

Eczema and ceramides: an update

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Summary

Although normal epidermal barrier function depends on numerous factors, including corneocytes, lipids, enzymes, pH, and calcium gradient, the key players are lipids and proteins in the stratum corneum. Atopic dermatitis is characterized by barrier abnormalities, such as the presence of filaggrin mutations in almost 50% of patients with moderate/severe atopic dermatitis, and lipid disturbances, mainly expressed as insufficient ceramides. In this review, with an emphasis on human studies, we consider the latest research on ceramides, on ceramides in different types of eczema and following various types of treatment. We also consider the genetic influence on stratum corneum lipids. The review is an update on research indexed in PubMed following the discovery of the filaggrin mutations in atopic dermatitis in 2006, but when newer publications cannot stand alone, we include publications from before 2006.

Key words: ceramides; eczema; occlusion and genetics; treatment.

Intact skin barrier function is essential to protect the body against dehydration and external hazards. The barrier function of the skin is located in the stratum corneum (SC), which consists of corneocytes embedded in lipids (1, 2).

The lipid bilayers of the SC consist predominantly of three different lipids: ceramides, cholesterol, and free fatty acids. The ceramides are further divided into 12 subspecies (ceramides 1–12) (3), and are thought to be critical in the organization of the lipid bilayer (4). The synthesis of the lipids takes place in the stratum granulosum, from where the lipids are delivered to the SC (5). The lipids surround the corneocytes, which are flat nucleus-free cells built of keratin filaments and surrounded by cross-linked proteins called the cornified envelope (6).

In healthy skin, the synthesis of lipids and the differentiation of keratinocytes are linked in a sophisticated

symphony regulated by enzymes, pH, and calcium gradient (6). Different enzymes are involved in the synthesis of ceramides, and they all have slightly different optimum pH values, theoretically rendering them highly susceptible to any changes in pH, irrespective of whether the changes come from the internal or external environment (7). The precise role of the calcium gradient in the ceramide composition of the SC is unknown, and needs further investigation.

The concentration of ceramides in healthy individuals differs according to anatomical location and environmental influences, such as humidity and ultraviolet (UV) exposure. During the winter, decreased levels are found in skin exposed to low humidity, for example the face (8). The fact that the lipid profile varies according to the conditions indicates that some ceramide levels and ceramide/cholesterol ratios are more favourable for barrier function than others. The evidence points to high levels of ceramides 1 and 3 and a high ceramide/cholesterol ratio as being favourable (7). This is supported by *in vitro* data showing that ceramide 1 is of special importance for the integrity of SC lipid bilayers, probably because of its very long carbon chain length (9). It has also been shown that the ceramide/cholesterol ratio is important for the structural arrangement of the SC lipids (10).

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Further division of the ceramide subgroups into > 100 different molecules according to the chain length is now possible (11, 12). The clinical relevance of chain length has been examined in only one human study, and the evidence points towards a positive influence of long chain length on the barrier function as evaluated by transepidermal water loss (TEWL) (13). This is supported by data generated from porcine skin (14).

The objective of this review is to summarize new findings from 'the post-filaggrin discovery period' of 2006 regarding SC lipids, with a focus on human clinical experimental research, eczema, and treatment regimens (15). We searched PubMed using the term stratum corneum ceramides, giving critical preference to human studies from 2006 and onwards whenever possible.

