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Altered sphingoid base profiles predict compromised membrane structure and permeability in atopic dermatitis

Nicolas Loiseau [a,b,c,d], Yasuko Obata [e], Sam Moradian [a,b,c], Hiromu Sano [e], Saeko Yoshino [f], Kenichi Aburai [f], Kozo Takayama [e], Kazutami Sakamoto [f,g], Walter M. Holleran [a,b,c], Peter M. Elias [a,b,c], Yoshikazu Uchida [a,b,c,*]

[a] Department of Dermatology, School of Medicine, University of California, San Francisco, San Francisco, CA, USA
[b] Veteran Affairs Medical Center, San Francisco, CA, USA
[c] Northern California Institute for Research and Education, San Francisco, CA, USA
[d] Integrative Toxicology and Metabolism, Pôle de Toxicologie Alimentaire, Laboratoire de Pharmacologie et Toxicologie, Institut National de la Recherche Agronomique INRA UR66, Toulouse, France
[e] Department of Pharmaceutics, Hoshi University, Tokyo, Japan
[f] Department of Pure and Applied Chemistry, Tokyo University of Science, Noda, Chiba, Japan
[g] Faculty of Pharmacy, Chiba Institute of Science, Chosi, Chiba, Japan

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ABSTRACT

Background: Ceramide hydrolysis by ceramidase in the stratum corneum (SC) yields both sphingoid bases and free fatty acids (FFA). While FFA are key constituents of the lamellar bilayers that mediate the epidermal permeability barrier, whether sphingoid bases influence permeability barrier homeostasis remains unknown. Pertinently, alterations of lipid profile, including ceramide and ceramidase activities occur in atopic dermatitis (AD).

Object: We investigated alterations in sphingoid base levels and/or profiles (sphingosine to sphinganine ratio) in the SC of normal *vs.* AD mice, a model that faithfully replicates human AD, and then whether altered sphingoid base levels and/or profiles influence(s) membrane stability and/or structures.

Methods: Unilamellar vesicles (LV), incorporating the three major SC lipids (ceramides/FFA/cholesterol) and different ratios of sphingosine/sphinganine, encapsulating carboxyfluorescein, were used as the model of SC lipids. Membrane stability was measured as release of carboxyfluorescein. Thermal analysis of LV was conducted by differential scanning calorimetry (DSC).

Results: LV containing AD levels of sphingosine/sphinganine (AD-LV) displayed altered membrane permeability *vs.* normal-LV. DSC analyses revealed decreases in orthorhombic structures that form tightly packed lamellar structures in AD-LV.

Conclusion: Sphingoid base composition influences lamellar membrane architecture in SC, suggesting that altered sphingoid base profiles could contribute to the barrier abnormality in AD.

1. Introduction

The outermost layer of the epidermis, the stratum corneum (SC), serves as the principal barrier against excessive transcutaneous water loss, while simultaneously blocking ingress of microbial pathogens, allergens and other xenotoxic agents [1,2]. The epidermal permeability barrier localizes to the extracellular domains of the SC, where stacks of broad lamellar bilayers, enriched in free fatty acids (FFA), cholesterol and ceramides (Cer), account for barrier competence [3–5]. Most abundant among these lipids are Cer, which account for about 50% of total SC lipid mass, and comprise at least ten different species, including epidermal-unique omega

Abbreviations: AD, atopic dermatitis; CFC, 2,5-carboxyfluorescein; Cer, ceramide; DSC, differential scanning calorimetry; FFA, free fatty acids; Sa, sphinganine; So, sphingosine; LV, unilamellar vesicles; SDS, sodium dodecyl sulfate; SC, stratum corneum.

* Corresponding author at: Dermatology Service (190); Veterans Affairs Medical Center, 1700 Owen Street, Room 326, San Francisco, CA 94158, USA; 4150 Clement Street, San Francisco, CA 94121, USA. Tel.: +1 415 575 0524; fax: +1 415 750 2106.
E-mail address: uchiday@derm.ucsf.edu (Y. Uchida).

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(ω)-O-acylated forms, which are critical contributors to the organization of these lamellar bilayers [6,7]. Much of this Cer heterogeneity is determined by differences in sphingoid base and amide-linked fatty acid structures [7]. Yet, it remains unknown how each lipid type influences barrier function.

Sphingoid bases are long-chain amino alcohols that are both immediate precursors, as well as catabolites of Cer, generated within the SC by one or more ceramidases [8–10]. While these molecules show amphiphilic, detergent-like properties that account for their potent *in vitro* antimicrobial activity [11,12], they also inhibit both protein kinase C [13,14] and phosphatidate phosphohydrolase [15] activities. Although the free sphingoid base content of nucleated mammalian cells, including keratinocytes, is relatively low [16], levels rise to 5–6 mol% of total lipids in SC, amounts still lower than the three major SC lipids (Cer, FFA, and cholesterol in Table 1). Prior studies demonstrated that supraphysiological levels of sphingosine (So) (>10 mol%), when added to phospholipid-cholesterol model membranes, increase phase transition temperatures and enthalpy [17], leading to our current hypothesis that sphingoid base content could also influence membrane stability in the SC. Yet, nothing is known about how sphingoid base profiles dictate function in normal SC; nor whether abnormalities in sphingoid base content and composition (distribution) contribute to the abnormal permeability barrier in atopic dermatitis (AD).

