The Effect of Bentonite and Glycolic Acid on the Stratum corneum

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Background **: Bentonite clay, which is a major component of mud pack, has been used for various purposes in cosmetics. Glycolic acid is known to be effective in the treatment of acne. Although those products are used widely, information on the mode of action and effects on the skin are little and controversial till now.**

Objective **: To investigate whether bentonite alone, or bentonite with glycolic acid in mixed formulation affect the stratum corneum leading to alteration on cutaneous barrier function and whether those products alter the lipid lamellae and desmosomes of corneocytes.**

Materials and Methods **: Mud pack-type ointment of bentonite, bentonite and 5% glycolic acid formulation, bentonite and 10% glycolic acid formulation were applied on the volar forearm of the five healthy men and flank skin of five 6-8 week old hairless mice. Transepidermal water loss and capacitance were measured. Electron microscopic examination after ruthenium tetroxide postfixation was performed on the flank skin of the mice.**

Results **: Transepidermal water loss(TEWL) increased immediately and normalized 4 to 6 hours later after removal of vapor permeable membrane in both mouse and human. Capacitance did not show any evidence of change in the water content of the stratum corneum. Electron microscopic examination revealed that lipid lamellae and desmosome of corneocytes were not degraded, but lamellar body secretion and partially electron-lucent material was increased in 10% glycolic acid and bentonite mixture-treated area.**

Conclusion **: Barrier function of stratum corneum is not disturbed by bentonite and glycolic acid formulations at the concentration used. Barrier structures are not disrupted, but lamellar body secretion and partially electron-lucent material was increased by bentonite and glycolic acid formulations at higher concentration.**

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Key Words : Bentonite, Glycolic acid, Capacitance, TEWL , Lipid lamellae

Recently, variable cosmetic pack products have been developed and frequently used at homes and beauty shops[1]. These products are utilized to ease the transportation of variable functional substances such as moisturizers, organic acids and plant extracts to the skin[2]. The classical type of these products is clay-based masks and among these, bentonite and hectorite clay is widely used in cosmetics and skin care products. Bentonite is native colloidal hydrated aluminum silicate with formula, $Al_2O_3.4SiO_2$. H_2O, which is utilized to increase the viscosities or as stabilizers or as adsorbents[2]. It is known to be less irritating and it does not cause

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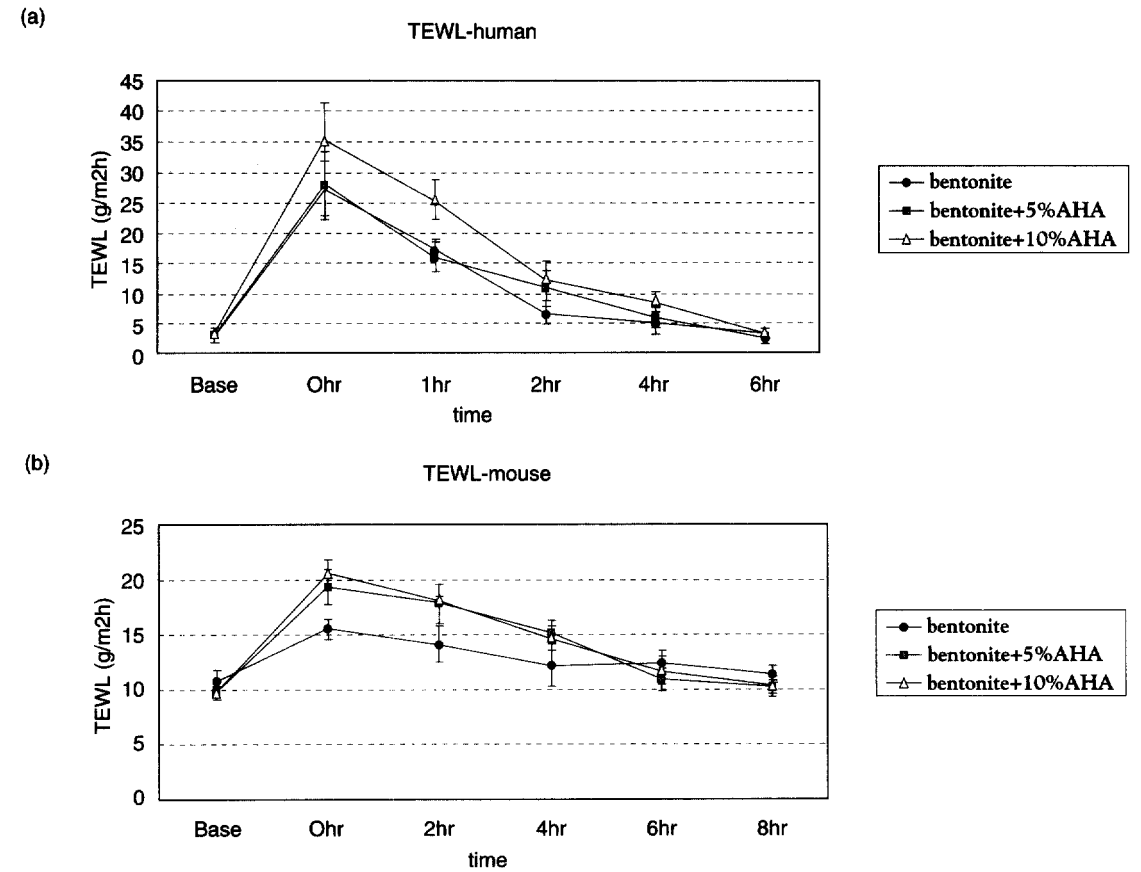

