Ceramides from Wool Wax

Sir:

Lanolin is a natural product with a complex and variable composition that is obtained by refining and purifying wool wax. Chemically, lanolin is composed mainly of mono- and diester waxes (75–90%) composed of (i) branched and unbranched FA as well as α- and ω-hydroxy FA and (ii) branched and unbranched alkanols, alkane-1,2-diols, sterols, and triterpene alcohols. Minor amounts of FFA (1–8%), free fatty alcohols and sterols (6–12%), and other more polar compounds, allegedly phospholipids (1), are also present (2). However, no ceramides have been reported in lanolin or wool wax to date.

The unique properties of lanolin have been valued for ages (2). Recent works comparing lanolin with stratum corneum (SC) lipids have found an interesting array of both physical and chemical similarities (3). Physically, both lanolin and SC lipids exist in liquid and solid phases at skin temperature. Both systems also tend to form liquid crystalline structures and multilamellar vesicles. Chemically, both comprise cholesteryl derivatives, cholesterol and other free sterols, free and esterified FA, wax esters, and hydrocarbons, even though their proportions are very different and the ceramides seem to be absent. Despite the inclusion of lanolin and its fractionated products in a variety of topical products, little is known about its effects on barrier function.

At least seven different families of ceramides, differing in headgroup structure and FA composition, have been found in the skin and hair of mammals. These fatty amides of sphingosine or phytosphingosine (4) are an important component in the outermost layer of the skin, the SC. They are embedded within an extracellular lipid matrix together with other lipids such as cholesterol, FFA, and cholesteryl sulfate (5) forming highly ordered lamellar lipid bilayers.

The ceramides play an essential role in maintaining and structuring the lipid barrier, which affords protection against external insults and water loss through the skin; thus, ceramides are directly related to the water-retaining properties of the skin (6–8). Extensive efforts have been made by the cosmetics and pharmaceutical industries to obtain human skin-identical ceramides. Nevertheless, given the extensive use nowadays of ceramides in the production of cosmetic and skin care products, it is necessary to find new natural extracts rich in ceramides similar to those in human skin from accessible and cheap materials. In this regard, internal wool lipids have been demonstrated to have a high ceramide content with a composition similar to that found in human SC (9). Different methodologies to extract internal wool lipids and their use in products for skin care and treatment have been described (9,10). These lipids, structured as liposomes, reinforce the barrier function and increase the water-holding capacity of healthy skin and accelerate recovery of water barrier functions of disturbed skin (10,11). Given that these ceramides have a natural origin similar to that present in the skin (both wool and SC are keratinized tissues), they enjoy a better acceptance than products of synthetic origin.

This work sought to determine the presence of ceramides in wool wax, not only to explain its effect as a skin repair product but also to be a new source of natural ceramides suitable for designing new pharmaceutical or cosmetic products for skin care and treatment.

Wool wax, industrially obtained by washing wool with detergents and diluted sodium carbonate solutions, was supplied by SAIPEL (Terrassa, Spain). A given amount of wool wax (between 20 and 60 g) was dissolved in 1 L of *n*-hexane. This solution was washed four times with 250 mL of methanol or 4:1 methanol/water using a separatory funnel with vigorous mixing. The nonpolar (Wool Wax Hexane fraction) and polar (Wool Wax MeOH fraction and Wool Wax MeOH/H_2O fraction) fractions were collected and evaporated to dryness.

TLC coupled with automated FID (Iatroscan MK-5 analyzer; Iatron, Tokyo, Japan) was used for quantitative analysis because it affords rapid separation and precise quantification without sample pretreatment (12). The eluents used had previously been optimized for analysis of ceramides from wool lipids (9). The samples analyzed were: Wool Wax; the two polar fractions (Wool Wax MeOH fraction and Wool Wax MeOH/H_2O fraction); purified anhydrous lanolin (Stellux AI); lanolin oil (Stellanol) supplied by Lanolines Stella (Mouscron, France); and lanolin oil (Lanor Crystal) and lanolin alcohols (Lanor A) supplied by Lanolines de la Tossée (Turgoing, France). Samples (15 μg) were spotted on Silica gel S-III Chromarods using an SES (Nieder-Olm, Germany) 3202/15-01 sample spotter. The rods were initially eluted to a distance of 10 cm with *n*-hexane/diethyl ether/formic acid (53:17:0.3, by vol) to separate polar and nonpolar lipids. A partial scan of 85% was carried out to quantify and eliminate the nonpolar lipids. Then a second development, again to a distance of 10 cm, was performed with chloroform/*n*-hexane/methanol/acetone (55:5:3:7, by vol). Thus, a suitable separation and quantification of the ceramides and other polar lipids were achieved (9). This procedure was also applied to palmitic acid and cholesterol standards from Merck (Darmstadt, Germany); type II and type IV ceramides, 7OH-cholesterol, galactoceramides, and cholesterol sulfate from Sigma (St. Louis, MO); and type III and type VI(II) ceramides from Cosmoferm (Delft, The Netherlands) to determine their calibration curves for quantification of each compound.

