The Effect of Multi-lamellar Emulsion (MLE) on Skin Barrier Function: Can an Improve Permeability Barrier Provide a Solution for Itching due to Skin Barrier Malfunction?

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Summary

Physiological lipid mixtures comprised of cholesterol, ceramide and free fatty acid better maintain epidermal homeostasis and have been recently used for dermatoses induced by skin barrier damage, for example for atopic dermatitis and xerotic skin. Itching and dry atopic dermatitis of the skin may be related to altered skin barrier function. In a previous study, the use of multi-lamellar emulsion (MLE), which is a lipid mixtures containing cholesterol, pseudoceramide and free fatty acid, has been shown to accelerate the recovery of the epidermal permeability barrier. In this study, we assessed the efficacy of MLE compared with a currently used anti-itch moisturizer (AIM), the active ingredients of which are menthol and camphor, on barrier recovery after barrier disruption. To clarify the effect of MLE and AIM after acute barrier perturbation, we measured the relation between transepidermal water loss (TEWL) and the barrier recovery rate at 3, 6, 24, and 48 hours after tape stripping hairless mice and then observed changes in the stratum corneum (SC), including the intercellular lipid structure and secretion of lamellar bodies, by electron microscopy. MLE treated skin recover skin barrier

function more rapidly, and AIM treated skin delayed barrier repair. Morphological changes in the epidermis, of MLE treated skin revealed well-conserved lipid multi-lamellar structures at 24 h after tape stripping, whereas AIM treated skin showed altered lamellar bilayers within the SC interstices at 48 h. In addition, MLE treated skin showed an increase in the number of LBs and in their secretions and a decrease in the number of SC layers versus AIM treated skin. These results suggest that MLE may accelerate the production of an epidermal permeability barrier in hairless mice by increasing the number and secretion of LB and improve the dryness and itch associated with an altered epidermal permeability barrier.

Introduction

Pruritus is a common symptom in various dermatoses in dermatitis, and is characterized by a dry skin, for example, senile xerosis, atopic dermatitis or seasonal xerosis in winter [1-2]. Some reports have implicated skin dryness, as characterized by a reduction in stratum corneum (SC) hydration, and barrier disruption, characterized by an increase in transepidermal water loss (TEWL), either by itself related to pruritus [3]. Other reports were unable to relate changes in SC hydration and TEWL to pruritus [4]. Atopic dermatitis is a chronic, relapsing and pruritic dermatitis. Dry skin is one of the prominent clinical features of atopic dermatitis. Intense itching is the most characteristic feature of atopic dermatitis, and causes scratching and eczematous lesions [5]. Dry skin with atopic dermatitis displays impaired skin barrier function as indicated by increased transepidermal water loss (TEWL) and diminished water-holding properties. The epidermal permeability barrier, localized to the SC interstices, is mediated by lamellar bilayers, which are rich in cholesterol, free fatty acids and ceramides [6]. These lipids are delivered to the intercellular spaces as a mixture of precursors as secretions of the epidermal lamellar body (LB). Following their secretion, the lipid precursors are metabolized within the extracellular spaces by colocalized, LB-derived hydrolytic enzymes into hydrophobic, lamellar basic unit structures, which mediate barrier function [7]. Prior studies have suggested that the skin barrier in terms of water loss is attributable to SC lipids [7-8]. Imokawa et al. [9] reported that intercellular lipids, especially ceramide, play a critical role in the water-holding properties of the SC. Recent studies have shown that topical mixtures of the 3 key SC lipids, i.e., ceramide, cholesterol, and free fatty acids, when applied in optimized proportions (i.e., a 3:1:1 molar ratio), accelerate barrier repair after a variety of external, acute, or sustained perturbations of the skin barrier [10-15]. Such barrier abnormalities correlate with a reduction in these stratum corneum lipids, along with a selective reduction in one or two of three main intercellular lipid species. Even though ceramide itself is known to be an effective component of skin barrier function, the use of ceramide alone on damaged skin was ineffective. In a previous study, lipid mixtures expressing a multi-lamellar

