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Effects of glucocorticoids on stratum corneum lipids and function in human skin—A detailed lipidomic analysis

Mads A. Røpke[a,*], Cristina Alonso[b], Sora Jung[c], Hanne Norsgaard[a], Claudia Richter[d], Maxim E. Darvin[c], Thomas Litman[a], Annika Vogt[d], Jürgen Lademann[c], Ulrike Blume-Peytavi[d], Jan Kottner[d]

[a] LEO Pharma A/S, 2750 Ballerup, Denmark
[b] OWL Metabolomics, Parque Tecnológico de Bizkaia, Derio, Spain
[c] Charité—Universitätsmedizin Berlin, Center of Experimental and Applied Cutaneous Physiology, Department of Dermatology and Allergy, Charité-Universitätsmedizin, Berlin, Germany
[d] Clinical Research Center for Hair and Skin Science, Department of Dermatology and Allergy, Charité-Universitätsmedizin, Berlin, Germany

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ABSTRACT

Background: Topical glucocorticoids (GCs) are known to induce atrophy of human skin including thinning of epidermal and dermal compartments by influencing keratinocyte proliferation and synthesis of extracellular matrix proteins. GCs are also known to reduce skin barrier integrity but little is known about the changes in lipid composition in human skin following topical administration of GCs.
Objective: This study investigated the effects of GCs on stratum corneum (SC) function and lipid profile of human skin *in vivo*.
Method: Over a period of 4 weeks, 16 healthy volunteers were treated on the forearms once daily with topical clobetasol proprionate (CP), betamethasone diproprionate (BDP) or vehicle. One day after last application (Day 29) SC lipids were collected by tape stripping and analysed by a high sensitivity liquid chromatography–mass spectrometry method. Gene expression was analysed in skin biopsies. The full skin, epidermal and SC thickness were assessed by ultrasound, optical coherence tomography and confocal microscopy, respectively, and barrier integrity was assessed by measuring transepidermal water loss (TEWL).
Results: Compared to vehicle controls, GCs induced significant alterations in SC lipid profiles. CP caused a reduction in 98 lipids of 226 analysed while BDP treatment only resulted in a significant change of 29 lipids. Most pronounced changes occurred among long chain, ester-linked, ceramide classes while other ceramide classes were much less affected. Almost the complete profile of triacylglycerols (TGs) was significantly decreased by CP while more modest changes were observed in free fatty acids. Topical GCs reduced the thickness of skin layers and increased TEWL. GC treatment also induced changes in expression of genes coding for extracellular markers and enzymes involved in lipid synthesis.
Conclusions: This study shows a reduction in specific SC lipid classes following topical GC treatment of human skin and contributes to the characterisation of the barrier disruption in human skin induced by topical steroids.

1. Introduction

The main function of the skin is to protect the body by providing an efficient barrier. The stratum corneum (SC) consists of dead keratinocytes (corneocytes) that have undergone terminal differentiation with degradation of the nucleus, loss of DNA, and formation of a unique cornified envelope that serves as a platform for a neutral lipid enriched extracellular matrix. This extracellular hydrophobic lipid matrix of SC provides a barrier to the movement

Abbreviations: SC, stratum corneum; GC, glucocorticoid; GR, glucocorticoid receptor; CSLM, confocal scanning laser microscopy; OCT, optical coherence tomography; US, ultrasound; CP, clobetasol proprionate; BDP, betamethasone diproprionate; FFA, free fatty acid; LC/MS, liquid chromatography–mass spectrometry; TG, triacylglycerols.

* Corresponding author at: Department of Translational Medicine, LEO Pharma A/S, Industriparken 55, 2750 Ballerup, Denmark.
E-mail address: mads.roepke@leo-pharma.com (M.A. Røpke).

