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Functional Changes in Human *Stratum corneum* Induced by Topical Glycolic Acid: Comparison with *All-trans* Retinoic Acid

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The effects of topical glycolic acid and *all-trans* retinoic acid on *stratum corneum* barrier function and hydration of human skin were investigated in 6 healthy volunteers utilizing non-invasive techniques. In addititon, changes in *stratum corneum* turnover time induced by the substances were examined using the dansyl chloride fluorescence test. Twelve percent glycolic acid in water and 0.1% retinoic acid in ethanol, respectively, were applied for 60 min once daily, over a period of 2 weeks (5 consecutive days weekly) on dansyl chloride-labelled skin and on untreated skin.

During a 10-day application period, both glycolic acid and retinoic acid similarly induced a significant increase in TEWL. However, after discontinuing treatment, TEWL in retinoic acid-exposed skin remained increased. Glycolic acid significantly reduced *stratum corneum* hydration from day 11 to day 18 ($p<0.05$), while retinoic acid induced skin dryness after 9 days of treatment, which persisted until day 18 ($p<0.005$). Whereas glycolic acid rapidly induced an intense erythema implying a direct non-specific inflammatory response, the retinoic acid-exposed skin gradually developed erythema. Retinoic acid caused scaling to a greater extent than did glycolic acid, even after treatment cessation. Both glycolic acid and retinoic acid significantly decreased *stratum corneum* turnover time and *stratum corneum* turnover time$_{50}$ (the time in days from labelling until approximately 50% of fluorescence disappeared), compared with the vehicle controls. However, glycolic acid shortened *stratum corneum* turnover time (12.8 ± 0.9 days) as well as *stratum corneum* turnover time$_{50}$ (7.3 ± 0.7 d) significantly more than did retinoic acid (15.8 ± 0.7 d and 9 ± 0.8 d, respectively). While ethanol (vehicle of retinoic acid) slightly but significantly decreased *stratum corneum* turnover time ($p<0.05$), water (vehicle of glycolic acid) did not.

This study showed that both glycolic acid and retinoic acid induced certain functional changes in *stratum corneum*, mirroring their irritation potential. However, changes at retinoic acid-exposed sites appeared longer-lasting, implying a distinct mode of action. An increase in *stratum corneum* turnover induced by the substances may be, in part, linked with their irritation properties. *Key words: alpha hydroxy acid; tretinoin; stratum corneum turnover; transepidermal water loss; stratum corneum hydration.*

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The mode of action of alpha hydroxy acids in various cosmetic and dermatologic conditions is not fully understood (1–4). Van Scott & Yu (2) suggested that the acids exert their desquamative effects by diminishing corneocytes cohesion at the innermost levels of *stratum corneum*. Alternatively, alpha hydroxy acids as acids may possess irritant properties which stimulate *stratum corneum* renewal and increase skin thickness. Some alpha hydroxy acids, however, seem effective without detectable clinical and histological signs of irritation (5). Currently, glycolic acid (GA) represents a widely used alpha hydroxy acid as chemical peeling agent and as a major component of skin care products (5).

Topical *all-trans* retinoic acid (RA) improves photodamaged skin (6–8). The precise mechanism by which improvement occurs remains unknown. The RA signal is thought to be transduced by nuclear receptors (the RAR and RXR families), which activate the expression of target genes via *cis*-acting transcriptional enhancer elements responsible for its biological effects (9, 10). However, the irritation property of RA could be, in part, accountable for some beneficial effects (11, 12).

Thus, GA and RA, two structurally unrelated compounds, seem to produce some relatively similar beneficial effects on certain dermatologic conditions (2). Whether the (similar or different) irritant properties of both acids are partly responsible for the effects has not been established. This study investigates and compares the effect of GA on water barrier function, hydration, and cell renewal time of human *stratum corneum*, using non-invasive bioengineering techniques and dansyl chloride fluorescence technique, with that of RA.