Fig. 1. Recovery rate of Transepidermal water loss as an indicator of stratum corneum integrity in human skin(a) and mouse skin(b).

hypersensitivity reaction or have any toxicity to the skin[3] and it is used not only in these products but also in many other topical therapies[2]. Recently, an effect of a product based on bentonite clay in preventing rhus dermatitis has been reported[4]. In addition, bentonite has been utilized to measure the amount of sebum secretion by its ability to adsorb the sebum[5,6].

Glycolic acid(GA) is one kind of alpha hydroxy acid(AHA) which loosen the cohesive attachment of the cornified cells and it is known to be effective in hyperkeratotic disease such as ichthyosis, acne, pigmentary disease and photoaging[7,8,9]. Thus, considering efficacy of each bentonite and GA, they would be effective in elimination of comedones and sebums from acne patients and stabilizing the skin surface. The influences of GA to stratum corneum have been studied recently and used more and more in several cosmetics.

Thus, to investigate the effect of mud pack-type formula of 12% bentonite, bentonite and 5% and 10% glycolic acid formulation to the stratum corneum, transepidermal water loss(TEWL) and capacitance were measured. Also, skin biopsies were done with electron microscopic examination after ruthenium tetroxide postfixation to intercorneal lipids.

MATERIALS AND METHODS

1. Patients

Five healthy men in their twenties who did not have any kind of skin diseases that might affect the test results took part in the study. Also fifteen of 6-8 weeks old hairless mice(Hr/Hr) were used. Hairless mice were divided into 3 groups with 5 mice in each as bentonite group, bentonite and 5% GA mixed formulation group, bentonite and