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of water and electrolytes [1]. This lipid matrix, accounting for about 10% of the tissue mass of the SC, has a unique lamellar organization and composition consisting of approximately 50% ceramides, 25% cholesterol, and 15% free fatty acids (FFA) with a low content of phospholipids [2,3]. After secretion, lamellar body-derived polar lipids are further metabolized in the SC extracellular spaces by enzymes that are co-secreted in lamellar bodies [4]. The FFAs present in native human SC are mainly saturated and range in chain length from C14 to C34. It has been reported that the presence of FFAs enhances the formation of the lateral packing order [5], which determines the skin barrier function. The basic structure of the main epidermal sphingolipids, ceramides, is a sphingoid base with fatty acid connected by an amide bond. Three types of fatty acids (esterified ω-hydroxy [EO], -α-hydroxy [A] and non-hydroxy [N] fatty acids) with four types of sphingoid bases (sphingosine [S], 6-hydroxysphingosine [H], dihydrosphingosine [DS] and phytosphingosine [P]) constitute 12 classes of ceramides, which have been identified in SC [6]. Ceramide species are differentiated by the length of the fatty acid or the sphingoid backbone. In general, topical application of glucocorticoids (GCs) is frequently applied for the treatment of inflammatory skin diseases. The most common adverse effect of topical corticosteroids is cutaneous atrophy which is characterized by a decrease in skin thickness and loss of elasticity causing cutaneous transparency, increased fragility and telangiectasia. On the histological level GCs reduce the size and proliferation of keratinocytes, which results in epidermal thinning. In the dermis they lead to atrophy *via* a reduced synthesis and induced degradation of extracellular matrix components [7,8]. Previous studies have mostly focused on effects of GC on dermal and epidermal compartments and there are only few reports on the influence on SC and barrier integrity. Increased transepidermal water loss (TEWL) has been reported in response to GC treatment [9], which indicates a disturbed skin barrier function [10]. Furthermore, the use of corticosteroids may lead to a decrease in the number of lamellar bodies, and thereby, a decrease in SC lipids [11,9]. Decreases in ceramide, cholesterol, and FFA synthesis were observed in the epidermis of animals and in human keratinocyte cultures treated with GC [10]. GC treatment resulted in abnormalities in permeability barrier homeostasis which were shown to be partly normalized by topical treatment with a mixture of the three key SC lipids [10]. Studies of GC effects on SC lipid composition have so far focused on changes of the main lipid families or on the main ceramide groups. Detailed information on lipid subclasses as well as the lipid chain length distribution in each of the subclasses has not been possible with traditional techniques, such as thin layer chromatography. The advent of high sensitive liquid chromatography–mass spectrometry (LC/MS) methods has allowed detailed lipidomic analysis of biological membranes such as the SC isolated *in vivo* by tape stripping [12]. In this study, we combined an in-depth lipid profiling with functional and morphological analyses of SC in order to gain detailed insights into the biochemical and functional aspects of the SC response to topical GCs.

2. Materials and methods

2.1. Study design

The study was a randomized, explorative, single-center, investigator-blinded, negative-controlled phase 1 trial with intra-individual comparisons. Subjects were recruited according to inclusion criteria from January 29, 2015 to February 27, 2015 at the Charité-Universitätsmedizin Berlin, Department of Dermatology and Allergy with the last subject finished on April 28, 2015.

2.2. Subjects and randomization

16 healthy male subjects were included in the study with a skin phototype I–III according to the Fitzpatrick classification [13]. Informed consent was obtained from all subjects. Prior to the first measurements, randomized assignment to two different measurement groups and treatment assignment to test sites was conducted. The four test sites A, B, C and D (Group 1: 28 cm^2, each; Group 2: 20 cm^2, each) were marked on both lower volar surfaces of the arms, with areas A and B on the right arm and areas C and D on the left arm.

2.3. Treatments

Included subjects were treated over a 28-day treatment period including weekends once daily with approx. 1.7 mg/cm^2 on the marked test sites to clobetasol propionate 0.05% (CP) (Dermoxin® ointment 0.05 %), betamethasone diproprionate 0.064% (BDP) (Diprosis® ointment 0.064 %), and petrolatum ointment (P) (Petrolatum ointment/Vaseline Salbe LAW), the vehicle used for the active treatments, while one test site remained untreated. A computer-generated random list was used for the allocation to the groups and treatments. Sealed randomization envelopes were used for successive randomization. All investigators involved in the study assessments were blinded to all treatment allocations.

2.4. Assessments

Ultrasound (US), confocal laser scanning microscopy (CLSM) and optical coherence tomography (OCT) were used for *in vivo* measurements on the marked areas A, B, C and D at baseline and day 29 (at end of treatment). All observers were blinded to the treatments during the analysis of obtained images and spectra. SC samples for lipid analysis and skin biopsies for gene expression analysis were collected at Day 29 from the four marked areas. To avoid interference, non-invasive assessments and tape-stripping were done at different skin areas but within the marked areas. Biopsies were collected after all other assessment procedures were completed.

2.5. Ethics

Prior to study start, approval by the independent Ethics Committee of the State Office of Health and Social Affairs Berlin (LAGeSo) was obtained. The study was registered at the European Union Drug Regulating Authorities Clinical Trials Database (EudraCT 2014-001450-42) and was conducted according to the Declaration of Helsinki (1996) and Guidelines of Good Clinical Practice. All subjects provided written informed consent.

2.6. Sonographic measurements of skin thickness

For assessment of the skin thinning potential of the corticosteroid ointments the changes in skin thickness in each of the test sites on the volar arms were measured non-invasively using high frequency ultrasound. Measurements were done *in vivo* with DermaScan C (Cortex Technology, Denmark) and a 20 MHz probe.

2.7. Optical coherence tomography (OCT)

Epidermal thickness was assessed *in vivo* using OCT. This non-invasive interferometry-based coherent imaging technique provides vertical images from the skin using the Swept Source OCT System VivoSight® (Michelson Diagnostics, Kent, UK) and a laser wavelength of 1305 nm as a light source. Cross-sectional images