A Possible Function of Structural Lipids in the Water-Holding Properties of the Stratum Corneum

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In order to clarify the possible role of lipids in the water-holding property of stratum corneum, the forearm skin of 6 healthy male volunteers was treated with acetone/ether (1/1) for 1, 5, 10, and 20 min. A prolonged treatment period of 5–20 min produced a chapped and scaly appearance of the stratum corneum without any inflammatory reactions. Under these conditions, there was a marked decrease in the water-holding capacity of the stratum corneum accompanied by a considerable and selective loss of intercellular lipids such as cholesterol, cholesterol esters, and phospholipids. These impairments persisted until day 4 after treatment. Electron microscopic observation of the altered stratum corneum revealed that naturally occurring intercellular materials were absent, leaving the area with the appearance of a vacant space. These findings suggest an additional and essential role of the specific structural lipids for the water-holding properties of the stratum corneum.

The important role of water content for the physical flexibility of the stratum corneum is generally acknowledged [1]. Available evidence suggests that water-soluble materials, such as free amino acids, organic acids, urea, and inorganic ions, are primarily responsible for the water-holding properties of the stratum corneum, and these materials have been termed the "natural moisturizing factor" [2]. Elias pointed out [3,4] the possible role in the skin-water permeability of lipids which construct lamellar structures in the intercellular spaces of the stratum corneum. These lipids have been thought to originate from the lamellar body and are composed mainly of cholesterol, glycolipids, phospholipids, and free fatty acids. Known removers of lipids, such as surfactants and organic solvents, induce dry skin which is specifically characterized by a reduction in water content [5,6], suggesting that the structural lipid may also play a considerable role in the water-holding potential of stratum corneum. In order to clarify that possibility, we studied the effects of impairment of structural lipids by acetone/ether treatment on the water-holding capacity of stratum corneum, together with accompanying changes in the extracted lipid composition. This is the first report to show the essential role of specific structural lipids in the water-holding property of the stratum corneum which is selectively impaired by prolonged treatment with acetone/ether.

MATERIALS AND METHODS

Treatment with Acetone/Ether

The forearm skin of 6 healthy male volunteers, aged 24–33 years, was used. Open-ended, 3 cm-diameter cylinders filled with 10 ml of acetone/ether (1/1) were pressed with occasional shaking onto the test areas with gentle pressure for 1, 5, 10, and 20 min intervals (day 0).

Measurement of Water-Holding Capacity of Stratum Corneum In Vivo

Water-holding capacity of the stratum corneum was measured according to Tagami et al [8]. The treated areas were hydrated by placing 25 μl of distilled water for 30 s and then the small amount of excess water was blotted with a pad of gauze. Ten minutes after hydration, conductance of the treated areas, which had become steady by this time, was measured by a capacitance conductance meter (Model IB-364, IBS Inc., Japan). Changes in water-holding capacity of the treated areas were measured daily for 4 successive days. During this experiment, volunteers were not allowed to take a bath. All measurements were carried out at 23°C and under relative humidity of 50–60%.

Statistics

The level of significance of the difference was calculated by Student’s t test for paired comparison.

Analysis of Lipids

Lipids that were extracted in acetone/ether mixtures were analyzed by using one-dimensional thin-layer silica gel (Wako B5 gel, Wako Chem., Japan) chromatograms, utilizing benzene/hexane (1:1) as the first developing solvent for neutral lipids (squalene, cholesterol-esters, waxes), and hexane:benzene:acetic acid (70:30:1) as the second developing solvent for polar lipids (cholesterol, triglycerides, free fatty acids, and phospholipids), as has been previously reported [9].

Electron Microscopic Observation

Four days after treatment, biopsy specimens were taken using a surgical knife, fixed with glutaraldehyde/osmium tetroxide, and embedded in Epon 812. Thin sections were observed by JEOEL 100-CX.

