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Alpha-hydroxy Acids: Consideration of the Biological Effects and Possible Role in Photococarcinogenesis

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ABSTRACT

The U.S. Food and Drug Administration nominated topically applied alpha-hydroxy acids to the U.S. National Toxicology Program for toxicological studies. The alpha-hydroxy acids are present at significant concentrations in many cosmetic products and are marketed as products to correct photoaging of skin. In this paper, we review the current knowledge concerning the effect of topical treatment of mice with creams containing alpha-hydroxy acids and the effect of ultraviolet radiation on treated and untreated mouse skin.

Key words: alpha-hydroxy acids, glycolic acid, phototoxicity, simulated solar light, SKH-1 hairless mice

INTRODUCTION

I. *Inclusion of Alpha-hydroxy Acids in Skin Care Products*

The alpha-hydroxy acids (AHA) are naturally occurring organic acids with a hydroxyl group alpha to the carbonyl carbon of the carboxylic acid group. Glycolic acid [2-hydroxyacetic acid; $CH_2(OH)COOH$] is the simplest AHA and is found naturally in sugar cane juice. A second naturally occurring AHA is lactic acid [2-hydroxypropanoic acid; $CH_3CH_2(OH)COOH$], which occurs in soured milk and in most fruits, tomato juices, wines and beer.

The most commonly used AHA in over-the-counter (*i.e.* prescription lotions) and cosmetic products is glycolic acid. The safety of products containing glycolic acid has been reviewed by a Cosmetic Ingredient Review Expert Panel[(1)]. Following its review, the expert panel recommended that skin care products contain no more than 10% glycolic acid at a pH no less than 3.5[(1)].

The AHA are keratolytic chemicals, and this property is evidently related to their relative acidity. The pKa for glycolic acid is 3.83. Therefore, a lotion that contains 10% glycolic acid at pH 3.5 actually contains 1.7 M glycolic acid very near its pKa. This provides a significant buffering capacity to the lotion and would have an effect on the pH of the skin after application[(1)].

II. *Use of AHA Containing Products*

Creams or solutions containing glycolic acid have been used for several decades by dermatologists to correct skin disorders including ichthyosis, acne, solar lentigos, melasma, xerosis, actinic keratosis, warts, seborrheic keratosis, and psoriasis[(2-4)]. The treatment of most of these conditions is under the care of a dermatologist and has been recently reviewed[(5)].

One commonly promoted use of products containing AHA is to correct photoaged skin[(6-12)]. This has resulted in reported widespread use among individuals who have photoaged skin either as the result of occupation or recreation choices (*e.g.* suntanning). It has been reported that application of creams and lotions containing AHA results in increased epidermal thickness, increased density in collagen, and altered skin elasticity. Investigators have reported a marked decrease in rough texture and wrinkling associated with photoaging and increased appearance of smooth and shiny skin after treatment with AHA.

III. *Manifestation of Use of AHA in Skin Care Products*

The exact mechanisms by which AHA elicit changes in the skin are not completely understood. It has been shown that application of creams containing AHA results in removal of the outermost layers of the strateum corneum[(2,13)]. The application of AHA containing creams or lotions to human skin results in increased epidermal thickness[(7)]. The epidermal thickness was increased by 25% when glycolic acid (25% solution) was used in a clinical study, while the increase in epidermal thickness was slightly less when 12% lactic acid was used[(14)]. The changes in epidermal thickness were accompanied by dispersal of melanin pigmentation and increased glycosaminoglycan and collagen synthesis[(14)]. Other studies have shown similar results, where clinical treatment of patients with 12% lactic acid[(15)]

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or 20% citric acid[16] resulted in a 12% and 40% increase in epidermal thickness, respectively. The increased epidermal thickness was accompanied by decreased skin wrinkles[11,12,15].

The use of AHA products has very few acute side effects. The most noticeable effects are prolonged erythema, stinging sensation, and postinflammatory hyperpigmentation[17,18].