emulsion (MLE) were shown to be able to accelerate skin barrier recovery [16]. Topical application of MLE on atopic dermatitis produced good results with respect to itching, erythema, and dryness et al. [17]. And the reason why the lamellar structure is effective in skin homeostasis had studied by X-ray diffraction [18]. The purpose of the present study was to determine whether lipid mixtures containing pseudoceramide are of greater benefit, in cases of barrier disruption, than anti-itch moisturizer (AIM). Moisturizers with an anti-itch function have been commercialized on the basis that itching is very typical symptom of atopic dermatitis. The moisturizer claim a cooling effect due to the presence of a menthol and a camphor and moisturizing effect. In this study, we wanted to determine whether an improved skin barrier provides a solution to itching due to skin barrier malfunction. Based on our previous results, skin dryness due to a defective skin barrier function can cause pruritus.

Materials and Methods

Animals

Hairless mice, 6-8 weeks old, were purchased from the animal laboratory of Yonsei University, and fed a standard mouse diet with water available *ad libitum*. Their ages ranged from 8 to 12 weeks at the time of the study.

Preparation of Emulsion

Multi-lamellar emulsion (MLE) was prepared by the previously reported method [16]. Anti-itch moisturizer (AIM, active ingredient: menthol and camphor, made in USA) was purchased in market.

Experimental protocols

Barrier disruption was achieved by repeated applications of cellophane tape (Scotch Crystal Clear Tape, 3M, St. Paul, MN) on mouse flank skin. The procedure was terminated when the transepidermal water loss (TEWL) levels reached 4 mg per cm^2 per h. TEWL was measured using a Tewameter TM210 (Courage and Khazaka, Germany). Immediately after barrier disruption 300μl of MLE was applied to the flank, and a commercial AIM was applied to $5cm^2$ areas of treated skin as a control. TEWL was measured before barrier disruption, immediately after barrier disruption, and at 3h, 6h, 24h, and 48h after barrier disruption. Barrier recovery results are expressed as percentage recovery. In each animal the percentage recovery was

calculated using the following formula: [1- (TEWL at the indicated time – baseline TEWL)/ (TEWL immediately after treatment – baseline TEWL)] X 100%. All data were compared with data from controls studied simultaneously.

Electron microscopic examination

All biopsies for light microscopy were fixed in 10% formalin. After routine processing and embedding in paraffin, 5μm sections were cut and stained with hematoxylin and eosin. Changes in the epidermal layers were evaluated under a light microscope.

Skin samples for electron microscopy study were minced to 0.5mm^3 and fixed in modified Karnovsky's fixative overnight, washed in 0.1 mol of cacodylate buffer, split, postfixed in 0.2% ruthenium tetroxide to evaluate intercellular multi-layers, postfixed in 1% osmium tetroxide (OsO_4) to examine the stratum corneum layers, and routinely processed and embedded in Epon-epoxy resin mixtures. Ultrathin sections (60-80nm) were then double-stained with uranyl acetate and lead citrate, and examined under an electron microscope **[19-20]**.

Statistical analysis

The Student's paired *t* test was used for the statistical analyses. $P < 0.05$ was considered significant.

Results

Barrier recovery after barrier perturbation

After MLE had been applied to hairless mouse skin and compared with the commercial AIM. We had also compared with non-treat (NT) skin to assess whether the MLE had influenced barrier recovery after the barrier disruption induced by tape stripping. Tape stripping removes not only intercellular lipids but also the cellular components of the SC. As seen in Fig. 1, following mechanical disruption of the barrier by tape stripping, application of MLE accelerated barrier repair versus AIM and NT, and this was significant ($p<0.05$). These results suggest that MLE effectively improves barrier recovery after barrier perturbation.

Figure 1

Histological and electron microscopic observation (Table 1)

Differences in epidermal thickness measured under the LM were compared for MLE and AIM application ($P<0.05$). The epidermal thickness of skin after MLE application was lower than that of skin after AIM application (Fig. 2). These results suggest that MLE application is effective at preventing the epidermal hyperproliferative response induced by barrier disruption. The degree of epidermal hyperplasia correlates with the level and duration of barrier disruption.