Skin Lipids in Various Forms of Eczema

Atopic dermatitis

Atopic dermatitis is the disease that initially highlighted the ceramide profile (16, 17). Comparisons of SC ceramides in healthy skin and atopic dermatitis skin were made by different groups in the 1990s, and showed lower levels of ceramides 1 and 3, as well as a lower ceramide/cholesterol ratio, for non-lesional atopic skin (16–20). Today, the loss of function mutation in the gene encoding filaggrin has made it possible to subdivide both atopic dermatitis patients and healthy controls in relation to filaggrin status. Ceramide status could be expected to differ between atopic dermatitis patients with and without filaggrin mutations, as breakdown products of filaggrin contribute to the acidic pH of the surface SC and thereby change the pH. The enzymes involved in SC lipid synthesis have different optimum pH values. Ceramides 1 and 4 both belong to the acylceramides, and are generated by the enzyme glucosylceramide deacylase, with a pH optimum near 5 (21, 22), rather than the pH optimum of 8 for cholesterol sulfatase, which is involved in cholesterol synthesis (23). The desquamation-enhancing serine proteases have a near neutral pH optimum (24). However, two recent studies subdivided atopic dermatitis patients according to their filaggrin status, and found no significant differences for the SC lipids regarding filaggrin status (25, 26). A difference would have been expected, owing to the aforementioned differences in enzyme pH optima, especially as the study by Jungersted et al. found significantly higher pH levels in the filaggrin-deficient groups. This indicates that skin surface pH, as measured in most studies, does not necessarily correlate with pH levels in the deeper layers of the skin, where lipid synthesis occurs (25). In continuation of the filaggrin

discovery, one research group studied atopic dermatitis skin, excluding patients with filaggrin mutations to ensure that the discoveries made were independent of the mutations, and correlated the ceramide composition with the lamellar lipid organization. They found significantly lower levels of ceramide 3 in atopic dermatitis individuals than in healthy controls, as well as a correlation between a low ceramide 3 level and lamellar lipid disorganization, despite the presence of wild-type filaggrin in both groups (27).

Other types of eczema

Little information is available for other types of eczema, and data have been obtained from single studies and case observations. The lipid profile in sensitive skin as compared with healthy skin has recently been examined. Ceramide levels in different body sites were analysed, and no differences were seen. However, the study highlighted the problem of the definition of sensitive skin being unclear (28).

Observations on skin barrier function in other diseases may facilitate understanding of the pathophysiology in eczema. Few reports on rare genetic eczematous diseases exist. Anhidrotic ectodermal dysplasia and Sjögren–Larsson syndrome are both characterized by dry skin and an impaired skin barrier. Anhidrotic ectodermal dysplasia patients have been compared with atopic dermatitis patients with regard to SC lipids; a significantly higher level of ceramide 1 was found in anhidrotic ectodermal dysplasia patients. The low ceramide 1 level in atopic dermatitis patients as compared with those with healthy skin suggests that the lipids of individuals with anhidrotic ectodermal dysplasia resemble those of healthy skin (29). In contrast, a report involving 1 patient compared ceramides in patients with the neurocutaneous disorder Sjögren–Larsson syndrome with those in healthy skin, and lower levels of ceramides 1, 6 and 7 were found in diseased skin (30).

Other skin diseases in which a different ceramide profile is expected are the rare genetic disorders harlequin ichthyosis, Niemann–Pick disease, and Gaucher's disease, where abnormalities in keratinization and/or calcium levels constitute part of the disease. However, although the theoretical reasons for ceramide deficiency are known, it has been examined only in a study on Gaucher 2 disease, where the SC ceramides were found to differ from those in healthy human skin (31–34).

Treatment

The skin as an organ has the advantage of being easily accessible for topical treatment, which is frequently used

in dermatology. The main topical treatments used for eczematous diseases are: moisturizers, corticosteroids, and, more recently, calcineurin inhibitors.

Moisturizers

Moisturizers are widely used by the public for both diseased skin and for cosmetic purposes, and still constitute the basic treatment for all eczematous diseases. Although moisturizers can contribute to the restoration of the barrier of eczematous skin (35, 36), the effect on intact skin is more debatable, as is the effect of moisturizers used as barrier creams against external exposure (37–39).

Despite the abundance of moisturizers, research comparing different combinations of the ingredients is scarce, and also difficult, as the list of ingredients in each moisturizer is long. One study in which different moisturizers were compared for their impact on SC ultrastructure and lipid organization concluded that they do not change the organization of the SC much, but form separate domains in the SC (40).

The ceramide composition of atopic dermatitis skin differs from that of healthy skin, with decreased ceramide 1 and 3 levels. It has been thought that adding ceramide to moisturizers, or making SC lipid-like moisturizers, might benefit atopic dermatitis skin. However, a recent study examining the effects of three different moisturizers, one of which was a ceramide-dominant cream, found no significant difference in disease severity with moisturizer as monotherapy for mild-to-moderate atopic dermatitis (41).