IV. *Basis for Concern*

The concern about the continued use of AHA skin care products arises from the possibility that the users of these products may simultaneously continue exposing their skin to sunlight. The stratum corneum is the primary barrier to ultraviolet radiation (UVR) from sunlight. Continued removal of this layer could result in increased penetration of UVR into the epidermis. In addition, clinical studies have shown that application creams or lotions containing AHA results in increased epidermal cell proliferation, the possibility exists that more rapidly proliferating epidermal basal cells would receive greater DNA damage from the increased penetration of UVR. This increased genetic damage could result in increased skin cancer rates. On the other hand, the increased thickness of the epidermis following treatment with AHA could reduce the dose of UVR that reaches the basal cells of the epidermis, thereby reducing the level of DNA damage that would occur.

We have been working on the hypothesis that quantitative determination of the effects of AHA on the skin of hairless mice and the response of these mice to UVR contained in simulated solar light (SSL) will allow us to predict the tumorigenic outcome in humans who continuously use AHA containing products on sun-exposed skin. In the following sections, we review the pertinent studies reported to date concerning the effects of AHA on hairless mouse skin.

V. *Response of Mice to AHA Creams*

As summarized above, treatment of human skin with AHA results in increased epidermal thickness. We have examined[19] the results following treatment of SKH-1 hairless mice with AHA creams to determine if the changes are consistent with the effects in humans[19]. Mice were treated with a cream that was formulated[19] to be representative of the creams and lotions used in skin care products. Glycolic acid was added to a final concentration of either 4% or 10%, and the pH was adjusted to either 3.5 or 4. The cream was applied five days per week at 4 mg/cm^2 to the dorsal area of the mouse. After 3.5 weeks of treatment, the control cream and creams containing glycolic acid increased the thickness of the epidermis by approximately 75%[19]. The proliferation of the epidermal basal cells was increased by approximately 90% following application of the cream base, and increased with increasing concentration of glycolic acid to an approximate 270% increase for skin treated with 10% glycolic acid[19].

The kinetics of basal cell proliferation and skin thickness were determined[19]. Mice were treated for 2.5 weeks with 10% glycolic acid cream (pH 3.5), and the epidermal thickness and basal cell proliferation were determined for up to 48 hours. Even after 2.5 weeks of treatment, there was a temporal increase in basal cell proliferation 12-16 hours following the last treatment, and a temporal increase in epidermal thickness at 16-20 hours[19]. Similar kinetic "waves" of cell proliferation have been reported 12-15 hours following treatment of SKH-1 hairless mice with UVR[20].

In a subsequent study[21], we have examined the effect of treatment of mice with AHA on the quantity of UVR required to elicit an acute toxic response (*i.e.* edema). The amount of SSL required to induce edema (or basal cell proliferation) in naive mice is approximately 90 mJ/cm^2 [mathematically weighted with the Commission Internationale de l'Eclairage (CIE) human erythema action spectrum] of SSL[21-23]. Since 10 mJ(CIE weighted)/cm^2 equals one Standard Erythema Dose (SED)[22-23], we can conclude that 9 SED's of SSL induce edema in naive mice[21]. Mice were treated 5 days/week for 6 weeks with 1.4 SED, a dose of UVR used in standard photocarcinogenesis protocols[24]. After exposure to SSL for 6 weeks, the mice were treated with acute doses of SSL to determine the minimal dose required to induce edema and epidermal basal cell proliferation. This dose was 18 SED. Additional groups of mice were treated 5 days/week with 2 mg/cm^2 cream containing 10% glycolic acid, pH 3.5, and 1.4 SED of SSL, and the amount of SSL required to induce edema and basal cell proliferation was determined to be 18 SED. These results confirmed that continued exposure of mice to UVR results in increased epidermal thickness. This adaptation of the skin results in more tolerance to UVR, in that more UVR is required to induce edema or epidermal basal cell proliferation. The cotreatment of the mice with AHA did not increase the magnitude of this adaptive response, suggesting that co-treatment of mice with AHA does not alter the parameters associated with injury from acute and intense doses of UVR. These results, however, do not address the probability of AHA altering the tumorigenic potential of UVR in a chronic exposure model.

