Study on the Relationship between Epidermal Barrier Functi on and Cornified Envelope (CE)-Bound Lipids

Takao Hattori, Midori Oyobikawa, Masami Suzuki

POLA Laboratories, POLA Chemical Industries, Inc.

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SUMMARY

The importance of cornified envelope (CE)-bound lipids to epidermal barrier function is increasingly being recognized. In the present study, we intentionally damaged the cornified layer of hairless mice by ultraviolet irradiation and sodium dodecyl sulfate (SDS) treatment, and assessed the changes in epidermal barrier function by measuring Trans Epidermal Water Loss (TEWL). We also measured changes in the amount of CE-bound lipids using thin layer chromatography (TLC). The results showed that both treatments increased TEWL and decreased CE-bound lipids (omega-hydroxy ceramide and omega-hydroxy acid). In addition, investigation of the chronological changes in TEWL revealed an inverse relationship between TEWL and CE-bound lipids, and a correlation between CE-bound lipids and epidermal barrier function. We then measured the amount of CE-bound lipids in the cheek and the medial side of the upper arm in humans. The results showed that because the cheek receives external stimulation on a daily basis, the amount of CE-bound lipids was significantly lower, while the level of TEWL was higher. These observations, together with those from the animal study, indicate that CE-bound lipids are related to epidermal barrier function.

INTRODUCTION

Corneocytes are covered by a protein membrane called the cornified envelope (CE). It has

been shown that skin-specific lipids are covalently bound to the CE, and one study reported that these lipids are involved in stabilization of intercellular lipids (1). There are two types of CE-bound lipids, namely omega-hydroxy ceramide and omega-hydroxy fatty acid, and both contain long chain fatty acids made of 30 to 36 carbons with a hydroxylated terminus (Figure 1). This hydroxyl group forms an ester bond with the carboxyl group in proteins (glutamate residue). In recent years, several studies have reported that UV irradiation decreased the amount of CE-bound lipids and that immunostaining of CE using the involucrin antibody changed with respect to maturation of the stratum corneum (2,3).

In an attempt to ascertain the relationship between epidermal barrier function and CE-bound lipids, the amount of CE-bound lipids was quantified in various types of damaged skin. The amount of CE-bound lipids was also measured in different types of human skin.

MATERIALS and METHODS

Animals

Nine weeks old hairless mice (Hr-/Kud, Kyudo, Japan) were used.

Preparation of Stratum Corneum from Damaged Skin of Hairless Mice

To the back of hairless mice, 180 mJ of UV-B irradiation was applied, and a skin sample was collected four days later. To a separate region, a 7.5% sodium dodecyl sulfate (SDS) solution was applied twice daily for four consecutive days, and a skin sample was collected on day 5 of the experiment. From each sample, a 3-cm Φ punch was used to collect skin discs. Each disc was treated with a Dispase solution (Godo Syusei Inc.) overnight at 5°C in order to obtain the epidermis, including the stratum corneum. The epidermis was treated with 0.5% trypsin at room temperature for one hour and washed using distilled water to collect a stratum corneum sheet. This sheet was dried under reduced pressure.

Human Volunteer Testing and Preparation of Human Corneocytes

In 21 healthy male volunteers, TEWL was measured using a Tewamater TM-210 in a

room maintained at 21°C and a relative humidity 50%. Corneocytes were then collected from the medial side of the upper arm, the lateral side of the forearm, and the cheek by tape stripping (approximately 10 cm x 10 cm) (Total: 200 cm^2). Corneocyte area was measured in certain samples. The remaining tape samples were finely cut and placed in glass centrifugation tubes. In each tube, xylene was added to separate corneocytes and the adhesive from the tape. After eliminating the tape, the residue was centrifuged. The supernatant was eliminated, and xylene was added for centrifugation. This procedure was repeated five times. After the final centrifugation, the supernatant was removed, and a small quantity of xylene was added in a glass test tube of known weight. The corneocytes were air dried and then dried under reduced pressure. Informed consent was obtained from all human volunteers.

