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A lamellar body mimetic system for the treatment of oxazolone-induced atopic dermatitis in hairless mice

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ABSTRACT

Background: Atopic dermatitis is a common skin disease characterized by a Th2 cell-dominant inflammatory infiltrate, elevated serum IgE levels and impaired epidermal barrier function. It is associated to abnormal epidermal lamellar body secretion, producing alteration in lipid composition and extracellular lamellar membrane organization.
Objectives: The oxazolone-induced atopic dermatitis in hairless mice was used to evaluate *in vivo* the effect of the application of a lipid system that mimics the morphology, structure and composition of epidermal lamellar bodies.
Methods: The skin barrier function was evaluated measuring TEWL and skin hydration *in vivo*. Inflammation was assessed by analysis of serum IgE levels and histological analysis. The microstructure of the intercellular lipid region was also evaluated before and after treatment.
Results: The skin condition was improved after 10 days of treatment indicated by decreased TEWL, decreased serum IgE levels, reduced epidermal thickness and reduced lymphocyte-dominated infiltrate. However, the treatment did no improve skin hydration.
Conclusions: The treatment with this lipid system seems to improve the skin condition by reinforcing the barrier function and reducing the skin inflammation. Therefore, the present study provides evidence that this lipid system combining appropriate lipid composition and morphology could be of interest for the development of future treatments for atopic dermatitis.

1. Introduction

Atopic dermatitis is a common skin disease characterized by inflammatory, pruritic eczema. It is a multifactorial disease which involves a complex interplay of environmental and genetic factors. Atopic dermatitis shows a Th2 cell-dominant inflammatory infiltrate, elevated serum IgE levels and impaired epidermal barrier function [1], indicated by an increased transepidermal water loss (TEWL) [2] and decreased water-holding capacity [3].

The cutaneous permeability barrier is mediated by extracellular lipids mainly ceramides, free fatty acids and cholesterol, which form extracellular lipid-enriched lamellar membranes between the corneocytes that block the movement of water and electrolytes [4]. These lipids are delivered to the extracellular spaces of the stratum corneum by the secretion of lamellar bodies. Atopic dermatitis is associated to abnormal lamellar body secretion, producing alteration in lipid composition, especially ceramides and free fatty acids [5,6], and in extracellular lamellar membrane organization, as well as dysfunction in antimicrobial defense. Moreover, in atopic dermatitis alkaline pH of the skin surface is another cause of increased skin susceptibility to bacteria, mainly *Staphylococcus aureus* [7].

One animal model that closely reflects these features is the oxazolone-induced atopic dermatitis in hairless mice [8]. When oxazolone is applied to the skin of hairless mice for a period of 3 weeks, mice develop symptoms characteristic for atopic dermatitis including barrier dysfunction, secretion of IgE, epithelial cell hyperplasia, fibrosis and infiltration of inflammatory cells into the dermis and epidermis and secretion of Th2 cytokines.

Bicosomes are lipid aggregates (200–400 nm) formed by disks encapsulated in lipid vesicles [9]. The interaction of these systems with the skin can modify the permeability of the barrier, reinforce

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the lipid structure and promote targeted delivery of molecules to specific skin layers, among others effects [10–12]. In a previous study, we designed a lipid system based on bicosomes that mimic the morphology, structure and composition of epidermal lamellar bodies [13]. In addition, we performed *in vitro* studies to evaluate the effect of this system on delipidized stratum corneum. We concluded that the treatment with the lamellar body mimetic system (LBms) re-establishes *in vitro* part of the lipid lamellar structure of delipidized stratum corneum, mainly the short periodicity phase described by Bouwstra et al. [14]. In other study, we evaluate *in vivo* the hydration-dehydration kinetics of human skin after treatment with the LBms [15]. We found that skin increased its ability to retain water. Thus, this lipid system seems to reinforce the barrier function.

Our previous results point to a high potential of the LBms to improve skin conditions. Thus, in the present study, we sought to evaluate the effect of the application of the LBms on oxazolone-induced atopic dermatitis mouse model. Accordingly, we evaluate skin hydration and TEWL to study the skin barrier function. Inflammation was assessed by analysis of IgE levels in serum. In addition, skin biopsies were taken the last day of the experiment for histological analysis by optical microscopy and for evaluating the microstructure of the intercellular lipid regions by freeze substitution transmission electron microscopy (FSTEM).

2. Material and methods

2.1. Chemicals

1,2-Dipalmitoyl-sn-*glycero*-3-phosphocholine (DPPC) and 1,2-dihexanoyl-sn-*glycero*-3-phosphocholine (DHPC) were from Avanti Polar Lipids (Alabaster, United States). Cholesterol from lanolin (chol), cholesteryl sulphate sodium salt (Schol), cholesteryl palmitate (Echol), stearic acid (SA), oxazolone, hematoxylin solution Gill 2, eosin Y alcoholic solution, lithium carbonate, xylene and DPX mountant for histology were from Sigma-Aldrich (Steinheim, Germany). Ceramide IIIb (cer3) and ceramide VI (cer6) were from Evonik (Essen, Germany). Lipoid S100, whose main component (>94%) is soybean phosphatidylcholine (PC) and Lipoid S LPC 80 whose main component (80%) is soybean lysophosphatidylcholine (lysoPC) were from Lipoid GmbH (Ludwigshafen, Germany). Chloroform, formaldehyde solution 4% and acetic acid were from Merck (Darmstadt, Germany). Acetone and ethanol absolute were from Panreac (Barcelona, Spain). Optimal cutting temperature compound (OCT) was from Sakura Finetek (Torrance, United States).