VI. *Photocarcinogenecity Studies with AHA Creams*

We are currently studying the effects of AHA-containing cream on the photocarcinogenicity of SSL. The protocol is designed to (a) quantify the SSL dose-dependent induction of skin tumors, and (b) the effects of co-treatment with AHA on SSL-induced skin tumors[24]. Mice are being treated 5 days/week at 2 mg/cm^2 with creams containing 0%, 4%, or 10% glycolic acid (pH 3.5) or 2% or 4% salicylic acid, a beta-hydroxy acid. The doses of SSL used were 0.7, 1.4, or 2.1 SED/day. The protocol design and the reasons for choosing SSL are outlined in Howard *et al.*[24].

Hong *et al.*[25] have reported the effects of AHA on UVR-induced skin tumors in SKH-1 mice. There are several differences between the ongoing study at our facility and

the study reported by Hong *et al.*[25]. These investigators applied glycolic acid twice each week at a dose of 8 mg glycolic per cm^2, whereas in our study the glycolic acid is applied 5 times each week at a dose of 0.2 and 0.08 mg/cm^2. Polyethylene glycol is used as the vehicle for the application of glycolic acid in Hong *et al.*[25], while we are using a cream formulation that is representative of the creams and lotions used by the public[19]. The mice in Hong *et al.*[25] were irradiated with UVR from a fluorescent light source, with a reported UVA:UVB ratio of 285:1. The dose of UVR was increased 10% per week. In our studies, the ratio of UVA:UVB (21:1) is very similar to sunlight, and the dose of UVR is kept constant throughout the study.

In the study of Hong *et al.*[25] UVR induced tumors starting after 9 weeks of treatment, and tumors occurred in 100% of the mice by week 20. The co-administration of glycolic acid reduced the onset of tumors to week 13, and only 80% of the mice had tumors by week 22. The results of this study indicated that co-treatment with AHA inhibited the development of tumors. Furthermore, the mean number of tumors per mouse was decreased in the mice treated with AHA and UVR when compared to the group receiving UVR alone.

CONCLUSIONS

AHA have been reported to reduce wrinkling in skin, and correct photoaging with concomitant increases in epidermal thickness in humans. We have demonstrated that treatment of SKH-1 mouse skin with the base cream or cream containing AHA results in increased epidermal thickness, and that basal cell proliferation was greatly enhanced with the creams containing AHA[19]. We have also shown that daily treatment of SKH-1 mice with AHA results in an increase in the amount of UVR required to induce toxicity (edema)[21]. The only published photocarcinogenesis study on AHA has suggested that AHA are photoprotective of UVR-induced skin tumors; however, an unpublished study has been used to suggest that topically applied lactic acid increased the carcinogenicity of UVR[26]. It is therefore unclear at this time whether topical application of glycolic acid with the established protocol used in this facility will result in increased or decreased UVR-induced skin tumors. The results of Hong *et al.*[25] are consistent with the observation from our laboratory that daily AHA treatment reduces the effect of an acute dose of SSL[21]. It is anticipated that the results from our current photocarcinogenesis study, along with clinical studies with AHA and UVR, can be used to determine the safety of continued use of AHA products on photoaged skin.

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Alpha-hydroxy Acids－皮膚之生物效應及光致癌效應之研究

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摘　要

在美國許多用於修補因光引發皮膚老化的化粧品中含有Alpha-hydroxy acids。然而是否有毒性效應並未有相關報告。因此美國Food and Drug Administration之毒理研究中心遂以有系統的動物模式，針對Alpha-hydroxy acids用於敷膚進行系列探討及毒理研究。本文主要提供當前對含有Alpha-hydroxy acids的化粧品相關研究之參考。

關鍵詞：Alpha-hydroxy acids，乙醇酸，光毒性，模擬太陽光，SKH-1 裸鼠