

Human epidermal lipids

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Abstract - Human epidermis is a very heterogeneous tissue and this is reflected in its lipid composition. The lower layers contain typical membrane lipids, but lipid synthesis and catabolism continue throughout the full thickness of the epidermis until, in the stratum corneum, most of the lipid consists of ceramide, cholesterol and free fatty acid; the lipid is present in the form of multiple layers between the stratum corneum cells where it forms a barrier to water and water-soluble compounds.

INTRODUCTION

Mammalian epidermis is a veritable gold-mine of lipids, containing many species which are found rarely, if at all, in other tissues. The organization of these lipids is unusual too: for example, epidermis contains membranes devoid of phospholipid. The most interesting and the most studied part of the epidermis is the stratum corneum, "the raison d'être of the epidermis" (ref. 1), and this review will be largely concerned with the formation, composition, organization and physical properties of this structure. Several good reviews of this field have appeared in recent years (refs. 2-10).

LIPID COMPOSITION OF EPIDERMIS

In a classic paper, Long (ref. 11) cut cow snout epidermis into six horizontal slices and analysed each slice for phospholipid, triacylglycerol, cholesterol and free fatty acid. Phospholipid, which was the most abundant lipid in the lowest part of the epidermis, was virtually absent from the stratum corneum. The other lipids that were assayed increased progressively in concentration in the outer layers. Long used cow snout epidermis for technical reasons: a similar study on human epidermis produced similar results (ref. 12). Epidermis can also be fractionated by sequentially removing cells from the underside of the epidermis with a protease (ref. 13). In an extension of this method, trypsin was used to remove the lower part of the epidermis and proteinase K was used to remove the upper part of the stratum corneum, yielding a preparation of stratum compactum (ref. 14). Superficial stratum corneum cells can also be removed by rubbing or brushing (ref. 15). In parallel with these mechanical methods, Elias and colleagues have used histochemical and cytochemical methods to identify and locate epidermal lipids (refs. 16-17).

The results of these various investigations can be summarized as follows. The basal cells of the epidermis have a complex lipid composition, characteristic of cells containing a full complement of subcellular organelles: phospholipid accounts for about two thirds by weight of the lipid present, with cholesterol, triacylglycerol, free fatty acid and glucosyl ceramide accounting for most of the remainder. In complete contrast, half of the lipid present in the outer layers of the stratum corneum consists of ceramide with the remainder being accounted for mainly by cholesterol and free fatty acid: phospholipid is completely absent. The change in lipid composition from the basal layer to the stratum corneum is not uniform: some lipids increase in concentration only to disappear again. One interesting example is phosphatidyl-(N-acyl)-ethanolamine. This unusual phospholipid accounts for less than 5% of phospholipid in the basal layer, for 25% in the granular layer (ref. 18) and is absent in the stratum corneum. It has been found in BHK cells (ref. 19) and in infarcted myocardium (ref. 20). The very high proportion of palmitic acid found in amide linkage with the ethanolamine moiety suggests that the phospholipid may be a precursor of palmitoyl ethanolamide, a compound reported to have anti-inflammatory properties (refs. 21,22). Epidermis may, therefore, contain an endogenous anti-inflammatory agent. A second lipid which waxes and wanes is glucosyl ceramide: the removal of the glucosyl group from this sphingolipid is thought to be an important step in the terminal differentiation of the epidermal cell (ref. 23). A third lipid that disappears as the surface of the stratum corneum is reached is cholesteryl sulphate. This lipid, which has been implicated in stratum corneum cell cohesion, is not broken down in patients suffering from recessive X-linked ichthyosis due to a deficiency of the enzyme steroid sulphatase (for review see ref. 24).