Lipids were quantified not only in samples of wool wax but also in lanolin, its refined and purified fraction, and its most important derivatives, lanolin oil (the liquid ester fraction of lanolin) and lanolin alcohols (the unsaponifiable fraction of lanolin) (2).

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TABLE 1
Analysis of Wool Wax and Lanolin Fractions (in percentage) by TLC/FID[a]

	Wool wax	Lanolin (Stellux)	Lanolin oil (stellanol)	Lanolin oil (Lanor Crys.)	Lanolin alcohol (Lnor A)	Wool wax (MeOH fr.)	Wool wax ($MeOH/H_2O$/Fr.)
Waxes	48.4	54.4	43.6	48.9	1.8	34.5	3.2
FA	10.3	12.7	17.9	20.7	10.0	11.7	2.3
Fatty alcohols	4.4	6.1	4.4	5.8	43.9	14.6	1.4
Sterols	2.6	3.4	3.4	3.9	30.6	6.3	8.2
Ceramide II	1.1	0.4	0.5	0.5	0.0	1.7	5.7
7OH-Cholesterol	1.3	1.1	1.2	1.3	4.4	2.4	6.0
Ceramide III/IV	1.0	0.8	0.8	0.9	1.2	1.8	5.3
Ceramide VI (b)	1.7	1.0	1.1	1.2	1.3	2.3	4.8
Cerebrosides	2.6	2.2	2.4	3.1	2.5	4.1	5.9
% Lipids analyzed	73.3	82.1	72.3	86.3	95.6	79.3	42.8
% Ceramides	3.7	2.3	2.4	2.6	2.5	5.8	15.8

[a]Stellux and Stellanol were supplied by Lanolines Stella (Mouscron, France); Lanor Crystal and Lanor A, by Lanolines de la Tossée (Turgoing, France).

The Wool Wax polar fractions, i.e., MeOH and $MeOH/H_2O$ fractions, accounted for 18 and 2% of the total initial wool wax, respectively. Analytical results are presented in Table 1 as the percentage of lipid identified and analyzed compared with the total amount of lipid determined. The remaining lipids correspond to cholesterol derivatives, some unidentified compounds, polar compounds, protein fractions, and so on.

Industrial wool wax was found to contain ceramides in relatively low proportions (<4% by weight) (Table 1). Commercially available lanolin and its derivatives, lanolin oil and lanolin alcohols, also contain ceramides but in even lower concentrations (<3% by weight). The polar lanolin fractions contain a markedly higher ceramide content than wool wax, lanolin, and lanolin derivative commercial fractions. Especially noteworthy is the high ceramide content of the fraction obtained by aqueous-alcoholic extraction, which exceeds 15% by weight. Thus, wool wax may be a new and cheap source of natural ceramides that are similar to those present in human SC. These findings lend support to the view that the ceramide-rich lanolin fractions are also suitable for designing new pharmaceutical or cosmetic products for skin care.

The presence of ceramides in wool wax and lanolin fractions could explain their effect as a barrier repair product. Elias *et al.* (13) have shown that the barrier repair effect of 3% lanolin in an inert propylene glycol/ethanol vehicle is comparable to the effect produced by optimized ratios of physiological lipids (ceramides, cholesterol, and FFA) and is superior to that of both glycerin and petrolatum. In the same studies, a 15% solution of lanolin in an inert vehicle accelerated the barrier repair even further. The explanation for this effect is unknown but perhaps the presence of low levels of ceramides could explain these results.

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