that Cer with a palmitoyl chain (e.g., CerNS16) cannot substitute the very long chain Cer (e.g., CerNS24). However, it is difficult to link this particular change in Cer chain length to higher skin permeability because of the multifactorial nature of atopic dermatitis.

Additional mechanistic insight into the role of Cer chain length in the epidermis can be provided by a study of model lipid membranes with a well-defined lipid composition. One of the first investigation of the artificial lipid membranes mimicking the human SC was published by Landman in 1984 [16]. Later on, the model lipid membranes have become widely used in the SC lipid barrier research [17–19] Several recent studies investigated biophysical aspects of model lipid membranes as a function of the Cer acyl chain length or the FFA chain length and reported that chain shortening led to a less tight lipid arrangement and a phase separation [20–29]. Only two studies focused directly on CerNS16 in comparison to CerNS24. The first reported that CerNS16 in the fully hydrated SC model was mostly found in the gel phase, while CerNS24 was crystalline [28].The second study showed that CerNS24 preferred an extended (splayed-chain) conformation in which the fatty acid was associated with the ceramide acyl chain. In contrast, the shorter CerNS16 and fatty acids were mostly phase separated [29].

The Cer with very short (C4-C6) acyl chains showed negative effects on the permeability of porcine skin and model membranes; however, Cer with C12 and C18:1 acyl did not significantly increase the permeability relative to very long acyl CerNS24 [26,27]. These findings cast doubt over the hypothesis that Cer with C16 acyl chains instead of very long C24 acyl chains would have a negative effect on the barrier properties of SC lipid membranes.

In light of the correlation between the impaired skin barrier function and the levels of Cer with C16 acyl in diseased skin, we decided to directly compare the effects of the long chain CerNS16 relative to the very long chain CerNS24 on the permeability and microstructure of model SC lipid membranes. The model lipid membranes were composed of an equimolar mixture of the studied Cer/FFA/Chol with 5 wt% CholS. Further variation was achieved using either lignoceric acid (LIG) or a mixture of FFA with C16, C18, C20, C22 and C24 acyl chains (FFA(16-24)), which is close to the FFA profile of the native SC [30]. In fact, skin lipids are structurally heterogeneous, in the case of FFA the differences are in the chain length. Therefore we decided to introduce a partial heterogeneity and compared the model membranes with the different FFA component. The membrane permeability was evaluated using three permeability markers: the water loss through the membranes, the steady-state flux of theophylline (TH) and the steady-state flux of indomethacin (IND). X-ray powder diffraction (XRPD) was used to reveal how the studied Cer influenced the periodical arrangement of the lipid membranes. The area per lipid in the monolayer at the air-water interface was studied by the Langmuir monolayers and the domain formation was visualized in the supported Langmuir-Blodgett monolayers by atomic force microscopy (AFM).

2. Material and methods

2.1. Chemicals and material

N-tetracosanoyl-D-*erythro*-sphingosine (CerNS24) and *N*-hexadecanoyl-D-*erythro*-sphingosine (CerNS16) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Cholesterol from lanolin (Chol), sodium cholesteryl sulfate (CholS), hexadecanoic acid,

[1] We use the nomenclature defined in Rabionet et al., in which long chains are defined as C14–C19, very long chains are defined as C20–C26 and ultra-long chains are those over 26C.