LIPID CONTENT OF EPIDERMAL CELLS

In 1964, Kligman (ref. 1) made the pregnant observation that whereas the stratum corneum weighs about the same as the viable part of the epidermis, it contains considerably fewer cells. This implies that a stratum corneum cell weighs much more than a viable cell. In 1975, using a count and weigh method, it was shown that the mean weight of a basal/spinous cell was 276 pg whereas the mean weight of a granular cell was 770 pg (ref. 13). In the following year, using a scanning microinterferometer, Yardley and Goldstein (ref. 25) showed that a typical human basal epidermal cell weighs about 100 pg, whereas a desquamated stratum corneum cell weighs up to 600 pg. Since the lipid concentration stays constant at about 10% of the dry weight, it is possible to compute the weight of each lipid component in each cell type. For example, a basal cell contains approx. 0.1 pg ceramide whereas a fully cornified stratum corneum cell contains approx. 30 pg ceramide. Similarly, a basal cell contains approx. 1 pg cholesterol, but this has increased to approx. 12 pg in a stratum corneum cell. These changes are not brought about by rearrangement of lipid components, since the total mass of lipid increases from approx. 10pg per cell to approx. 60 pg per cell. Rather, these observations demonstrate that lipid synthesis (and protein synthesis for that matter) takes place throughout the whole thickness of the epidermis. Recently, (ref. 26) lipid synthesis in the stratum granulosum has been demonstrated directly; indeed, the rate of incorporation of substrate into lipid was greater in the stratum granulosum than in the stratum basale/stratum spinosum.

ORGANIZATION OF EPIDERMAL LIPID

In the lower part of the epidermis, lipids are found almost exclusively in cellular membranes and this is reflected in the predominance of phospholipid and cholesterol. In the stratum corneum, there are membranes - but no phospholipid. For many years, this presented a problem, since it was thought that phospholipids were essential components of biological membranes. In 1970, Weinstock and Wilgram (ref. 27) suggested that glycolipids could substitute for phospholipids, but it was not until 1979 that Gray and White (ref. 28) showed that liposomes could be made without phospholipid. It is a commentary on the thinking of the time that the late Dr Gray could not get his manuscript accepted by a reputable biochemical journal: this explains why this seminal paper appears as an unrefereed publication. Dr Gray's discovery has been abundantly confirmed since by Downing and his co-workers (refs. 29-32): his group has shown that small unilamellar liposomes can be made from a mixture of ceramide, cholesterol, palmitic acid and cholesteryl sulphate (ref. 31). These unilamellar structures can be made to flatten and fuse by the addition of epidermal acylceramides and/or acylglucosylceramides (ref. 32).

Epidermal acylceramide is a remarkable lipid: it consists of a sphingosine base in amide linkage with a very long ω -hydroxy fatty acid. In human epidermis, this ω -hydroxy fatty acid usually contains 30 carbon atoms and is saturated. To the ω -hydroxy group, a nonhydroxy fatty acid is joined via an ester linkage: a high proportion of this ester-linked fatty acid is normally linoleic acid (ref. 33). Epidermal acylglucosylceramide consists of acylceramide described above with β -D-glucose in glycosidic linkage with carbon atom 1 of the sphingosine moiety (ref. 34). It should be noted that erroneous structures for epidermal acylglucosylceramide have been previously reported (refs. 35,36). Epidermal acylceramide and acylglucosylceramide are extremely long molecules: the ω -hydroxyacid alone is long enough to span a typical lipid bilayer and might tend to make the bilayer more stable (ref. 36). In addition, it has been proposed that the other fatty acid linked to the ω -hydroxyl group can interdigitate with another lipid bilayer, thus suggesting a mechanism for the fusion of lipid bilayers seen when epidermal acylceramides are added to unilamellar liposomes (ref. 36). For this reason, Downing has coined the phrase "molecular rivets" to describe the function of acylceramides and acylglucosylceramides (ref. 31). However, another school of thought suggests that linoleic acid in acylceramides has a chemical rather than a physical function (see below and ref. 37).

The multilamellar structure revealed by electron microscopy in liposomes made from stratum corneum lipids is also seen in the intercellular spaces of stratum corneum. These multilamellar structures originate in distinctive subcellular structures variously known as lamellar bodies, membrane-coating granules, Odland bodies, keratinosomes and cementosomes. As cornification proceeds, these lamellar bodies move to the plasma membrane of granular cells, fuse with the membrane and discharge their contents into the intercellular space. In addition to the "molecular rivets" that are thought to fasten the lipid bilayers together, Downing et al. (ref. 38) has produced evidence that ω -hydroxyacylsphingosines are ester-linked to γ -glutamyl residues on the surface protein of stratum corneum cells. The stratum corneum, then, can be likened to a brick wall (ref. 5) with the bricks representing the corneocytes and the mortar representing the intercellular lipid. It is thought that the "mortar" plays a critical role both in water barrier function and in desquamation.

