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Review

The important role of stratum corneum lipids for the cutaneous barrier function ☆

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ABSTRACT

The skin protects the body from unwanted influences from the environment as well as excessive water loss. The barrier function of the skin is located in the stratum corneum (SC). The SC consists of corneocytes embedded in a lipid matrix. This lipid matrix is crucial for the lipid skin barrier function. This paper provides an overview of the reported SC lipid composition and organization mainly focusing on healthy and diseased *human* skin. In addition, an overview is provided on the data describing the relation between lipid modulations and the impaired skin barrier function. Finally, the use of in vitro lipid models for a better understanding of the relation between the lipid composition, lipid organization and skin lipid barrier is discussed. This article is part of a Special Issue entitled The Important Role of Lipids in the Epidermis and their Role in the Formation and Maintenance of the Cutaneous Barrier. This article is part of a Special Issue entitled The Important Role of Lipids in the Epidermis and their Role in the Formation and Maintenance of the Cutaneous Barrier. Guest Editors: Kenneth R. Feingold and Peter Elias.

1. Introduction

Two main functions of the skin are to act as an effective barrier against unwanted environmental influences as well as to prevent excessive water loss from the body. The skin consists of the epidermis and dermis as well as the subcutaneous fat tissue [1]. The epidermis is the outermost layer of the skin and consists of four distinctive layers. Each layer displays one of the sequential differentiation stages of the keratinocytes, the major cell type in the epidermis. The layers include the superficial stratum corneum (SC), stratum granulosum (SG), stratum spinosum (SS), and the inner most stratum basale (SB). The SG, SS and SB are part of the viable epidermis (thickness: 50–100 μm), whereas the SC (thickness: 10–20 μm) is part of the non-viable epidermis and the final differentiation product. The SB contains the proliferating keratinocytes. After the keratinocytes escape from the SB, they transiently migrate towards the SC, after which they are finally released from the skin surface, a process called desquamation. During this migration the keratinocytes differentiate: They flatten out and finally adopt the dimensions which are characteristic for the dead cells of the SC, the corneocytes. The keratinocytes in the SG contain a high number of membrane-coating granules referred to as the lamellar bodies (LBs) in which lipids are stored, such as glucosylceramides (GlcCERs), sphingomyelin and phospholipids. These are precursors of the SC lipids, and are enzymatically processed into their final constituents: ceramides (CERs) and free fatty acids (FFAs). CERs and FFAs are, together with cholesterol (CHOL), the main lipid classes in the SC. By means of exocytose, the lipid content of the LBs is released together with hydrolytic enzymes into the intercellular space at the SG/SC interface. Human SC contains 10 to 25 corneocyte layers that are oriented approximately parallel to the skin surface and are embedded in a lipid matrix [2–4]. The structure of the SC is often referred to as a "bricks in mortar" structure, in which the corneocytes are the bricks and the lipids are the mortar [2]. The corneocytes are filled with water and microfibrillar keratin that is surrounded by a cornified envelope which consists of a densely crosslinked layer of proteins such as filaggrin, loricrin and involucrin (see articles by Elias, Rabionet and Radner, elsewhere in this issue). A monolayer of non-polar lipids (ω-hydroxylated CERs and FFAs) referred to as the lipid envelope is esterified to the cornified envelope, mainly to glutamate residues of involucrin [3–6]. This so-called lipid envelope is suggested to form a template for the formation of the intercellular

Abbreviations: AD, atopic dermatitis; CD, Chanarin–Dorfman; CEMOVIS, cryo-electron microscopy of vitreous skin section; CER, ceramide; CHOL, cholesterol; ED, electron diffraction; ELA, elastase; ELOVL, elongation of very long chain fatty acids; FFA, free fatty acid; FLG, filaggrin gene; FTIR, Fourier transform infrared spectroscopy; FTS, full thickness skin; GC, gas chromatography; GLC, gas/liquid chromatography; GlcCER, glucosylceramide; HSE, human skin equivalent; IV, ichthyosis vulgaris; LB, lamellar body; LC, liquid chromatography; LI, lamellar ichthyosis; LPP, long periodicity phase; MS, mass spectrometry; MUFA, mono-unsaturated fatty acid; NTS, Netherton; PUFA, poly-unsaturated fatty acid; PPAR, peroxisome proliferator-activated receptor; SAXD, small angle X-ray diffraction; SB, stratum basale; SC, stratum corneum; SCD, stearoyl CoA desaturase; SCS, stratum corneum substitute; SG, stratum granulosum; SPP, short periodicity phase; SS, stratum spinosum; TAG, triacylglycerides; TEWL, transepidermal water loss; TLC, thin layer chromatography; WAXD, wide angle X-ray diffraction

