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A breached barrier : analysis of stratum corneum lipids and their role in eczematous patients

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Citation

Smeden, J. van. (2013, June 20). *A breached barrier : analysis of stratum corneum lipids and their role in eczematous patients*. Retrieved from <https://hdl.handle.net/1887/20998>

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Issue Date: 2013-06-20

CHAPTER 2

AIM AND OUTLINE OF THIS THESIS

Background

Human skin forms a protective barrier against penetration of exogenous compounds into the body and prevents excessive transepidermal water loss (TEWL)¹⁻³. This skin barrier function is primarily located in the uppermost layer of the skin, the stratum corneum (SC). Human SC consists of corneocytes embedded in a highly structured lipid matrix. The SC lipid matrix is comprised of ceramides (CERS), free fatty acids (FFAs) and cholesterol (CHOL)^{4,5}. The lipids are crucial for the skin barrier function. In atopic eczema (AE), the skin barrier is impaired, and allergens and irritants can penetrate through the SC into the lower epidermal layers thereby provoking an immune response⁶⁻⁸. Previous studies demonstrated that SC lipid composition in AE patients is changed, although the role of the lipids is not fully understood.

Objectives

The primary aim of this thesis is to study in detail the SC lipid composition and organization as well as their role in the skin barrier function in eczematous patients. This is achieved by pursuing the following challenges:

1. Developing robust methods that enables quantitative analysis of all main SC lipid classes in detail.
2. Determine the comprehensive SC lipid composition of both lesional and non-lesional skin in AE patients and compare the lipid profile to healthy controls.
3. Establish how changes in the SC lipid composition of AE patients are related to changes observed in the lipid organization, and how those affect the skin barrier function.
4. Determine the relationship between SC lipid

composition and organization with respect to other SC sources that show a reduced skin barrier function, i.e. Netherton syndrome (NTS) and human skin equivalents (HSEs).

Outline

This thesis is divided into three parts that will address the aforementioned objectives:

- Part II (Chapters 3-4) covers the development of a novel method for analysis of CHOL, CERs and FFAs, by means of liquid chromatography coupled to mass spectrometry (LC/MS).
- Part III (Chapters 5-8) describes the use of the developed LC/MS methods to examine the SC lipid composition in AE patients and control subjects. The observed changes in the SC lipid composition in skin of AE patients will be related to changes in the lipid organization and reduced skin barrier function.
- Part IV (Chapters 9-10) focuses on the relationship between lipid composition and lipid organization of two different skin sources: SC from NTS patients and SC from HSEs.

Chapter overview

Chapter 3 describes the development of a normal phase LC/MS method for analysis of all CER subclasses and their chain length distribution in human SC. A short validation assay is presented and comparisons are made between *ex-vivo* human SC and SC harvested from tape strips. It also reports on the identification of a new CER subclass by means of fragmentation studies (MS/MS) and high mass accuracy MS.

LC/MS method development continues in **Chapter 4**, in which the analysis of FFAs is described. Reverse phase LC/MS in negative ion mode is used. Method validation shows the robustness, linearity, and sensitivity of the method, and demonstrates the use of chloride adducts for increased ionization efficiency. The FFA method was successively coupled to the method for CER analysis explained in the previous chapter, which was improved to enable analysis of CHOL as well. The combined method was then applied on SC samples from different skin sources: human SC from *ex vivo* skin, human SC harvested by tape stripping, porcine skin, and skin obtained from HSEs.

Part II focuses on the application of the developed LC/MS methods for analysis of the SC lipid in AE patients. **Chapter 5** describes a short study in 6 AE patients and 6 healthy subjects. The CER composition is examined and related to the lamellar lipid organization studied by small-angle x-ray diffraction (SAXD).

In **Chapter 6**, data are presented on the CER composition of non-lesional SC of 28 AE patients and healthy subjects. The CER composition is related to the lamellar lipid

organization (SAXD studies) and lateral lipid organization (Fourier transform infrared spectroscopy (FTIR) studies). In addition, changes in the CER composition and lipid organization were associated to changes in skin barrier function (monitored by TEWL) and disease severity (SCORAD). Finally, the influence of loss-of-function mutations in the flaggrin gene as well as the level of natural moisturizing factor is studied in relation to the SC lipids and skin barrier function.