Figure 2

Morphological changes of the SC layers induced by the acute barrier disruption were examined after OsO_4 postfixation under an electron microscope. No remarkable differences in the number of SC layers were observed for MLE and AIM application. However, the thickness of the SC skin layers of the MLE treated skins was lower than that of the AIM treated skins (Fig. 3).

Figure 3

The number of LBs was slightly higher in skin treated with MLE than in skin treated with AIM (Fig 4). Decreased LB production was also evidenced by diminution in the quantities of newly secreted extracellular lamellae in skin treated with AIM (Fig. 5)

Figure 4

Finally, we examined the structure of the lipid multi-layers using RuO_4 postfixation under the electron microscope. At 6 h, as shown in Figs. 5A, B, a, b, the intercellular domains of the stratum corneum revealed abnormal bilayer structures, the basic unit structure of the intercellular bilayer system had been obliterated in both treated with MLE or AIM. However, the MLE treated skin showed almost normal intercorneocyte lipid multi-layer structures at 24 h (Fig. 5C), whereas the AIM treated skin showed disordered intercorneocyte lipid multi-layer structures 48 h after tape stripping (Fig. 5d). These results suggest that MLE application may accelerate epidermal permeability barrier recovery in hairless mice by increasing the number of LB's and of their secretions and that MLE use is associated with relatively well-conserved lamellar bilayers 24 h after barrier perturbation.

Figure 5

Discussion

Impaired barrier function is a feature of several skin disease, possibly due to the differences in SC lipid composition and the abnormal lamellar structure of intercellular lipids. A dry and itching skin such as atopic dermatitis and severe xerosis have an impaired skin barrier function as indicated by increased TEWL, diminished water-holding properties and decreased ceramide levels. Thus, preparations of accelerating barrier recovery after barrier disruption, as induced by tape stripping, would be effective in the treatment of several skin disease induced by barrier damage. A recent study confirmed that an altered permeability barrier maybe contribute to the susceptibility of sensitive people to various stimuli [21]. Sensitive skin is also associated with increased TEWL, increase penetrability, and a higher susceptibility to irritants. These parameters can be measured independently and, taken together, may be used to indicate that sensitive skin is a clinical state associated with impaired barrier function. Thus preparations of accelerating barrier recovery would be also effective in the treatment of sensitive skin.

In present study, we found that MLE, which consists of pseudoceramide, cholesterol and fatty acid, accelerates barrier repair versus AIM after barrier disruption by tape stripping. This observation not only confirms the ability of this MLE to enhance barrier recovery but it also demonstrates the potential use of treatment for more clinically relevant levels of dysfunction. Occlusive moisturizers, such as petrolatum, have been reported to form a non-permeable barrier on damaged skin [22-24], a barrier that improves the skin's water-holding capacity [25]. However, other reports suggested that occlusion with water-impermeable membranes could not prevent epidermal hyperplasia [26]. The degree of epidermal hyperplasia is correlated with the level and duration of barrier disruption. The epidermal mitotic index also increases with repeated disruption, indicating that hyperplasia could be ascribed to increased cell proliferation. Thus, a better preparation, in which the TEWL value decreases more rapidly, might also help prevent epidermal cell layers thickening. In our study the epidermal thickness of skin after MLE application was lower than that of skin after AIM application. Prior studies suggest a correlation between accelerated skin barrier recovery and improved epidermal hyperplasia [27-28]. An improvement of epidermal hyperplasia by the topical application of MLE might be partially the result of accelerated barrier recovery. The skin barrier is the main structure protecting the body, which it achieves by separating the internal and external environments. The SC is the primary structure of the skin barrier, and has vitally important functions. It is made up of intercellular lamellar layers of abundant hydrophobic lipid and corneocytes, which are enveloped by the lipid layers. SC intercellular lipid layers are multi-lamellar structure composed of hydrophobic and hydrophilic layers. These lamellar structures are formed and maintained by LBs that supply lipids and enzymes for the synthesis of ceramide and free fatty acid. Our electron microscopic

findings showed a higher number of LB's and of their secretions in skins treated with MLE than in skins treated with AIM. An increased number of LBs in the epidermis means that MLE probably accelerates the recovery of intercellular lamellar layers and increases the resistance to irritants and, therefore, prevents skin dryness, itching, et al.