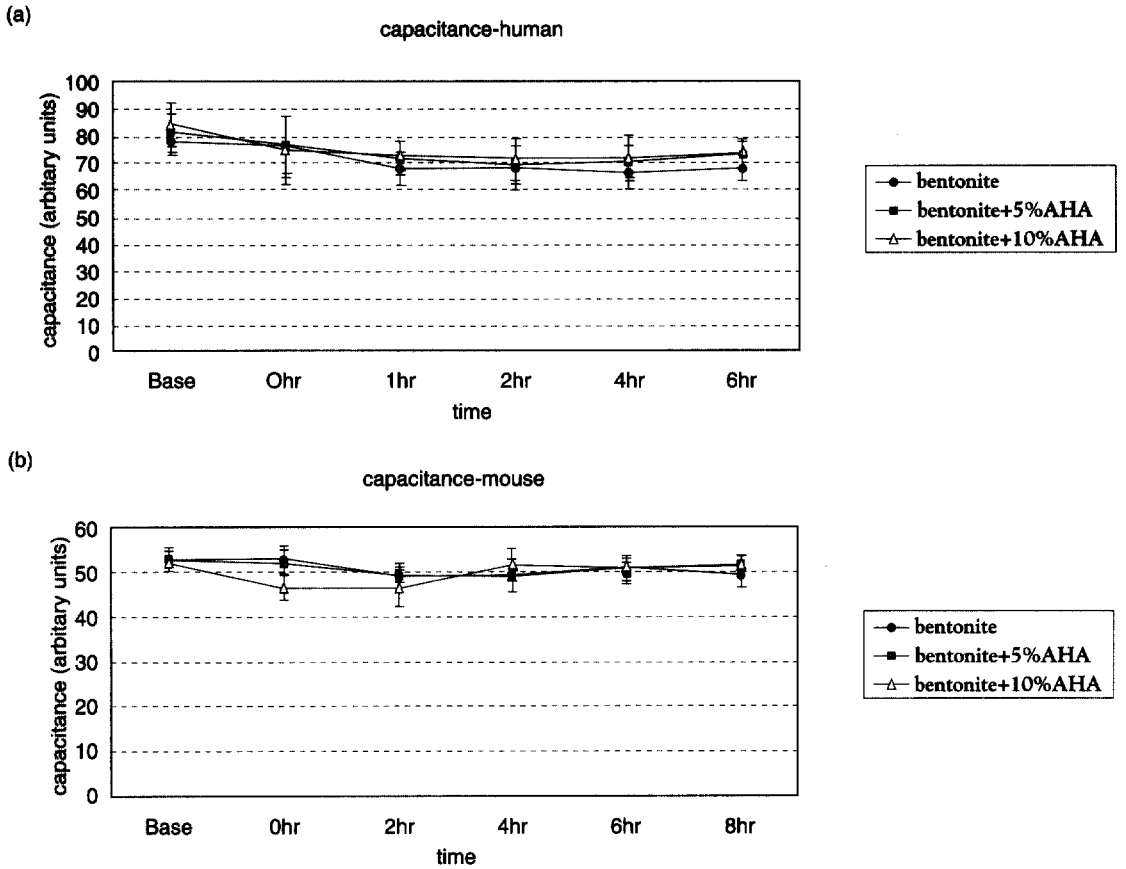

Fig. 2. Electrical capacitance as an indicator of stratum corneum hydration in human skin(a) and mouse skin(b).

10% GA mixed formulation group.

2. Measurement and methods

1) Measurement of TEWL and capacitance

TEWL and capacitance were measured at 0 hour, 1 hour, 2 hours, 4 hours and 6 hours by Tewameter TM 210(Courage-Khazaka, K ln, Germany) and Corneometer(CM 420, Schwarzhaup, K ln, Germany) after removal of vapor permeable membrane (Tegaderm®) which was applied for 2 hours occlusively on the volar forearm of the five men and flank skin of hairless mice after application of 12% bentonite, 12% bentonite and 5%, 10% GA mixed formulation(pH= 7.1). The study was performed at the same place where the temperature was maintained at 24-26℃ and humidity at 70%.

2) electron microscopic examination (Ruthenium tetroxide postfixation)

Skin biopsy was done at flank skin of hairless mice 15 minutes after removal of occlusive dressing. Each specimen went marcellation to the thickness less than 1 mm under stereomicroscope immediately and were fixed in modified Karnovsky solution which is formulated with 2% paraformaldehyde, 0.06% calcium chloride and 0.1M sodium cacodylate buffer pH 7.4. The fixed specimens were kept at room temperature for 1 hour and then refrigerated at 4℃ for 18 to 24 hours. Then, Karnovsky solution was removed and the specimens were washed 3 times in 40 minutes with 0.1 M cacodylate buffer. After that, they were postfixed with both 1% OsO_4, 0.1M cacodylate buffer at room temperature with light protection for 1 hour and 0.25% RuO_4, 0.1M cacodylate buffer with light protection for 45 minutes. After postfixation, they were washed with 0.1M cacodylate buffer

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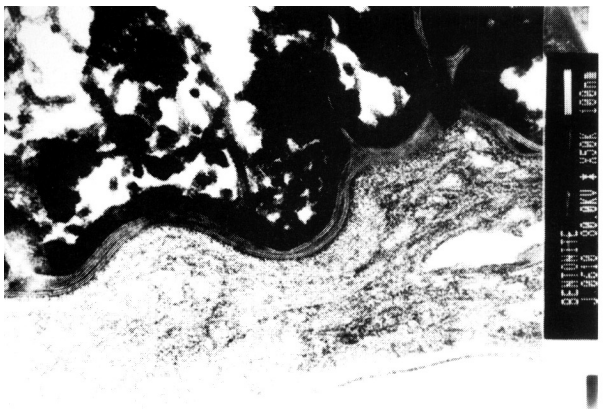

Fig. 3. After bentonite application. Lipid lamellae and desmosomes of corneocytes were not degraded at the junction of S. corneum and S. ganulosum. (Ruthenium tetroxide staining, Bar=100 nm).