Chapter 7 reports the continuation on examining the SC in AE, in which both the FFA and CER composition was determined and compared to healthy subjects. In addition, the effect of inflammation on the lipid parameters is discussed, as both lesional and non-lesional skin sites are examined. Changes observed in the SC FFAs and CERs are discussed in relation to the lipid organization and skin barrier function, and finally linked to epidermal lipid metabolism.

In contrast to the previous three chapters, which report on the relative levels of the various lipids in SC lipid in AE patients, **Chapter 8** demonstrates the importance of the lipid/protein ratio with respect to the SC barrier function. This is examined by Raman spectroscopy, whereas the barrier is monitored by TEWL.

Part III reports on SC lipid studies from two different skin sources. **Chapter 9** focuses on the SC lipids in patients with NTS, a severe skin disease in which a mutation in the SPINK5 gene leads to hyperactivity of epidermal proteases resulting in SC detachment. The observed changes in lipid composition are compared to changes in lipid organization. These changes are also discussed in view of the results obtained from AE patients described in the previous chapters.

In **Chapter 10**, data are shown on the lipid composition of SC obtained from HSEs. The lipid composition, examined by the newly developed LC/MS method, is compared to the lipid composition using thin layer chromatography. In addition, the lipid organization and the activity of several enzymes involved in epidermal lipid synthesis are determined and discussed with respect to the lipid composition.

Chapter 11 summarizes the results, presents overall conclusions, and elaborates on the perspectives.

An overview of the main parameters described in this thesis as well as the respective analytical methods used to collect these data are presented in Figure 1: The SC lipid composition, lipid organization, and (to a less extend) lipid amount are the most prominently discussed parameters in relation to the SC barrier function. In addition, the disease severity and flaggrin content are also related to the SC barrier function in several chapters.

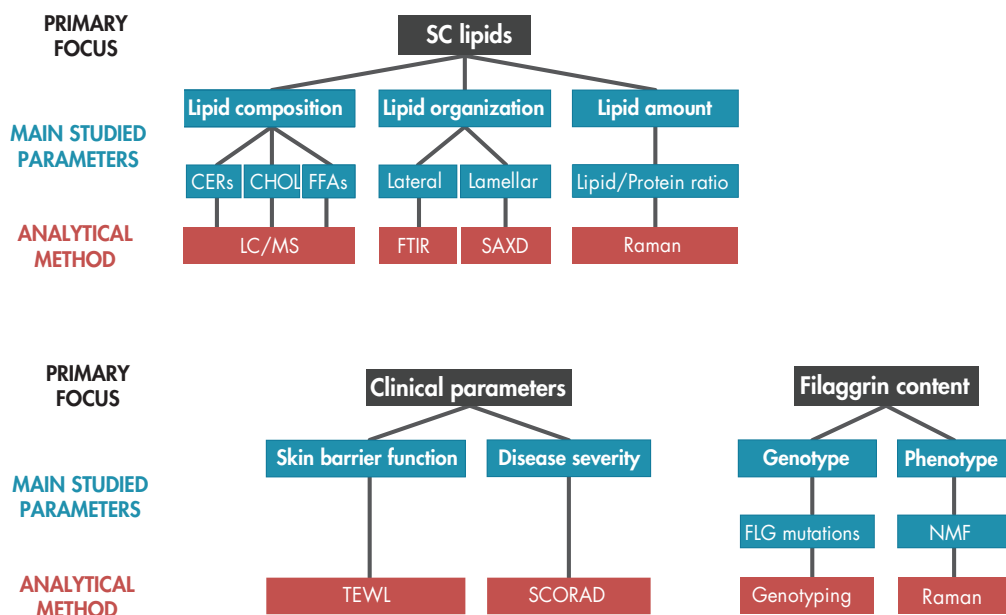


Figure 1: Schematic overview, illustrating the primary focus of the studies described throughout this thesis (in black); the main studied parameters (in blue); and the analytical methods used to examine these parameters (in red).

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