2.2. Preparation and characterization of the system

2.2.1. Discoidal structures preparation

Four types of discoidal structures were prepared: (a) DPPC/DHPC, q = 3.5 (q = DPPC/DHPC molar ratio) including 5 mol% cer3 and 5 mol% cer6; (b) DPPC/DHPC, q = 3.5 including 5 mol% chol; (c) DPPC/DHPC, q = 3.5 including 5 mol% Schol and (d) DPPC/DHPC q = 3.5 including 5 mol% Echol.

For each of the discoidal systems, appropriate amounts of lipids were mixed in chloroform and evaporated to dryness using a rotary evaporator. The systems were hydrated with distilled water to obtain a 10% (w/v) total lipid concentration and then subjected to several cycles of sonication, freezing and heating until the samples became transparent.

Next, different volumes of these samples were selected to obtain a mixture in which the molar ratio of cer, chol, Schol and Echol was similar to that found in the skin. To obtain 1 ml, 440 μl of (a), 440 μl of (b), 88 μl of (c) and 32 μl of (d) were mixed. This mixture was used to prepare the LBms.

2.2.2. LBms preparation

A chloroform solution containing 95 mg/ml PC, 5 mg/ml lysoPC and 1.6 mg/ml SA was rota-evaporated to remove the chloroform and thus form a lipid film. The film was hydrated with the previously prepared mixture of discoidal structures.

The total lipid concentration in the final system was 20% (w/v) with a cer:SA:chol:Schol:Echol molar ratio of 1:1:0.5:0.1:0.04.

The LBms was analysed 24 h after preparation by dynamic light scattering (DLS) and cryogenic transmission electron microscopy (Cryo-TEM).

2.2.3. DLS

The hydrodynamic diameter (HD) was determined by DLS with a Zetasizer nano ZS (Malvern Instruments, UK). The DLS technique measures the diffusion coefficient (D) of the particles corresponding to Brownian motion, and converts this to size by using the Stokes-Einstein equation,

$$HD = kT/3\pi hD$$

where HD is the hydrodynamic diameter, D is the translational diffusion coefficient (m^2/s), k is the Boltzmann constant ($1.3806503 \times 10^{-23}\ m^2\ kg\ s^{-2}\ K^{-1}$), T is the absolute temperature (298 K), and η is the viscosity of the dispersant at 25 °C (water, 0.8872 mPa s). The particles sizes were determined from the scattered light that was detected at an angle of 173° when a laser beam of 633 nm wavelength crossed the sample. Measurements were performed in triplicate.

2.2.4. Cryo-TEM

The morphology of the LBms was evaluated by Cryo-TEM. A thin aqueous film was formed by dipping a glow-discharged holey carbon grid in the suspension and then blotting the grid against a filter paper. The resulting thin sample film spanning the grid holes was vitrified by plunging the grid into ethane, which was maintained at its melting point with liquid nitrogen, by use of a Vitrobot (FEI Company, Eindhoven, The Netherlands). The vitreous sample film was transferred to a Tecnai F20 TEM (FEI Company, Eindhoven, The Netherlands) by use of a cryotransfer holder (Gatan, Barcelona, Spain), and the sample was observed in low-dose mode. Images were acquired at 200 kV at a temperature between −170 °C and −175 °C, under low-dose imaging conditions. Ten overviews and approximately 50 detailed electron micrographs were taken.

2.3. Animals and study protocol

The study protocol was approved by the Animal Experimentation Ethics Committee of the University of Barcelona according to the regulations of the local government (Decree 214/1997, July 30th). Female hairless mice SKH-1 (n = 8; Charles River, Germany) of 8–9 weeks old were housed in two cages (n = 4) in a room with controlled temperature and humidity. Food and water were provided *ad libitum*. Atopic dermatitis was induced by topical application of oxazolone in acetone. The design of the study is summarized in Fig. 1. The animals were sensitized on dorsal skin with 10 μl of 5% oxazolone in acetone (D0). A week later, the dorsal skin of the mice was treated with 60 μl of 0.1% oxazolone in acetone for 20 days (from D7 to D26). In addition, one group was treated with 30 μl of LBms (treated-dermatitis group) whereas the other was treated with 30 μl of water (dermatitis group) for 10 days (from D17 to D26). Treatment with LBms or water was applied one hour after oxazolone application. Twenty four hours after the last treatment, animals were euthanized and biopsies from the dorsal skin of the mice were taken (D27).

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