Isolation of CE-bound lipids

Each dried stratum corneum sheet (dried corneocytes) was extracted a total of three times for 2 hrs each using 2 ml of a chloroform/methanol (2:1, 1:1 and 1:2 v/v) solvent. After delipidization, the stratum corneum sheet (corneocytes) was dried and weighed (delipidized stratum corneum (corneocyte) weight). To the delipidized stratum corneum (corneocyte), 1 N methanol sodium hydroxide was added and heated at 60°C for 1 hr. After neutralizing the resulting sample to approximately pH 3 using 2 N HCl, a small quantity of chloroform was used to extract bound lipids three times, and the three chloroform layers were combined and dried under nitrogen.

Analysis of CE-bound lipids (analytical methods)

Lipids were quantified using the method of Dowing et al. (4,5), with some modifications. According to the method described in (6), CE-bound lipids (omega-hydroxy ceramide and omega-hydroxy fatty acid), which were isolated and purified from the porcine stratum corneum, were used as standard substances. Extracted CE-bound lipids were dissolved in

20 μl of a chloroform/methanol (2:1) solvent, and then 10 μl was applied to a TLC plate (Merch, Germany). After developing the plate using a chloroform/methanol/acetate (190/9/1) solution, 50% sulfuric acid mist was sprayed and the plate was charred at 220°C. The resulting TLC plate was scanned using a photodensitometer.

RESULTS AND DISCUSSION

CE-bound lipids from damaged skin of hairless mice

Skin samples were collected from hairless mice with damaged skin, and CE-bound lipids were extracted and quantified in order to determine quantitative changes. With UV-B irradiation, the amount of CE-bound lipids decreased to about one third. Furthermore, with SDS application, the amount of CE-bound lipids decreased to about a half. There were no marked changes in the two types of CE-bound lipids (omega-hydroxy ceramide and omega-hydroxy fatty acid) between UV-B irradiation and SDS application.

Chronological changes

Chronological changes in TEWL and CE-bound lipids following UV-B irradiation were investigated (Figure 3). When compared to day 0 of the experiment, TEWL increased by 2.5-fold on day 3, and then gradually decreased after that. Changes in CE-bound lipids and TEWL were complementary, suggesting correlations with epidermal barrier function.

Measurement of TEWL and CE-bound lipids in Humans

In 21 healthy volunteers, TEWL and corneocyte area were measured on the medial side of the upper arm, the lateral side of the forearm, and the cheek. TEWL for the cheek was found to be significantly higher than in other measured areas, while the corneocyte area for the medial side of the upper arm was the highest, followed by the lateral side of the forearm and the cheek, in this order. There were significant differences among all three measurement sites (Figures 4 and 5). From corneocytes collected from volunteers by tape stripping, CE-bound lipids were extracted and quantified (Figure 6). The results showed

that the amount of CE-bound lipids for the medial side of the upper arm was significantly higher than that for the lateral side of the forearm and the cheek. There were no marked differences in the amount of the two types of CE-bound lipid (omega-hydroxy ceramide and omega-hydroxy fatty acid) among the medial side of the upper arm, the lateral side of the forearm, and the cheek.

CONCLUSION

The results of the present study demonstrate that when the skin of hairless mice is damaged by UV irradiation or SDS application, the amounts of CE-bound ceramide and omega-hydroxy acid decreases. Furthermore, in humans, the amounts of CE-bound omega-hydroxy ceramide and omega-hydroxy acid in the medial side of the upper arm were found to be significantly greater than in the lateral side of the forearm or the cheek. The results of CE-bound lipid measurements in animals with experimentally damaged skin and at different measurement sites on human skin suggest a correlation between CE-bound lipids and epidermal barrier function.