MATERIAL AND METHODS

Chemicals

GA (hydroxyacetic acid), *all-trans* RA (tretinoin), and dansyl chloride (5-dimethylamino-1-naphthalenesulphonyl chloride) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). RA was stored under protection from UV light. Ethanol (ethyl alcohol denaturated) was from Fisher Scientific (Pittsburgh, PA, USA). Deionized water and ethanol served as vehicle controls.

Subjects and test procedure

Six healthy volunteers (3 females and 3 males, age range 28 to 40 years) provided informed consent.

Prior to treatment the *stratum corneum* was labelled with fluorescent dansyl chloride according to the method described by Jansen et al. (13). Briefly, dansyl chloride was finely triturated into white petrolatum at 5% (w/w) and applied to one volar forearm of the volunteers under semi-occlusive dressing for 24 h. Subsequently, after removal of any excess material with soft tissue paper, test substances were applied. The clearance of the fluorescence was examined daily under UV illumination. *Stratum corneum* turnover time (SCTT) was the time in days between staining (day 0) and fluorescence disappearance.

One hundred μl of 12% GA in water (pH 7.0) and 0.1% RA in ethanol (pH 7.3), respectively, were applied to both dansyl chloride-labelled and untreated volar forearms using polypropylene chambers (19 mm diameter, Hilltop Laboratories, Cincinnati, OH, USA) on paper adhesive tape (Scanpor, Norgesplaster, Norway) for 60 min once daily (5 consecutive days weekly for 2 weeks). Occlusive application was necessary to avoid spread of the solutions into the adjacent skin. Patches with an erythema score of 3.0 or greater (see below) were not reapplied (further erythema score was arbitrarily assigned a

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3.0). Deionized water and ethanol, respectively, served as vehicle controls. The distribution of the chambers was randomized between panelists. Untreated skin served as a control site.

Instrumental measurements

Before and during the exposure period, each site was examined prior to reapplication of test substances on days 0, 2, 4, 9 and 11, and after discontinuing treatment, on days 16 and 18. TEWL, as an indicator of *stratum corneum* integrity, was measured with an evaporimeter (Servo Med, Stockholm, Sweden). TEWL measurements were conducted at ambient conditions (45%–65% relative humidity; 20°C–22°C); volunteers rested at least 15 min before measurements (14). Electrical capacitance as an indicator of *stratum corneum* hydration (15) was measured in duplicate with a capacitance meter (Corneometer CM820 PC, Courage & Khazaka, Cologne, Germany).

Clinical scoring

With the same time schedule as noted above, each test site on the unstained forearm was examined and graded for erythema and scaling by the same investigator according to a visual scoring system (16). *Erythema:* 0 = no erythema; 0.5 = equivocal reaction; 1 = slight erythema, either spotty or diffuse; 2 = moderate, uniform erythema; 3 = intense erythema; 4 = fiery redness with edema. *Scaling:* 0 = no scale; 1 = minimal, fine; 2 = moderate; 3 = large flakes, intense peeling.

The level of fluorescence on the stained forearm was assessed daily in the dark under UV illumination using an arbitrary scale of 0-10, where 10 = brightest fluorescent subsequently after staining (100%) and 0 = no longer fluorescent visibly (0%). SCTT represents the time in days between staining (day 0) and fluorescence disappearance, whereas '$SCTT_{50}$' describes the time in days from labelling until the level of remaining fluorescence of approximately 50%.

Statistical analysis

Differences in clinical scores, TEWL, electrical capacitance, SCTT, and $SCTT_{50}$ between the treatments were examined for statistical significance using the non-parametric Friedman test. This test affords a two-way analysis of variance by ranks for matched samples. When the Friedman test revealed significant differences between the treatments, multiple comparisons of all groups were conducted by the Wilcoxon-Wilcox tests.

RESULTS

Erythema and scaling

GA caused a considerable increase in erythema from day 4 to day 11 as compared to its vehicle ($p<0.005$); RA significantly produced more erythema than did ethanol (vehicle) from day 9 to day 18 ($p<0.005$) (Fig. 1A). GA markedly induced more erythema than did RA from day 9 to day 11 only ($p<0.005$). Noteworthy, GA induced few follicular erosions with edema after 5–6 days of exposure in all volunteers. The vehicle controls had no significant influence on erythema.