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lipid layers [7–9] (see article by Breiden in this issue). The cornified envelope, together with the lipid envelope, minimizes the uptake of most substances into the corneocytes and allows proper formation of the lipid matrix. Indeed, a deficient lipid envelope results in a defective skin permeability function and an irregular lipid matrix [10–13]. This lipid matrix acts as the main barrier for diffusion of substances through the skin.

2. Lipid composition and organization in healthy human skin

2.1. Lipid composition in healthy human skin

Human SC lipids consist of FFAs, CERs and CHOL in an approximately equimolar ratio [14]. FFAs and CERs contain respectively 1 and 2 carbon chains that differ in their molecular structure: FFAs are predominantly saturated whereas CERs consist of a sphingoid base and a fatty acid (acyl) chain. Both FFAs and CERs show a wide distribution in their carbon chain lengths. Research on the SC lipid composition goes back as early as 1962, when Reinertson and Wheatley [15] published the chemical composition of human epidermal lipids. He demonstrated the presence of fatty acids and CHOL as well as other (non-specified) lipids that would later on be referred to as sphingolipids [16] and CERs [17]. The level of detail on this lipid composition has improved rapidly.

Regarding the FFAs, in 1970 Ansari et al. reported on the composition of human epidermis by means of gas/liquid chromatography (GLC). For the first time, information on the variation in chain lengths (12–30 carbon atom range) and degree of unsaturation was provided. Besides saturated FFAs, they demonstrated the presence of mono-unsaturated fatty acids (MUFAs), poly-unsaturated fatty acids (PUFAs) and hydroxyl-FFAs [18]. Although at that time they were already able to detect wide distribution in FFA chain length, the level of very long chain FFAs (≥24 carbon atoms) was underestimated. For example, the level of FFA with 24 carbon atoms long was estimated around 13%, whereas current reports indicate values around 30% [19,20]. Also Lampe et al. describe the FFA composition of human SC and shows regional differences in FFA chain length. However, they also overestimate the short chain length FFAs and did not identify FFAs with chains longer than 22 carbon atoms. The overestimation of the level of shorter chain fatty acids may be one of the reasons that other groups select lipid mixtures with short chain FFAs for studying the physical interaction between the main lipid classes [21,22]. This is surprising, as Wertz et al. already reported in 1987 that the FFA composition in human epidermal cysts are mainly 22 and 24 carbon atoms long [23,24], a very important observation. It is not before 1998 that Norlèn et al. extended the knowledge on the FFA composition by introducing a novel gas chromatography coupled to mass spectrometry (GC/MS) method, illustrating that human SC FFAs contain chain lengths as long as 36 carbon atoms, with C18, C24 and C26 being the most prominent FFAs [20]. This FFA composition has been confirmed by Ponec et al. using GC [25] (2002), and recently by our lab using liquid chromatography coupled to MS (LC/MS) [19]. In the latter study it was observed that in healthy human SC the fraction of hydroxy FFAs and MUFAs are low (both around 20%) compared with the saturated FFA, which is in agreement to the first report on the FFAs by Ansari mentioned above [18].

Knowledge on the CER composition in human SC has also rapidly progressed. In 1978 a very important publication of Gray and White demonstrated, by using quantitative thin layer chromatography (TLC) in combination with GLC, that human SC contains several CER subclasses. They reported that the sphingoid base can be a sphingosine or dihydrosphingosine (also referred to as sphingenine and sphinganine, respectively) and the acyl chain can be non-hydroxylated or hydroxylated [26]. In these early days it was already shown that the chain length of in particular the acyl chain varied between 14 and 30 carbon atoms, whereas the sphingoid base was mainly 16–20 carbon atoms long. Wertz et al. extended this knowledge and demonstrated that there are a total of 6 different CER subclasses, mentioning for the first time an ω-hydroxy esterified CER (CER [EOS]) [27], again a crucial observation as CER [EOS] plays an important role in the characteristic properties of the SC lipid matrix.