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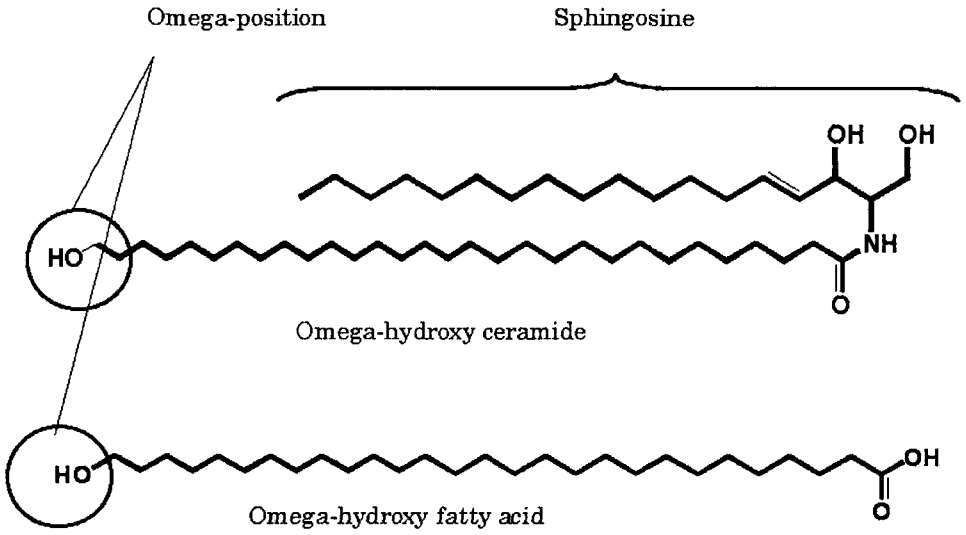

Fig-1 Structure of CE-bound lipids

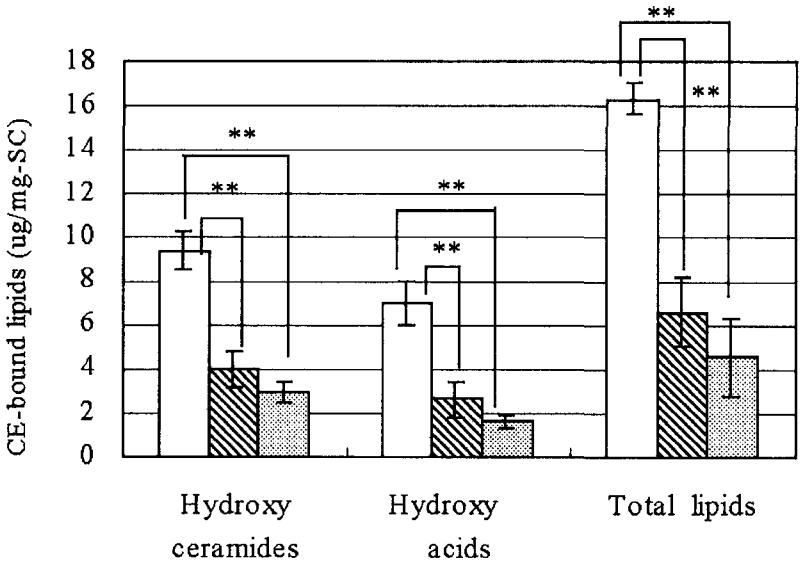

Fig-2 CE-bound lipids from various damaged skin of mice.
□ : Control. ▨ : SDS-damaged.
▩ : UV-B damaged.