Another interesting aspect of moisturizers is the influence of the different ingredients and their impact on upregulation or downregulation of ceramide synthesis. It has been shown that, depending on the specific moisturizer used, levels of mRNA expression for enzymes involved in skin barrier lipids change (42, 43). Some of the seemingly relevant ingredients are urea, canola oil, ammonium lactate, and linoleic acid. However, more research is needed before making specific recommendations on different ingredients (42–44).

Topical corticosteroids

Topical corticosteroids have been used in dermatology since the 1950s, and remain the first-line treatment for most eczematous diseases. Their effects are primarily anti-inflammatory. There is good evidence that moisturizers and topical corticosteroids work well together (45). Nevertheless, long-term use of corticosteroids leads to atrophy and thinning of the epidermis. However, barrier function is mainly impeded by inhibition of epidermal

lipid synthesis (46–48). One group investigated the effect of corticosteroids on mice ($n = 4$) and on *in vitro* human keratinocytes ($n = 5$) after 3 days of treatment (46), and found a decrease in both total ceramide and cholesterol content. However, no differentiation was made between the ceramide subgroups, and no ceramide/cholesterol ratio was given. Another group found inconsistency in the lipid bilayers after treatment with betamethasone (49). The negative influence of a topical corticosteroid has recently been shown by a reduction in the expression of enzymes involved in lipid synthesis (50). This is in contrast with a human study, where the effect of topical corticosteroids was compared with emollient treatment, and a statistically significantly higher ceramide/cholesterol ratio for corticosteroid-treated skin than for emollient-treated skin was found. An increased ceramide/cholesterol ratio is seen as a barrier-positive increase, on the basis of the decreased ratio found in atopic dermatitis (18). However, no significant changes in ceramide subgroups were found in corticosteroid-treated skin (51).

Topical calcineurin inhibitors

During the last decade, two new topical drugs have been introduced to treat atopic dermatitis: tacrolimus and pimecrolimus. These are calcineurin inhibitors without the side-effects of topical corticosteroids regarding skin atrophy. The impact of tacrolimus on barrier function, and especially the lipids, has been examined in two studies (51, 52). The study by Paslin et al. indicated a decrease in the ceramide 4 level after treatment of patients with atopic dermatitis, but showed no effect on the overall ceramide/cholesterol ratio (52). The change in ceramide 4 was not confirmed in the other study, which found no differences in ceramide subgroups between tacrolimus-treated skin and skin treated with a moisturizer. Nonetheless, a higher ceramide/cholesterol ratio was found in tacrolimus-treated skin (51).

Effect of systemic treatment

Data on systemic treatment regimens and skin barrier function are rare, and only the effects of corticosteroids and alitretinoin have been studied systematically.

In the study by Kao et al. mentioned in the previous section on topical corticosteroids, the effect of systemic corticosteroids on the barrier function of murine skin was studied. The data suggested a negative effect on barrier function after 1 day of systemic treatment, assessed as the response of TEWL following tape stripping (46).

The effects of systemic alitretinoin on barrier function and ceramides in a human study were reported not to

significantly influence the SC lipids. The study involved hand eczema patients following 2 months of treatment with alitretinoin (53).

UV treatment

UV treatment is another treatment option that is often used for a broad range of eczematous diseases.

The effect of UV light is multifactorial and not fully understood. UV treatment increases the thickness of the SC; UVA induces T lymphocyte apoptosis; and UVB decreases the number of skin surface bacteria on the skin surface. *In vitro* studies have also indicated an increase in the synthesis of ceramides after UV radiation (54). One group has shown an increase in SC lipids per square centimetre as a response to UV light (55). However, as the study did not account for UV light increasing the SC thickness, the result is difficult to interpret. Another group recently found no convincing differences in the ceramide profile following 18 UV treatments of human skin (56). The effect of the thickened SC could explain the decreased susceptibility of the skin to irritants found after UV treatment (55–57).