In summary, we examined the effects of MLE or AIM on skin barrier malfunction. Abnormalities in barrier function could be ascribed to alterations in lamellar membranes, widening of the SC interstices, and deterioration in the secreted contents of LBs. The present study demonstrates that MLE can contribute to the better recovery of skin barrier with less epidermal proliferation than AIM. Thus it suggests that MLE could be a clinical use in chronic skin diseases of barrier abnormality potentially such as atopic dermatitis and severe xerosis and useful for daily care like sensitive skin to prevent skin troubles.

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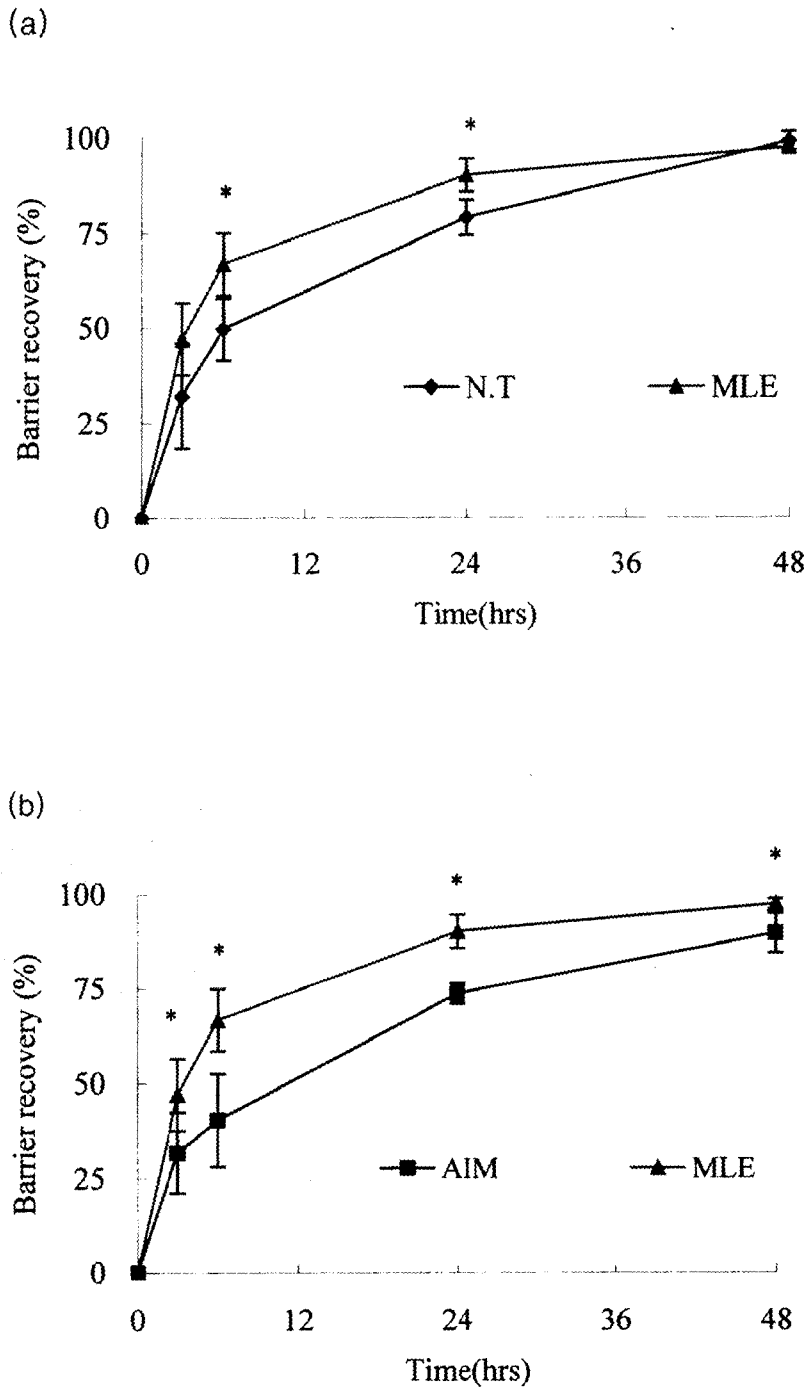