Hereafter, the CER subclass diversity expanded, and a new nomenclature based on the structure of the individual CER chains was introduced by Motta et al. [28], illustrated in Fig. 1, which is also used in this review. Robson et al. and Stewart et al. introduced a 7th and 8th CER subclass, respectively, using TLC in conjunction with NMR to elucidate the newly identified 6-hydroxy-4-sphingenine base structure, linked to either an α-hydroxy acyl chain (CER [AH]) or non-hydroxy acyl chain (CER [NH]) [29,30]. It took several years until Ponec et al. reported in 2003 the discovery of a 9th subclass, CER [EOP], using a combination of TLC and NMR [25]. The introduction of LC/MS led to the (re) introduction of 3 additional subclasses by Masukawa et al. (CER [NdS], CER [AdS]) [31], and van Smeden et al. (CER [EOdS]) [32]. Note that the former two have been mentioned by Gray and White in 1978 as sphingenine-CERs, but never considered as subclasses and were usually excluded in later papers. These advancements have led to the discovery of a total of 12 CER subclasses that are present in the human SC lipid matrix. As can be observed in Fig. 1, the [EO] subclasses (from here on referred to as acyl-CERs subclasses) possesses a unique structure; these CERs consist of an ω-hydroxy fatty acid chain to which linoleic acid is esterified.

The introduction of LC/MS has led to several improvements. First, its unmatched sensitivity led to the discovery of additional lipid subclasses as well as much smaller sample sizes necessary for lipid quantification, especially important for clinical studies. Second, without cumbersome sample preparation techniques, it is possible to quantify the sample and also obtain structural information thanks to the possibility of fragmentation (MS/MS), all within a very small time frame. Although the analysis of multiple lipid classes at once has been performed using TLC back in the early 80s, the amount of structural information obtained using this technique is limited, and additional NMR or GC studies were necessary to identify the molecular structure (lipid chain lengths and head group architecture). The frequency that LC/MS is used for SC lipid analysis has increased over the last decade, which led to multiple different applicable methods [32–35], greatly enhancing the knowledge on the SC lipid composition. Also in terms of quantification (usually one of the main difficulties of MS) several improvements have been made. Masukawa et al. report on a fully quantitative LC/MS method for CER analysis and demonstrate its similarity to quantification by TLC in two Asian subjects [34] (Table 1). Additionally, lipid profiling of 15 healthy Caucasian subjects using a totally different LC/MS method displayed comparable results [19]. In general, CER [NP] is the most abundant subclass whereas the ultra-long acyl-CER subclasses ([EOdS], [EOS], [EOP], and [EOH]) contribute to a total of around 8–13% of the CERs. By means of fragmentation MS (MS/MS), Masukawa et al. performed a comprehensive analysis on the individual chain lengths (i.e. sphingoid base and acyl chain) of each CER subclass, although they were not able to include MS/MS on the very short chain CERs (<40 carbon atoms) [34]. They reported that the acyl chain of the CERs varies between C14 and C32, whereas the sphingoid base varies in carbon chain length between C14 and C28. The very long sphingoid bases with C24–C28 are mainly detected in CER [NS] and CER [NdS], whereas the very long acyl chains (C28–C32) are mainly detected in the acyl-CERs, but also CER [NS] and [NP] exhibit some very long acyl chains.

2.2. Lipid organization in healthy human skin

2.2.1. Lamellar lipid organization

The intercellular lipid matrix in human SC shows a unique lamellar arrangement. In the seventies it was demonstrated for the first time using freeze fracture electron microscopy that the lipids were organized in lipid sheets [36–38]. The visualization of the lipid stacks using embedded tissue appeared to be very difficult, and no lamellar stacks could be visualized by using OsO_4, which was often used for fixation of lipids. It was not until 1987 with the introduction of RuO_4 post-