Chemical Irritation or Experimentally Induced Eczema

The skin is exposed to many chemical irritants in both work and domestic settings. In experimental research, the standardized irritant normally used is sodium lauryl sulfate (SLS), which can be used in Finn Chambers® or as a repeated open application test (ROAT). The use of SLS in Finn Chambers® produces a standardized irritation, whereas the ROAT better resembles the daily exposure to soap. SLS is a corrosive irritant, and application therefore results in impairment of skin barrier function. Nonanoic acid, another irritant that is used experimentally, differs from SLS in being a non-corrosive irritant; consequently, it impairs the skin barrier only slightly (58, 59).

It has been shown that both short-term and longer-term use of alcohol-based disinfectants irritates the skin far less than detergents do (60–62).

The effect of irritants on the production of SC lipids has not been examined. It has been shown that SLS affects the SC by increasing intercellular oedema and altering the parallel arrangement of the lipid bilayers, rather than by depleting the lipids (63). The effect of alcohol-based disinfection on SC lipids has not been examined. A high concentration of alcohol removes water from the skin, but as the procedure of applying alcohol disinfection does not remove any substances from the

skin (nothing is washed off), it is thought not to influence the SC lipids.

Occlusion on Healthy and Diseased Skin

The role of occlusion in dermatology is variable. Although occlusion is a risk factor for developing irritant contact dermatitis, as seen clinically when occlusive gloves are used in the healthcare sector (64–66), it is used to promote skin healing (67, 68). The effects of occlusion on skin are multiple, and influence parameters such as temperature, moisture, pH, and desiccation (69, 70). Occlusion promotes moisturization of the skin and increases the pH of the surface SC (71, 72), probably influencing the penetration of chemical substances through the skin. However, how these parameters interact and affect the SC lipids and other parameters of the skin barrier has only recently been investigated. The increased pH following occlusion is expected to influence the level and composition of the lipids, as previously explained (section on eczematous skin diseases). A study found that 1 week of occlusion did not influence the levels of ceramide subgroups or the ceramide/cholesterol ratio (73). An explanation could be that the 1-week occlusion was too short to show enzymatic changes. Another explanation could be that the increased pH was on the SC surface, and lipid synthesis takes place at the SC–stratum granulosum interface; therefore, it might not be influenced by the surface pH.

Genetics

A possible relationship between filaggrin mutations and SC lipids has been evaluated in two studies, by examining atopic dermatitis patients and healthy individuals with and without filaggrin mutations. No statistically significant differences between the groups were found for total ceramide levels or specific ceramide groups (25, 26).

Filaggrin mutations differ between different ethnic groups (74). It has been the general opinion that the prevalence of eczema, and especially atopic dermatitis, is higher in industrialized countries. However, the newest International Study of Asthma and Allergies in Childhood (ISAAC) cohort data challenge this opinion, by showing that the highest prevalence of atopic dermatitis is now on the African continent, and the lowest prevalence is in Asia (75). Whether these differences can be ascribed to the environment or genetics is unknown. Experimental data on barrier function between ethnicities differ. For pH, two studies suggested a higher pH in white individuals, and one study suggested the opposite, with a higher

skin surface pH found in black individuals (76–78). To overcome environmental differences, a study was undertaken examining different ethnicities living in the same city. It was found that the ceramide/cholesterol ratio differed significantly between ethnicities, with Africans showing the lowest level, Caucasians a medium level, and Asians the highest level (79). Interestingly, this reflects the same pattern as found for the distribution of atopic dermatitis in the ISAAC cohort (75).

Conclusion

The research of recent years focusing on the skin barrier has provided new data on factors important for epidermal barrier function. Key players in the barrier are proteins and lipids, especially ceramides, but there

is no evidence of a link between filaggrin and ceramide production. However, genetic factors other than filaggrin may influence ceramide production, which has been reported to differ between ethnic groups. The barrier response to irritant exposures differs from one irritation regimen to another, and the effect on the barrier of topical regimens known to improve barrier function is not homogeneous. The data presented advance our understanding of the processes in the SC involved in barrier function, but also indicate a complex situation that is dependent on internal and external factors, all of which influence barrier function.

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