Figure 1: The effect of MLE on barrier recovery following disruption of the barrier compared with (a) air-exposed non-treated (NT) skin, and (b) disrupted AIM treated skin; P values were calculated by using the Student's t-test. *P<0.05

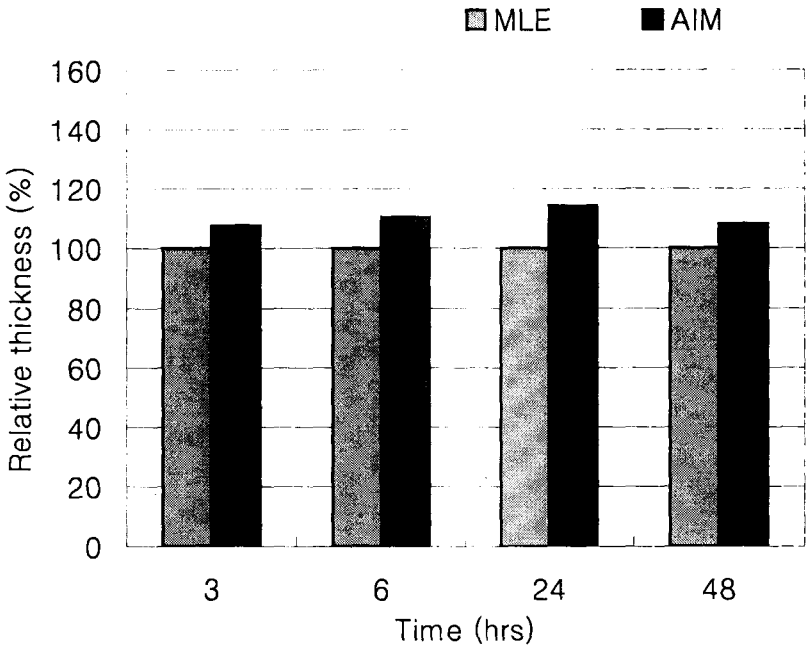

Figure 2: The effect of MLE on epidermal cell thickness after acute barrier disruption. The relative thickness of AIM treated skin was calculated against MLE-treated skin thickness.