RA produced scaling to a greater extent than GA from day 9 to day 18 ($p<0.005$) (Fig. 1B). At day 18 scaling tended to increase at RA-treated sites. The vehicles alone did not produce significant scaling.

TEWL

Comparable to visual scores obtained, TEWL at RA-treated skin remained increased after treatment cessation (Fig. 2). RA markedly increased TEWL more than did GA on days 16 and 18 ($p<0.005$). At day 18, TEWL at the exposed sites maintained significantly higher levels than that of untreated skin ($p<0.005$), implying that water barrier function of *stratum corneum* was not yet recovered 7 days after discontinuing treatment. The vehicles had no significant effect on TEWL, although cumulative application of ethanol tended toward elevated TEWL.

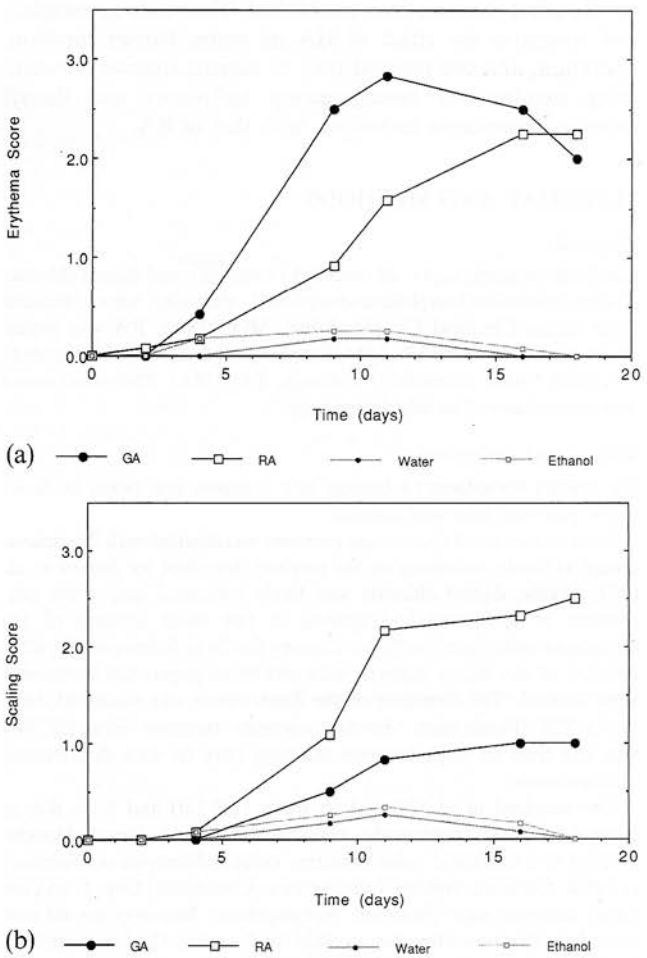

Fig. 1. Visual scoring after cumulative application of GA and RA, respectively, compared to the vehicle controls (water and ethanol).

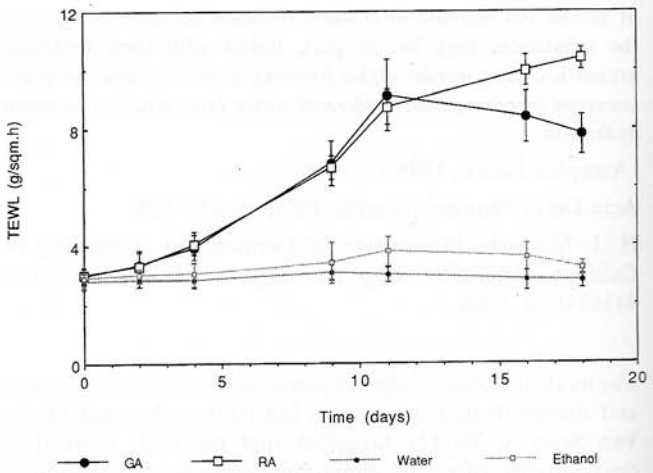

Fig. 2. Transepidermal water loss (TEWL) as an indicator of *stratum corneum* integrity.

