The Effect of Alpha Hydroxy Acids (Glycolic and Lactic acid) on Hairless Mouse Skin

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Background **: Alpha hydroxy acid containing products are now widely used as cosmetics or skin protectives because it is believed to have a favorable effect against the aging process of skin.**

Objective **: The study aimed to find the effects of AHAs (glycolic acid, lactic acid) on the skin of hairless mice.**

Methods **: Glycolic acid (10 %, pH 3.9), lactic acid (10 %, pH 6.0) and vehicle control were applied topically to the back skin of hairless mice for two weeks. The thickness of the skin was measured by histometric analysis in addition to Masson-trichrome staining, immunohistochemical staining for TGF-beta and a Northern blot assay for pro α-1(I) collagen mRNA.**

Results **: The change of the skin after topical treatment showed decreased mean epidermal thickness in the AHAs treated group, but the thickness of the dermis increased greatly compare to the controls (glycolic acid > lactic acid > control). Staining with Masson-trichrome and TGF-beta showed a relatively increased expression in the AHAs treated specimens. These effects were correlated to the increased expression of pro α-1(I) collagen mRNA from glycolic acid treated skin.**

Conclusion **: It is suggested that the favorable effects of AHAs treatment are achieved by increased dermal thickness associated with prominent collagen synthesis.**

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Key Words : Alpha hydroxy acid, Glycolic acid, Lactic acid, Collagen synthesis

Recently, attention to AHA (alpha-hydroxy acid) containing products and cosmetics has been increasing[1]. AHA represents a group of organic acids found in natural foods such as sugarcane (glycolic acid), milk (lactic acid), apples (malic acid) and oranges (citric fruits acid). It is well known that fermented fruit acids have long been used to improve the skin regardless of their unknown biologic effects. However, earlier studies by Van Scott and Yu[2-5] demonstrated that AHA treatment on the skin diminished the adhesiveness of the corneal layer at the lower portion of the stratum corneum. Consequently they tried lactic acid for the treatment of some hyperkeratotic disorders such as ichthyosis. Nevertheless, it is still uncertain whether alpha hydroxy acid has a direct modulatory action on the dermal components of skin[6-9].

In this present study, we demonstrated the direct effect of AHAs (glycolic and lactic acid) on the skin of hairless mice with semiquantitative histometric analysis, special tissue stains for collagen (Masson trichrome), immunohistochemical staining for TGF-beta and Northern blot hybridization of pro α-1(I) collagen mRNA.

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MATERIAL AND METHOD

1) Animals and AHAs

Hairless male mice (Hr+/kud), aged between 8

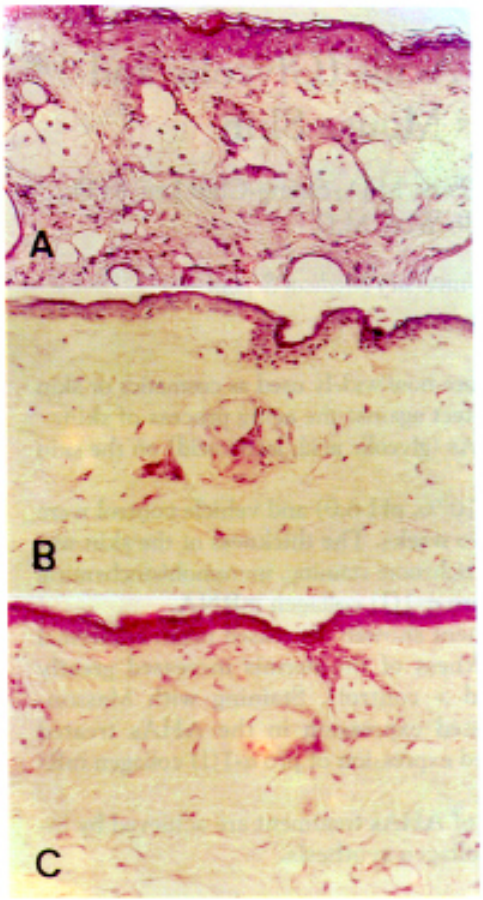

Fig. 1. Histological changes after two weeks of treatment: control (A), glycolic acid (B), lactic acid (C) (H & E ×200).