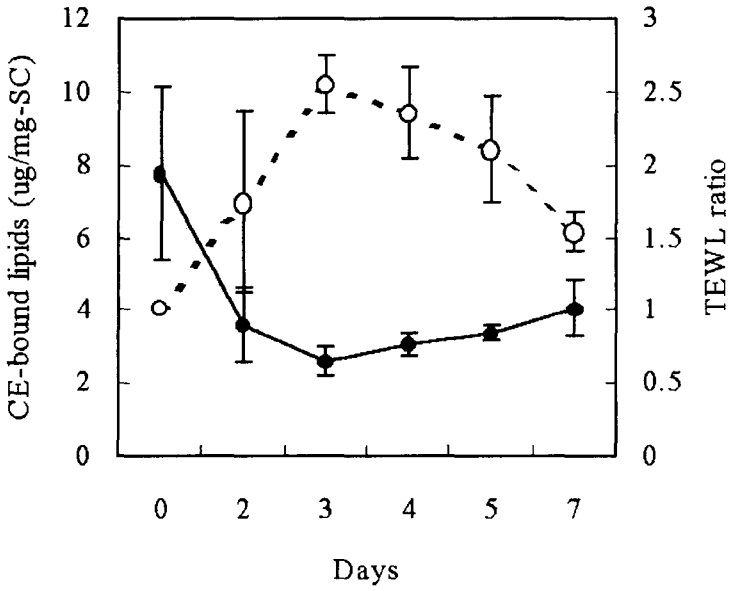

Fig-3 Relationship between CE-bound lipids and TEWL ratio ; irradiated 180mJ UV-B on 0day, ● change of CE-bound lipids, ○ change of TEWL ratio.

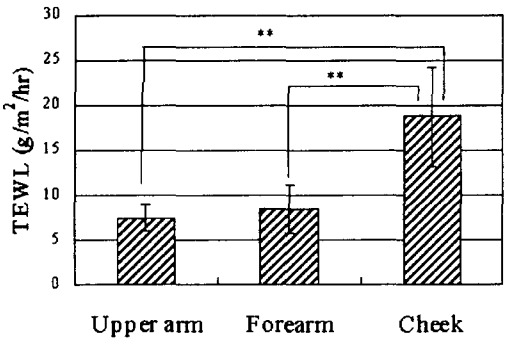

Fig-4 TEWL for various sites of human skin. (N=21)

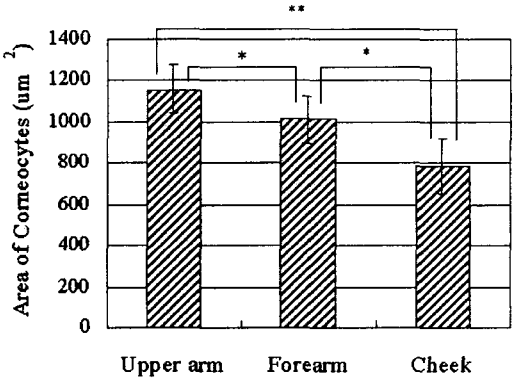

Fig-5 Corneocytes of various sites of human skin. (N=21)

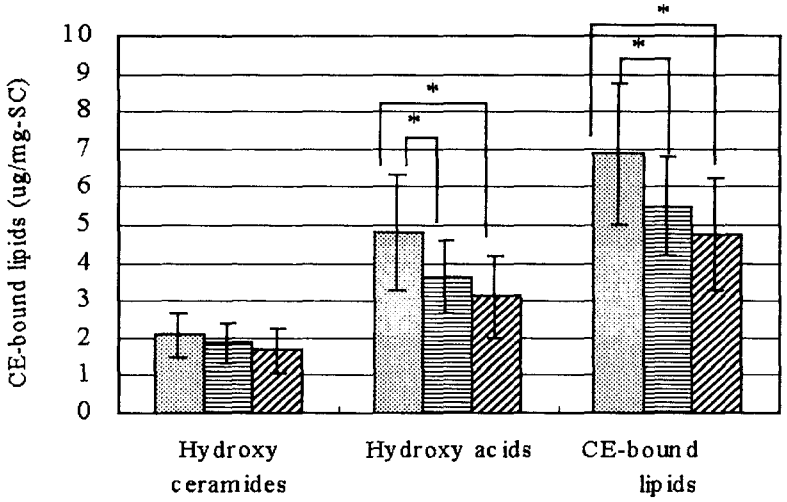

Fig-6 CE-bound lipids of various sites of human skin. (N=21)
: Upper arm : Forearm
: Cheek