EPIDERMAL BARRIER LIPIDS

If the stratum corneum is the *raison d'être* of the epidermis, then the *raison d'être* of the stratum corneum is the epidermal barrier. It is this barrier that keeps water in the body and harmful substances out. Ideas on the location and nature of the barrier, well reviewed by Elias and Feingold (ref. 8), have undergone great changes in the last two decades. It is now believed that the barrier is located in the stratum compactum (or stratum lucidum) in the lower part of the stratum corneum: this has been demonstrated by injecting water soluble substances into the dermis and observing their limit of travel. Stratum compactum has been prepared (ref. 39) by treating human epidermis successively with trypsin and proteinase K; the resulting preparation was about 5 μm thick and contained 5 - 7 layers of flattened cells with the intercellular space filled with layers of lipid.

The nature of the "barrier lipid" has received much attention in recent years. Sphingolipids have been popular candidates (ref. 40) for many reasons, one of which is that they contain very long, highly saturated fatty acids. The hydrocarbon chains of these saturated fatty acids would be expected to pack closely together and present a barrier to water molecules, unlike a normal biological membrane which contains many unsaturated fatty acids which do not pack closely together and do not present an appreciable barrier to water (ref. 41). A second reason for the popularity of sphingolipids as the barrier lipid has to do with its content of linoleic acid: as described above, this essential fatty acid is often esterified to the ω -hydroxyl group of epidermal ceramides and deficiency of linoleic acid in the diet leads to increased water loss through the skin (ref. 42). The literature on linoleic acid and epidermal water permeability (well reviewed in ref. 9) is conflicting and confusing. Houtsmuller and Beek (ref. 43) have shown that any n-6,9 fatty acid will substitute for linoleic acid in restoring the epidermal water barrier and it has been suggested (ref. 37) that linoleic acid is important, not because of its structural properties, but because it possesses the stereochemical configuration necessary for hydroxylation by a specific lipoxygenase to monohydroxyoctadecadienoic acid and trihydroxyoctadecenoic acid with concomitant production of peroxides which may activate enzymes involved in the cornification process.

In the last five years, Elias's group have looked at other putative epidermal barrier lipids. Their approach (reviewed in ref. 8) has been to damage the barrier by applying a lipid solvent, and then follow lipid biosynthesis in the recovering epidermis: damage to the barrier was followed by a burst of sterol and fatty acid biosynthesis. Their most recent work (refs. 44,45) has implicated the water molecule itself as the mediator of barrier lipid synthesis, because application of an occlusive membrane to damaged epidermis reduced the the expected increased rate of lipid biosynthesis. To further confuse the issue, it has (ref. 37) been suggested that low molecular weight polar and hydrophilic compounds play an important role in the maintenance of the epidermal permeability barrier, but discussion of this area is beyond the scope of this paper.

LIPIDS AND DESQUAMATION

At one time, it was thought that degradation of intercellular lipid led to desquamation of stratum corneum cells. Certainly, retention of cholesteryl sulphate in the intercellular lipid leads to pathological retention of stratum corneum (ref. 24), but this does not mean that hydrolysis of cholesteryl sulphate is the only mechanism at work in desquamation. The theory that stratum corneum is held together by layers of lipid received a setback when it was shown that desquamated stratum corneum cells retain several layers of lipid (ref. 46). A further blow to the theory came when it was shown (ref. 47) that extraction of stratum corneum intercellular lipids with chloroform methanol mixtures resulted in a preparation that was more resistant to disruption by detergents: this and related observations have led to the hypothesis that stratum corneum cells are held together by interdigitation of the envelope lipids that are covalently bound to the surfaces of stratum corneum cells.

CONCLUSION

As well as the lipids commonly found in biological tissues, mammalian epidermis is a rich source of lipids found almost nowhere else, including N-palmitoyl phosphatidylethanolamine and cholesteryl sulphate, both of which disappear before desquamation. It also contains unusual ceramides that contain very long fatty acids, some of which are esterified to essential fatty acids via a ω -hydroxyl group: these acylceramides can link together lipid bilayers to form multilamellar structures, both in vivo and in vitro. Their presence between stratum corneum cells in vivo has helped to explain the observed water barrier properties of epidermis and in particular, how it reacts to treatment with lipid solvents and detergents. To date, the multilamellar structures which can be made in vitro have not been exploited, but it remains entirely possible that they will find specialized applications, perhaps in drug delivery.

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