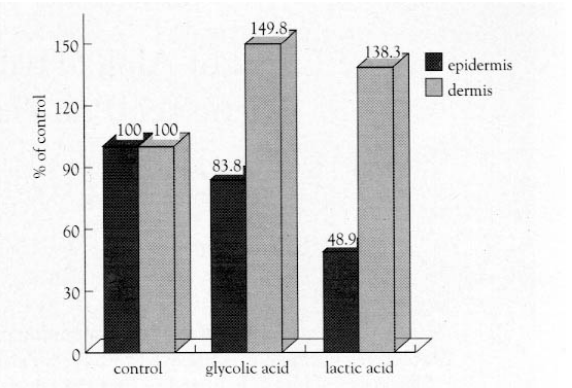

Fig. 2. Effects of glycolic and lactic acid treatment on epidermal and dermal thickness.

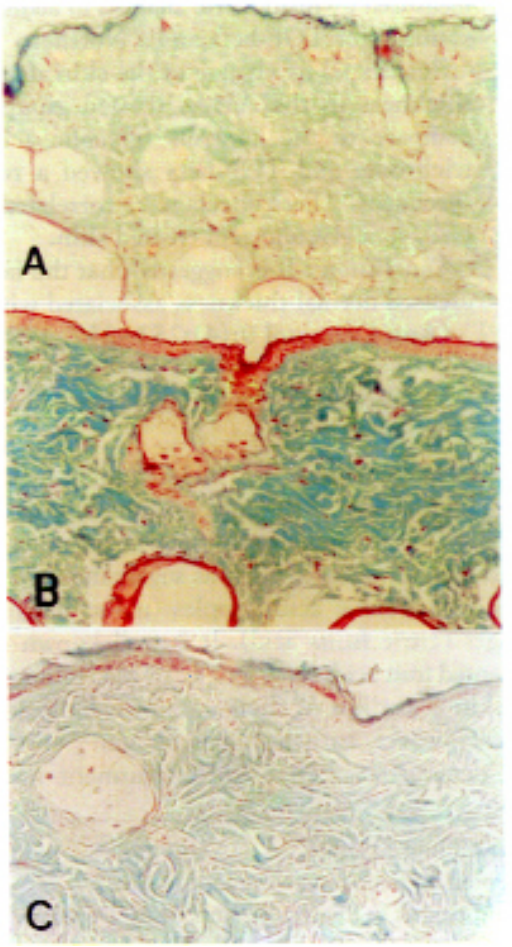

Fig. 3. Masson-trichrome staining : control (A), glycolic acid (B), lactic acid (C).

weeks and 10 weeks and ranging from 25-30 gm in weight were used. Animals were kept in clean rooms with controlled temperature and humidity and with 12 hour light/darkness cycles.

The diluted solution of AHAs (glycolic and lactic acid) were prepared in a vehicle composed of distilled water, ethanol and propylene glycol (2: 2: 1). The concentration and pH of the glycolic acid was 10 % (w/v), pH 3.9 and that of lactic acid 10 % (w/v), pH 6.0. All reagents were purchased from Sigma (St. Louise, U.S.A).

The mice were treated with 200 μl of each agent on the three standard areas (2×2 cm) of the dorsum, alternatively selected, twice a day for two weeks and sacrificed by cervical dislocation. Thereafter skin tissues were obtained for histometric analysis, special staining, and the Northen blot assay.