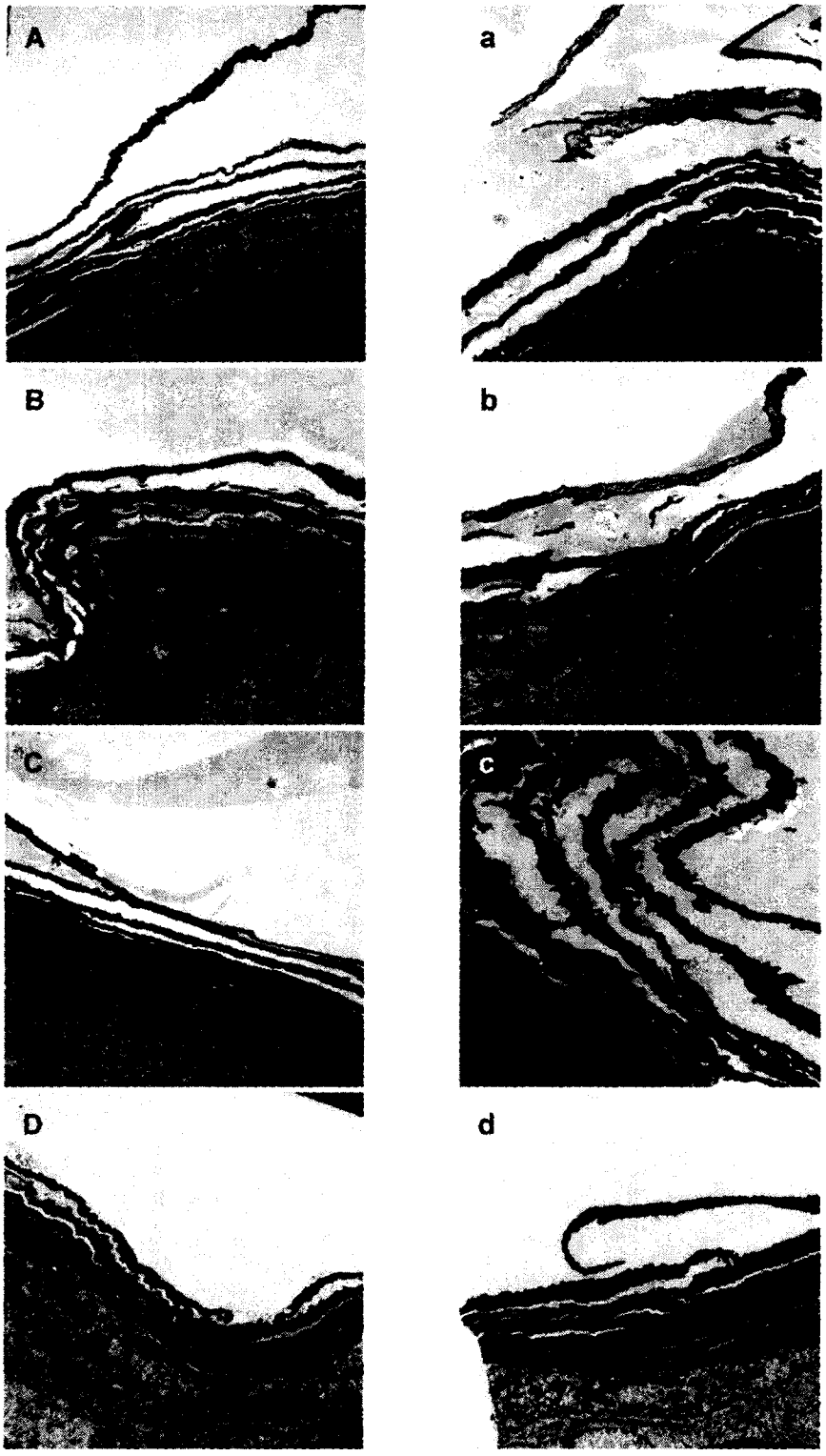

Figure 3: Electron microscopic finding of SC arrangement as MLE-treated and AIM treated skin following acute barrier disruption (X6, 000). A-D: 3, 6, 24, 48 hours after MLE application, respectively. a-d: 3, 6, 24, 48 hours after AIM application, respectively. Samples were stained with OsO_4.