RESULTS AND DISCUSSION

Application of acetone/ether to human forearm skin for extremely prolonged periods of 5–20 min, as compared to the usual procedure for the extraction of skin surface lipids, induced an enduring (more than 4 days), chapped and scaly appearance of the stratum corneum without any inflammatory reaction (Fig 1). Under these conditions, a significant decrease of conductance in the treated areas was observed when compared to the untreated control areas (Fig 2). This decreased conductance barely returned to the normal level until more than 4 days after treatment, with exception of the 5-min treatment in which the previous normal level of conductance was almost attained by 4 days with disappearance of a scaly skin. In contrast, such a persistent scaly skin accompanied by a significant decrease in conductance value could not be induced after only 1 min of treatment.

In order to clarify the cause of this change in the water-holding capacity of stratum corneum as evidenced by the decrease of conductance values, we compared the composition of extracted lipids in relation to varying periods of time of solvent treatment. It can be seen (Fig 3) that even after 1 min of treatment, the amounts of sebaceous gland lipids such as squalene, triglycerides, and wax esters almost reached a plateau. Additional or prolonged treatments induced no further substantial release of these lipids. On the other hand, stratum corneum lipids such as cholesterol, cholesterol esters, and phospholipids were successively solubilized by the solvent treatment from the stratum corneum in a time-dependent manner. Inconsistent with most sebaceous lipids, free fatty acids were also
extracted to almost the same extent as stratum corneum lipids in these experimental conditions. This may be due to the multiple origins of free fatty acids from both sebaceous glands and keratinocytes. Electron microscopic analysis of the interrupted water-holding status of treated stratum corneum revealed that intercellular materials that should normally exist in between the horny cells were absent, leaving the area with the appearance of a vacant space (Fig 4). These intercellular impairments continued even up to day 4 after treatment, having not yet been filled up by lamella constructing lipids.

Elias reported on the important role of intercellular lipids in the regulation of the stratum corneum barrier function against water [3,4]. It is generally documented that scaly skin shows a functional defect in both hygroscopicity and its water-holding capacity [8]. A similar situation is found in the case of essential fatty acid deficiency, which produces a defect in water barrier function in such a way that specific lipid components are eliminated from the stratum corneum [7].

Although we cannot rule out the possibility that acetone/ether treatment may interrupt the keratin protein-water interaction, present evidence for lipid extraction and water reservoir function suggests an additional and essential role of compartmentalization of the intercellular lipids for the development of the water-holding properties of the stratum corneum. Our findings are not consistent with the currently accepted theory that water-soluble materials are solely responsible for holding

**Fig 1.** Clinical appearance of chapped and scaly skin occurring 4 days after 20 min of acetone/ether (1/1) treatment.

**Fig 2.** A significant decrease of conductance values induced by acetone/ether (1/1) treatment. The forearm skin of 6 healthy male volunteers was treated with acetone/ether for 1, 5, 10, and 20 min, respectively (day 0). The treated areas were then hydrated with distilled water at 37°C for 30 s. Ten minutes after hydration, conductance values of the treated areas were measured daily for 4 successive days after treatment. *: p < 0.05, **: p < 0.01.

**Fig 3.** Lipid composition of materials released by acetone/ether treatment as shown by thin-layer silica gel chromatograms. s = Squalene; c = cholesterol, t = triglycerides, w = waxes, ce = cholesterol esters, f = free fatty acids, p = phospholipids.

**Fig 4.** Ultrastructure of the stratum corneum of 20-min treated (A) and untreated (B) areas. Biopsy specimens taken 4 days after treatment were fixed with glutaraldehyde/osmium tetroxide and were then embedded in Epon 812. JOEL 100 CX. Bars = 1 µm.
the water in the stratum corneum [1,2]; they do, however, appear to be corroborated by the fact that acetone/ether treatment could not induce a substantial release from the stratum corneum of any hygroscopic materials such as free amino acids or lactic acid. Blank [10] and Middleton [6] reported that, on human callus or animal corneum, extraction of lipid from stratum corneum enhances the susceptibility of water-soluble materials to water extraction. However, it seems unlikely that our hydration process subsequent to acetone/ether treatment could release water-soluble materials sufficient to induce the significant decrease in conductance value as seen immediately after treatment. The precise mechanisms whereby compartmentalized intercellular lipids within the stratum corneum exert their water-holding potential are now under investigation.

REFERENCES