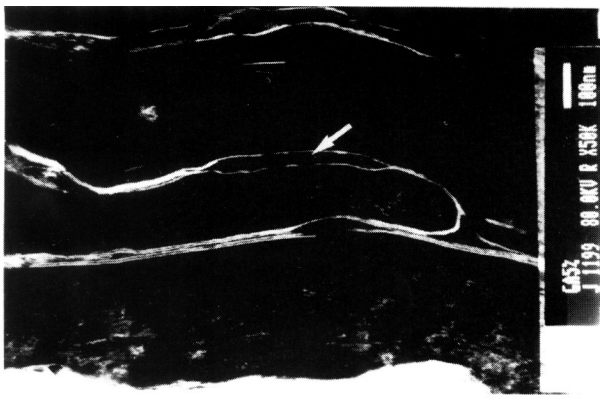

Fig. 4. After bentonite and 5% GA mixture application. Expansions of intercellular spaces by amorphous materials(arrow) were observed at the junction of S. corneum and S. ganulosum. (Ruthenium tetroxide staining, Bar=100 nm).

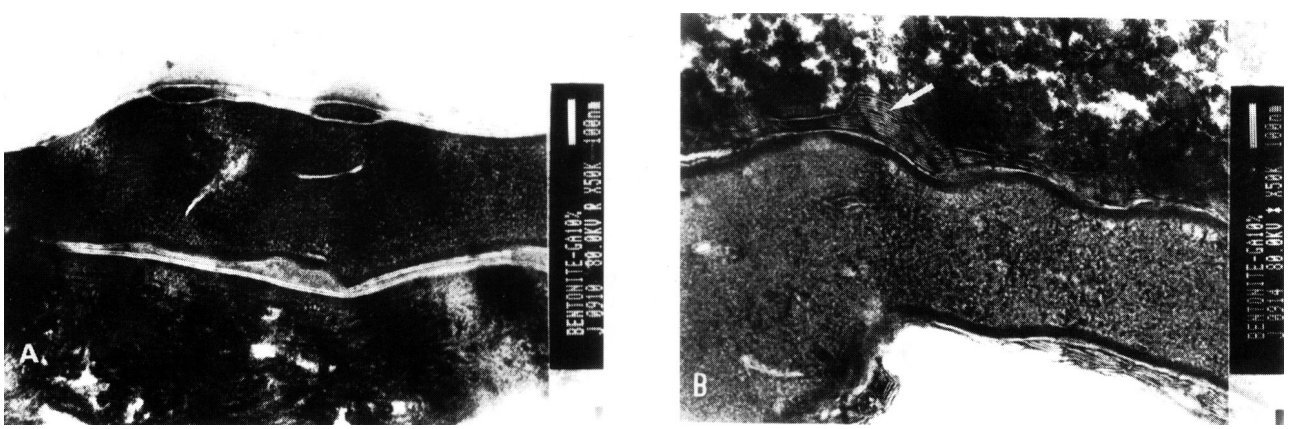

Fig. 5. After bentonite and 10% GA mixture application. Expansions of intercellular spaces by amorphous materials were more pronounced(A). Lamellar body(arrow) secretion was increased(B) at the junction of S. corneum and S. ganulosum. (Ruthenium tetroxide staining, Bar=100 nm).

once for 10 minutes, dehydrated twice with 50% ethanol, 70% and 95% ethanol for 10 minutes each, and 4 times with 100% ethanol for 20 minutes. After dehydration, 100% propylene oxide was penetrated to the specimen twice for 15 minutes each time and embedded to epon, then they were incubated for 36 hours in 78℃ incubator for polymerization of the resins. They were, then, sectioned to 1-5㎛ thickness and stained with uranyl acetate and lead citrate and studied with transmission electron microscope.