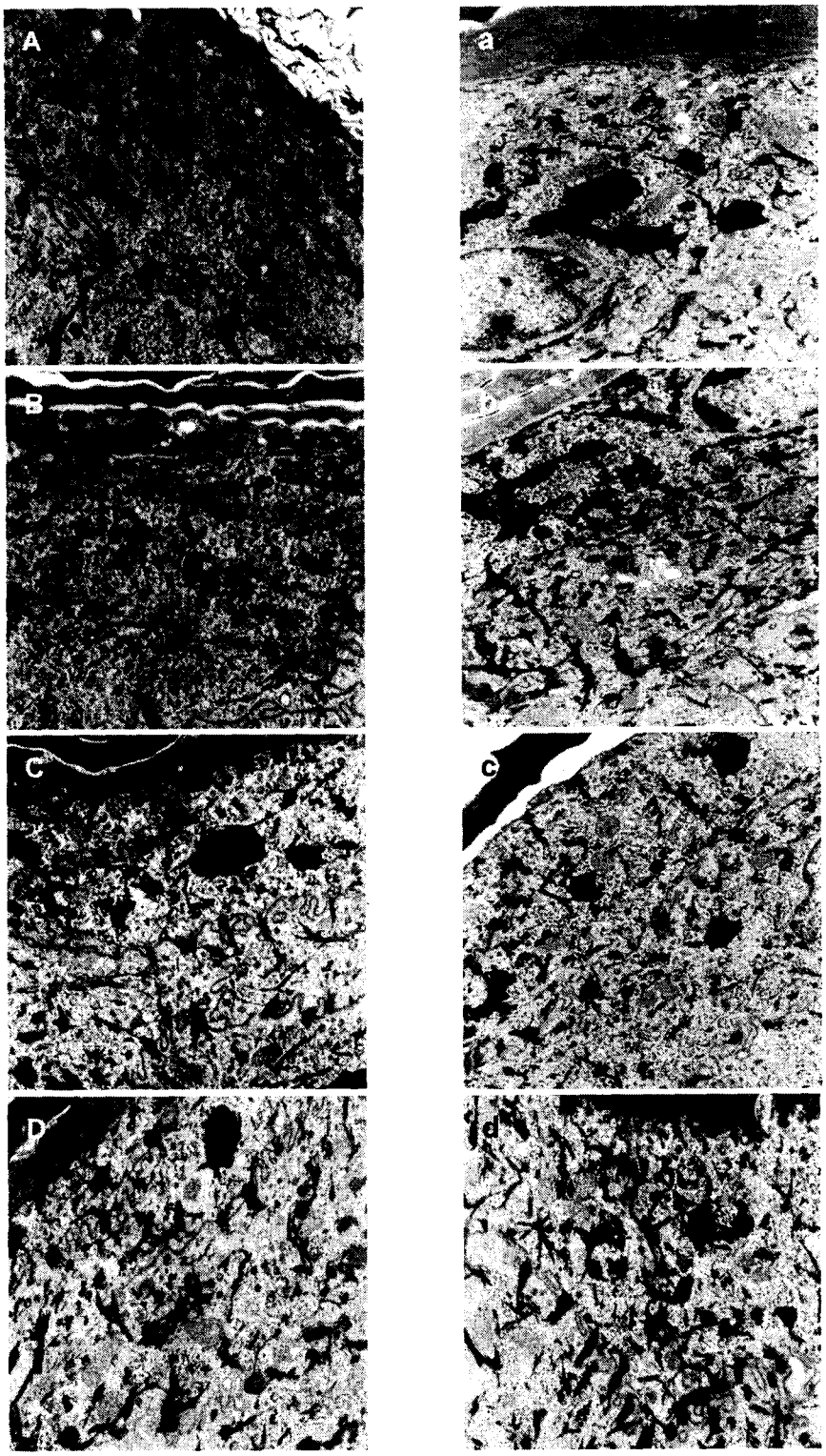

Figure 4: Electron microscopic findings of the stratum granulosum showing lamellar bodies after MLE or AIM application (X20, 000). A-D: 3, 6, 24, 48 hours after MLE application, respectively. a-d: 3, 6, 24, 48 hours after AIM application, respectively. Osmium tetroxide postfixation.