AD is a chronic, inflammatory skin disease that until recently was attributed to abnormalities in adaptive and innate immunity [18]. Recent evidence suggests that AD instead is often initiated by inherited abnormalities that compromise either SC structural or enzymatic proteins [19,20]. The diminished content of specific species of EOS (Cer 1) and NP (Cer 3) are characteristic downstream features that contribute to the abnormal permeability barriers not only in human [21–23], but also in canine [24] AD. Conversely, Cer replenishment appears to improve barrier function and clinical status in AD [25,26]. In addition, sphingosine (So) content and acidic (but not alkaline) ceramidase activity decreased in a part of the SC fraction in human [27]. This prior study assessed neither whole SC nor another sphingoid base species, *i.e.*, sphinganine (Sa) content. We previously reported that epidermal specific alkaline ceramidase is highly expressed in the late stages of differentiation, and its activity persists in the SC [28]. Moreover, ceramidase activities are elevated in AD patient skin [29], while pertinently glycerophospholipids derived from *Staphylococcus aureus* activate ceramidase generated from colonized *Pseudomonas* in AD patients. Both of these microbial pathogens often colonize in AD patients [30]. Hence, it is unclear whether decreased acidic ceramidase activities significantly influence ceramide hydrolysis in the SC of AD.

Given that prominent alterations in sphingoid base content occur in AD [27], we hypothesized that modulations in sphingoid base levels and/or ratios could influence lamellar membrane structure, thereby contributing to altered permeability barrier function in AD. However, reported lipid biochemical data for humans with AD have relied upon topical solvent extracts or tape/cyanoacrylate strippings, which may incompletely sample the SC. Prior studies demonstrated that alterations of lipid lamellar structures become evident in the lower stratum corneum [31], suggesting that abnormal barrier structures in the lower part of the stratum corneum affect barrier function. Therefore, it is important to use whole SC rather than a part of the SC for obtaining basal sphingoid base profile to assess the influences of sphingoid base composition on membrane structures. We analyzed whole SC instead of a part of the stratum corneum to obtain basis of sphingoid profile in order to study the roles of sphingoid bases in lamellar membrane structures in the SC. We first investigated whether the alterations in epidermal lipid content and composition that have been reported for human AD occur in lipid extracts of whole SC from our AD mouse model [32–34]. After showing that the mouse model replicates reported lipid biochemical abnormalities in human AD, we analyzed and quantitated sphingoid base levels in these whole SC extracts, and then investigated the role of sphingoid base variability in membrane permeability and organization, in unilamellar vesicles (LV) reconstituted with lipids that reflect normal or AD SC. Our studies show that (i) sphingoid bases influence membrane permeability, and (ii) altered ratios of So to Sa attenuate lamellar membrane stability and disturb lamellar membrane organization in AD SC.

Table 1
Major lipid and sphingoid base content in the normal and atopic dermatitis (AD) model mouse stratum corneum.

	Mass concentration (μg/mg dry weight)	
	Normal	AD
Cholesterol	17.2 ± 0.78	24.9 ± 0.10*
Free fatty acids	19.3 ± 2.92	15.1 ± 1.38
Total ceramides	11.0 ± 0.80	11.5 ± 0.30
EOS (Cer 1)	2.19 ± 0.78	1.04 ± 0.20*
NS (Cer 2)	3.87 ± 0.28	3.36 ± 0.11
NP (Cer 3)	1.59 ± 0.15	0.74 ± 0.12*
AS (Cer 5)#	3.33 ± 0.22	2.86 ± 0.31
AS (Cer 5)##	1.33 ± 0.12	1.63 ± 0.12
C18-Sphingosine	1.52 ± 0.34	2.40 ± 0.55
C18-Sphinganine	0.28 ± 0.06	0.16 ± 0.08**
Ratio C-18 So to Sa	5.43	14.3*

Cer structures are according to Motta et al. [52] and Robson et al. [53]: EOS (Cer 1), esterified ω-hydroxy (OH) FA with sphingosine; NS (Cer 2), non-OH FA with sphingosine; NP (Cer 3), non-OH FA with phytosphingosine; AS (Cer 5), α-OH FA with sphingosine. AS# are more hydrophobic species of AS, *i.e.*, containing longer amide-linked fatty acids, compared with AS##. The data are indicated as the mean value ± SD (n = 4–6 mice). See details in Section 2.

* $p > 0.01$.

** $p > 0.02$ *vs.* normal.

2. Materials and methods

2.1. Materials

N-Octadecanoyl-D-*erythro*-sphingosine (N-stearoylsphingosine) was purchased from Matreya (Pleasant Gap, PA). Cholesterol, palmitic acid and sphingoid bases were purchased from Matreya or Sigma–Aldrich (St. Louis, Mo.) or generously supplied by Takasago International (Tokyo, Japan). Other chemicals used were reagent grade.

2.2. AD model mice

AD model mice are prepared as described previously [32]. All animal procedures were approved by the Animal Studies Subcommittee of the San Francisco Veterans Affairs Medical Center and performed in accordance with their guidelines.

2.3. Lipids extraction

SC was isolated from skin by the incubation with trypsin in phosphate-buffered saline as described previously [35], followed by extracting lipids by Folch's method [36]. Lipid extracts were fractionated into cholesterol, FFA and ceramide by high-performance thin layer chromatography [37]. Lipids were visualized after treatment with cupric acetate–phosphoric acid, and heating to 160 °C for 15 min followed by quantitation by scanning densitometry as we described previously [37]. Lipid content was reported as μg of total lipids/mg dry SC weight. Molecular weights used for calculation are EOS (Cer 1), 1011.96; other ceramides, 649.64; stearic acid for FFA, 284.27; and cholesterol, 386.35 to prepare LV.