Capacitance

GA initially elevated *stratum corneum* hydration on day 4 but significantly caused skin dryness from day 11 to day 18 ($p<0.05$) (Fig. 3). RA reduced *stratum corneum* hydration more than did ethanol (vehicle) after 9 days of exposure only ($p<0.05$) but continued to decrease until day 18 ($p<0.005$). However, cumulative application of ethanol caused a significant decrease in *stratum corneum* hydration on day 11 ($p<0.05$), whereas water did not at all stages compared with the untreated skin (control site).

SCTT

Both GA and RA significantly reduced SCTT (12.8 ± 0.9 days and 15.8 ± 0.7 days, respectively) more than did their vehicles (water: 18 ± 1 d; ethanol: 17.1 ± 1.3 d) ($p<0.005$ and $p<0.05$, respectively). However, GA shortened SCTT more than did RA ($p<0.005$) (Fig. 4). While daily application of water did not alter SCTT (18 ± 1 d), cumulative application of ethanol (17.1 ± 1.3 d) slightly but significantly reduced SCTT when compared with that of untreated skin (control site) (18.3 ± 0.9 d) ($p<0.05$).

$SCTT_{50}$ values did not absolutely match half of the SCTT (Fig. 4), suggesting a different velocity of fluorescence removal at early time points. GA decreased $SCTT_{50}$, more than did RA ($p<0.05$). $SCTT_{50}$ at water-exposed skin (10.5 ± 0.8 d) and ethanol-exposed sites (10.3 ± 0.5 d) did not differ significantly from that of untreated skin (10.7 ± 0.7 d).

DISCUSSION

This study investigated variances in functional changes in *stratum corneum* between GA-exposed and RA-exposed sites. The frequently used concentrations of alpha hydroxy acids range from 5% to 15% (3). The chosen dose of 12% (w/v) GA approximates the highest suggested effective alpha hydroxy acid concentration for dry skin and analogous conditions (3). 0.1% RA corresponds to the therapeutic concentration used in practice (17). However, note that the ethanol formulation –which was necessitated due to the poor aqueous solubility of RA –may enhance the percutaneous absorption of RA over the cream commonly employed in clinical practice. Hence, this circumstance should be taken into account when comparing and interpreting the results.

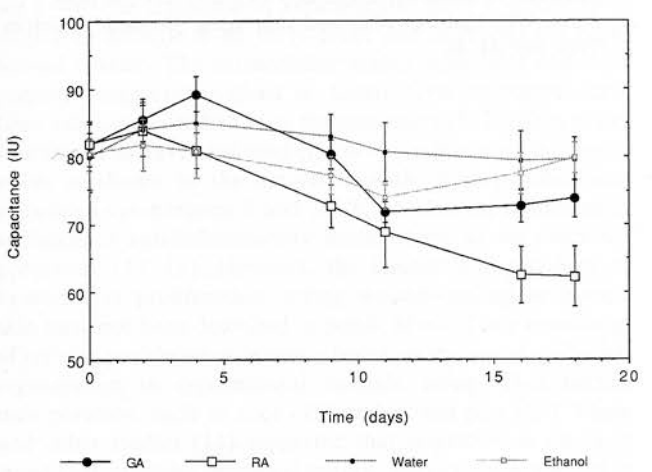

Fig. 3. Electrical capacitance as an indicator of *stratum corneum* hydration.