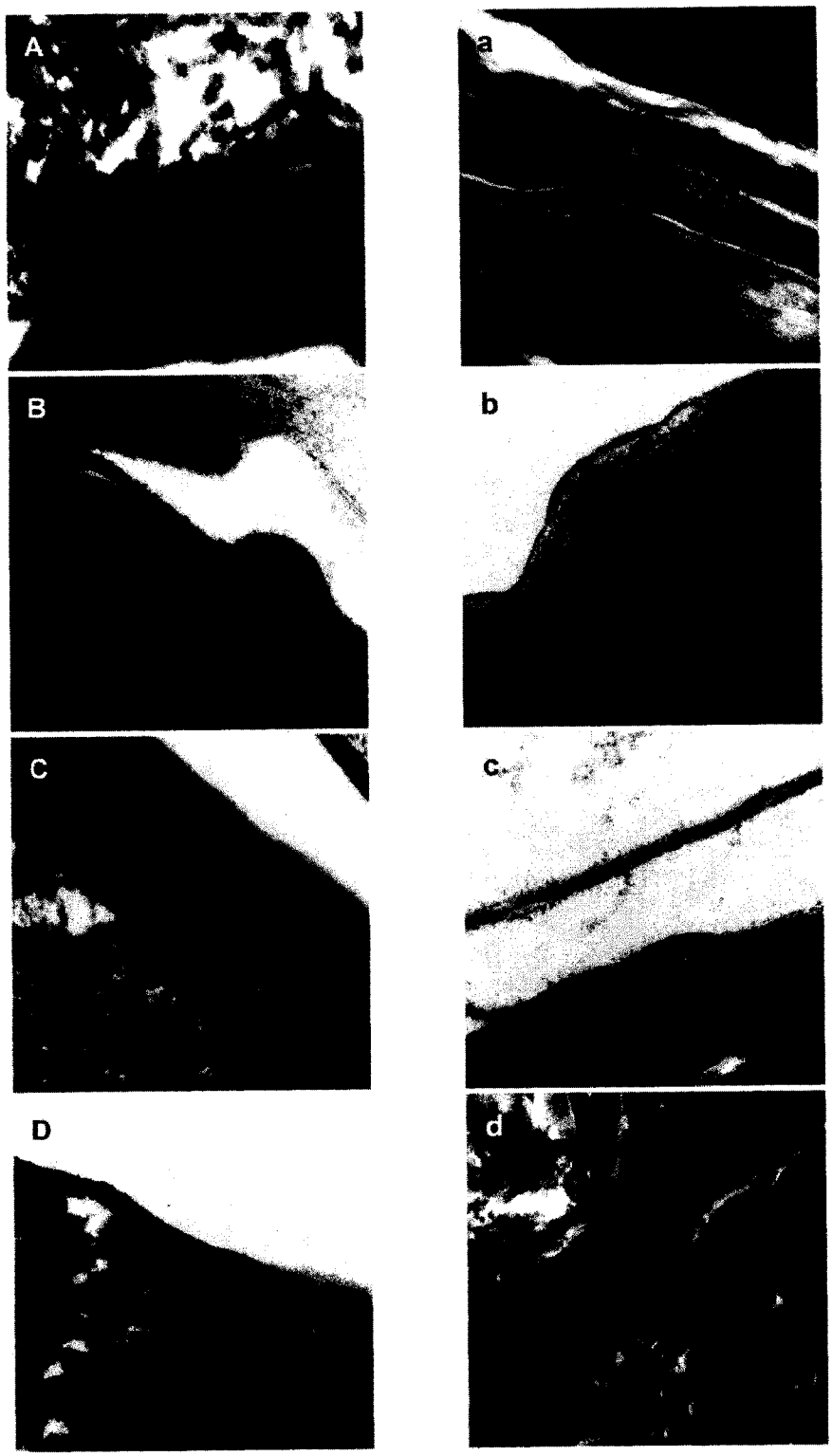

Figure 5: Electron microscopic finding of intercellular lipid layers stained with RuO_4 following acute barrier disruption (X100, 000). A-D: 3, 6, 24, 48 hours after MLE application, respectively. a-d: 3, 6, 24, 48 hours after AIM application, respectively. The intercorneocyte lipid lamellar layer structures shows a well-ordered structure 24 hours after MLE application, whereas disorganized intercorneocyte lipids in widened intercellular spaces are seen in the AIM treated skin.

Table 1: Comparison of electron microscopic findings after MLE and AIM applications

		LB numbers[a]	LB secretion	Lipid bilayer of the SC	Number of SC layers[a]
MLE	3h	41.1 ± 0.0	+++	aNL	5.3 ± 1.53
	6h	37.8 ± 0.0	+++	aNL	5.5 ± 1.29
	24h	25.2 ± 0.0	++	NL	6.3 ± 1.15
	48h	28.8 ± 0.0	++	NL	7.3 ± 2.31
AIM	3h	26.6 ± 0.0	++	aNL	6.0 ± 0.00
	6h	27.4 ± 0.0	++	aNL	4.0 ± 1.73
	24h	25.4 ± 0.0	++	aNL	6.3 ± 0.58
	48h	25.8 ± 0.0	++	aNL	7.0 ± 2.00

[a]Mean ± SD, LB: lamellar body, SC: stratum corneum, aNL: abnormal, NL: normal