3. Statistical analysis

TEWL and capacitance of each group were compared and ANOVA tests were done to evaluate statistical significance and the level of significance was less than 0.05.

RESULTS

1. TEWL and capacitance

TEWL increased immediately and normalized 4 to 6 hours later in all 3 groups of human and mice(Fig. 1). The amount and rate of increase in TEWL did not have significant differences inbetween each group($p>0.05$). Moreover, capacitance of all 3 groups did not show any evidence of change in the water content of the stratum corneum before and

after the occlusion(Fig. 2)($p > 0.05$). After application of these formulations, any other clinical evidence of irritation symptoms such as erythema, edema, scaling or vesicle were not observed in all three groups.

2. Electron microscopic examination

Electron microscopic examination revealed that lipid lamellae and desmosome of corneocytes were not degraded in all 3 groups.

In the group with application of bentonite alone showed no degradation of Lipid lamellae and desmosomes of corneocytes(Fig. 3). The increase in lamellar body secretion at the junction of stratum corneum and granulosum was evidenced in the group with application of bentonite and 5% GA mixed formulation(Fig. 4). Finally, electron microscopic examination demonstrated a more extensive increase in all these features in the group with application of bentonite and 10% GA mixed formulation(Fig. 5).

DISCUSSION

Bentonite clay, It's been used to eliminate the sebum or effete materials from the skin and thus conserve the cleanness and dryness of the skin. Although these products are used widely, information on the mode of action and effects on the stratum corneum are little and controversial till now. Administration of low concentration(4-10%) of GA is known to have effects on moisturizing and softening of the skin, photoaging and acne which allows it to be widely added in cosmetic products lately[10,11]. Past studies[12,13,14] were performed with application of GA lotion for 2-4 weeks, however, in the present study, bentonite and 5% and 10 % GA mixed formulation were added occlusively to evaluate the acute structural and functional changes in stratum corneum.

After application of these formulations, erythema or any other clinical evidence of irritation symptoms were not observed in all three groups. TEWL was normalized 4-6 hours after removal of vapor permeable membrane without continuous increase, although there was slight increase which might have been due to the effect of occlusion. Capacitance did not show any changes as well. Thus, there was no signs of functional barrier damages.

Electron microscopic examination after ruthenium tetroxide postfixation revealed that there were no significant changes except slight expansion of lipid lamellae, and desmosomes of corneocytes were not degraded. However, lamellar body secretion and partially electron-lucent material was increased and lacunae was found in 10% GA and bentonite mixture-treated area. Nevertheless, it has not been evaluated whether continuous application would cause desquamation. Therefore, long-term use of these formulations should be estimated.

Fartasch et al[12] studied the change of stratum corneum after application of 4% GA lotion for 3 weeks. They reported that there were no differences in TEWL and capacitance and electron microscopic examination showed no degradation in desmosomes but more increase in desquamation and degradation of desmosomes in upper corneal layer than the control groups. In the same study, there was no change in the secretion of lamellar bodies. In normal skin, continuous application of AHA increase in water content of epidermis in some reports[9], but others report that it rather decreases the water content[13] or no changes[12]. In our study, capacitance which reflects the water content, did not alter significantly. Rather than this, there were some changes in the secretion of lamellar bodies according to GA concentration which suggests that the occlusive therapy of pack types have influences somehow.

Berardesca et al[14] reported that AHA is not a skin irritant by itself, but it increases the tolerance of stratum corneum to the irritants and surfactants, which results in preventing skin diseases due to functional barrier damage or irritant contact dermatitis. Yu et al[15], and Johnson et al[16] presume that the frequent problems we experience clinically, such as irritant reactions in low concentration of AHA are probably due to the problems in the solvent itself or in formulating procedures. Moreover, Effendy et al[13] suggest that the irritating effect of ethanol solvent used in AHA formulations from their experiment cannot be excluded from the cause of irritating reaction they have encountered. In this study and the study of Fartasch et al[12], irritating symptoms such as erythema did not appear and any symptoms due to bentonite did not happened as well. However, Shin et al[17] proposed that irritating reaction might occur in facial area where the AHA or bentonite is most frequently applied.

They also presented that there might be distributional differences and differences due to the occlusion or longer duration of application, since they found the increase in erythema index and TEWL in 10% of the patients after applying 5% and 10% GA diluted in distilled water for 24 hours.

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