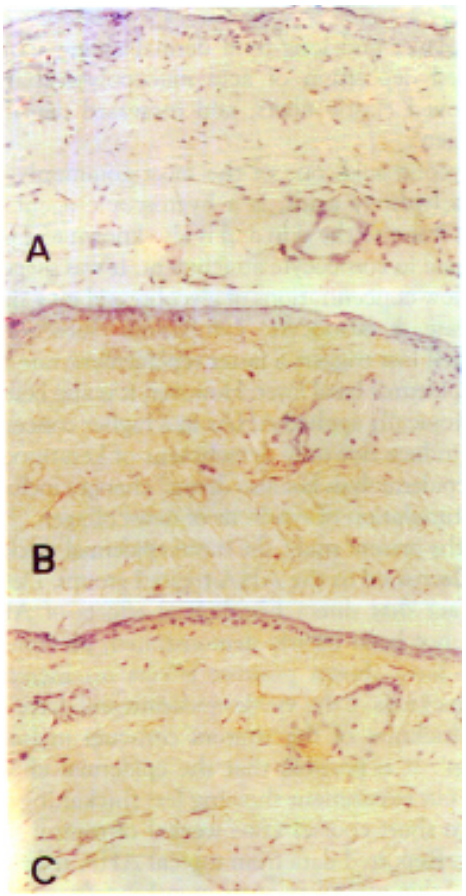

Fig. 4. Immunohistochemical staining with anti-TGF beta antibody shows relatively increased expression in glycolic(B) and lactic acid(C) treatment than control(A).

2) Histological evaluations

Twenty four hours after final treatment, the 5 mm sized punch biopsy specimens were taken to estimate the thickness of the epidermis and dermis in a routine light microscopic examination (stained with hematoxyline and eosin). The thickness from the upper layer of the stratum corneum to the upper border of the subcutaneous fat was measured by an eyepiece micrometer (American Opticals, U.S.A.) on a random access basis.

The other sections were stained with Masson-trichrome for collagen and TGF-beta with a standard technique of immunohistochemical method (ABC; avidin-biotin peroxidase complex system, DAKO, Denmark).

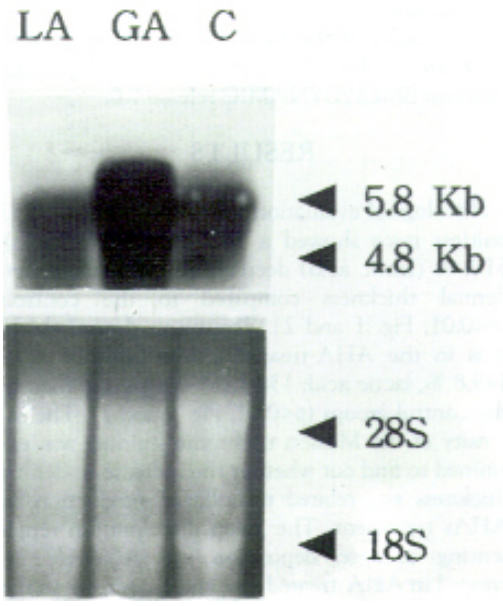

Fig. 5. Northern blot analysis of pro α1 (I) collagen mRNA transcripts from control (C) and glycolic acid (GA) or lactic acid (LA) treated skin.

3) RNA extraction and Northern blotting

The total RNA was isolated from hairless mice skin using the methods of Chomczynski and Sacchi[10]. The total RNA was lysed directly by adding a Guanidium-thiocyanate buffer, followed by phenol extraction and ethanol precipitation. RNA pellets were suspended in DEPC (diethylpyrocarbonate) treated water and the concentration of RNA was determined by measuring the absorbance at 260 nm and the purity of the nucleic acid preparation was assessed by the 260/280 nm ratio.

The total RNA was analysed by 1% agarose gel electrophoresis after denaturing the samples with formaldehyde and formamide[11]. The obtained RNA transcripts were transferred to the nitrocellulose filter in 20 X SSC overnight at 4℃[12]. The filter was prehybridized with a prehybridization mixture for 12 hours at 42℃, then hybridized with ^{32}P-labeled cDNA (pro α-1 (I) collagen) by nick translation at 42° for 24 to 36 hours. After hybridization, washing and autoradiography was performed in an X-ray film (Kodak, U.S.A.).

Statistics

The results of the histometric estimation were analyzed by the t-test from a statistical package program BMDP/DYNAMIC release 7.0.

RESULTS

Histological evaluation from 36 specimens of 12 hairless mice showed a 16.2 % (glycolic acid), 51.1 % (lactic acid) decrease in the average epidermal thickness compared to the controls ($p<0.01$, Fig. 1 and 2). The mean dermal thickness in the AHA-treated groups (glycolic acid; 149.8 %, lactic acid; 138.3 %) was greater than in the control groups ($p<0.01$, Fig. 1 and 2). The intensity of the Masson trichrome staining was examined to find out whether the increase in dermal thickness was related to collagen production by AHAs treatment. The green discoloration representing collagen deposition was relatively increased in AHA-treated specimens compare to the controls. There was some increase in thickness of the collagen fibers in the AHA-treated specimens as well (Fig. 3). The immunohistochemical expression of TGF-beta also revealed stronger staining intensity in the AHA-treated specimens than in the control group and the staining pattern was diffuse throughout the dermis (Fig. 4).

In the Northern blot hybridization from hairless mice skin with ^{32}P labelled pro α-1(I) collagen cDNA probes hybridiation revealed two mRNA transcripts, whose sizes were 5.8-kb and 4.8-kb. Steady-state levels of type I collagen mRNA increased in the AHA-treated groups, especially with glycolic acid, compared to the controls (Fig. 5).

DISCUSSION

Van Scott and Yu[2] first demonstrated the effect of topical AHAs in the treatment of ichthyosiform dermatoses. Subsequently, they tried AHAs clinically in xerosis, acne, seborrheic keratosis, actinic keratoses, intrinsically and extrinsically aged skin, and the prevention of skin atrophy caused by potent topical glucocorticosteroids[3-5,15].

Recently, attention has focused on the AHAs as a treatment agent of photodamaged skin and as chemical peeling agents[16-18]. The possible mechanisms of improvement in photodamaged skin were increased epidermal thickness by activation of the basal layer and long-term dermal changes by increased deposition of acid mucopolysaccharides, improved elastic fibers, and increased density of collagen[6].

Glycolic acid, one of the most commonly used alpha hydroxy acids, is a hygroscopic agent that binds water to the skin and is also known to cause a decrease in corneocyte attachment. It was suggested that low concentrations of glycolic acid may simply increase keratinocyte exfoliation, subsequently this cell loss triggers a faster replacement rate from the epidermal basal layer. However, it is also believed that topically applied AHAs in a higher concentration induce not only detachment of keratinocytes and epidermolysis but also dermal changes including the stimulation of synthesis of new collagen.[5,13,19]

In the present study, the mean epidermal thickness was decreased in the AHA treated groups. Despite the fact that direct histological effects of AHAs have not been clearly demonstrated, previous reports have shown positive effects on increasing the thickness of the viable epidermis and decreasing the thickness of the stratum corneum in human beings[8]. It is possible that the epidermal changes from our experiment showing less thickening were due to short courses (two weeks) of topical treatment. Reported data from topical AHA treatment on the skin was taken at six months[6], four weeks[8] by different concentrations and pHs, and three months[13] in which the epidermal thickness was prominent.

However, the increase of dermal thickness is apparent in AHAs treated groups. These effects were explained by increased synthesis of glycosaminoglycan, collagen and possibly elastic fibers, but not by edema formation[6,8]. Interestingly, there was no histological evidence of inflammatory cell infiltration which usually accompanied topical retinoic acid treatment. This result was comparable to the anti-inflammatory effects of topical glycolic acid against UVB irradiation which was demonstrated by Perricone and Dinardo[1]. Although the experimental design was different, the antiinflammatory action of AHA may play a role in less inflammatory cell infiltration.

In the present study we also analyzed the degree of staining intensity after using the Masson trichrome method. We found an increase in collagen deposition in AHAs treated tissues. TGF-beta is a well-known cytokine stimulating fibroblast that

produces many components of the extracelluar matrix including collagen and fibronectin[14, 20]. The intensity of the TGF-beta expression was relatively increased in the dermis by AHAs treatment suggesting such effects. The Northern blot hybridization yielded a higher densitometric value for pro α-1(I) mRNA in the AHAs treated groups. This finding is almost in accordance with the results of histological and immunohistochemical demonstrations of increased collagen synthesis.

What mechanism was involved in this effect of AHA ? It is still uncertain but increased dermal thickness with high expression of TGF-beta and pro α-1(I) mRNA suggested the favorable effect on the skin by prominent collagen synthesis.

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