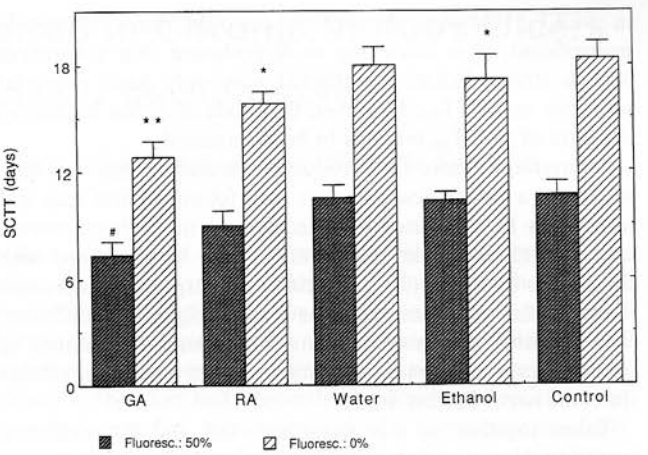

Fig. 4. Stratum corneum turnover time (SCTT) as indicator of cell renewal. $SCTT_{50}$ represents the time in days from labelling until approximately 50% of fluorescence disappeared.

GA, like RA, gradually impaired *stratum corneum* water barrier function when applied cumulatively. Hence, GA seems to hold a similar ability to alter *stratum corneum* as RA does. RA-induced changes in TEWL obtained during the exposure period were consistent with the findings of Tagami et al. (18). However, the present study revealed that after discontinuing treatment TEWL in RA-exposed skin continued to increase, suggesting an extended disruption of *stratum corneum* barrier. Similarly, RA persisted in a declined *stratum corneum* hydration. Thus, the lengthened increase in TEWL, as well as the long-lasting decrease in *stratum corneum* hydration induced by RA, may reflect a secondary, delayed cutaneous reaction. This phenomenon suggests a distinct mechanism of action between GA and RA.

The rapidly developing intense erythema and, particularly, follicular erosions with edema followed by GA demonstrated its strong irritancy potential. Whether the aqueous vehicle may be responsible for the convincing reactions is not yet clear. An intense scaling induced by RA is comparable to the effect associated with topical RA in acne therapy (17). Perhaps, the profound scaling provides an RA-specific effect on the skin.

The SCTT of untreated skin (18.3 ± 0.9 days) observed here accords with other studies (see 19 for ref.). A significantly shortened SCTT induced by GA and RA indicates that both acids have a substantial influence on *stratum corneum* cell renewal. However, cumulative application of ethanol also markedly decreased SCTT, indicating that changes in *stratum corneum* turnover may be, in part, irritant-specific (19, 20). Since SCTT in GA-exposed skin was markedly shorter than that at RA-exposed sites, GA seems to be a convincing accelerator of *stratum corneum* cell renewal. Either increasing concentration (from 0.1% to 10%) or decreasing pH (from pH 9 to pH 3) of GA may enhance its ability to stimulate cell renewal (21). Nonetheless, it was not clear in the report whether or not the possible distinct irritant effects observed in the two different groups were related to the cell renewal rates.

The $SCTT_{50}$ revealed a rapid fluorescence elimination induced by GA and RA at the early period. Since differences

in $SCTT_{50}$ between the vehicle controls were statistically insignificant, it is intriguing to hypothesize that substances with a strong irritation potential may only have a crucial influence on $SCTT_{50}$. However, the mode of action leading to the data of $SCTT_{50}$ remains to be determined.

Interestingly, since GA produced less scaling than did RA, scaling as a visible desquamative or exfoliative effect may not necessarily be associated with an increase in *stratum corneum* cell renewal. However, the results cannot be compared with the studies by Smith (21) reporting a corresponding increased number of corneocytes, since the study designs were different. Whether objective measurements of squamae or number of corneocytes under similar conditions would result in a similar outcome needs further study.

Taken together, in this assay both GA and RA exhibited irritation. However, RA-induced functional changes in *stratum corneum* lasted even after discontinuing treatment, suggesting a different mechanism. The irritation properties of the compounds may be, in part, responsible for an increase in *stratum corneum* cell renewal. Nevertheless, the data from the forearm may not extrapolate to the face – the typical application site of both substances.

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REFERENCES

1. Carrey FA. Organic chemistry. New York: McGraw Hill, 1992: 772–776.
2. Van Scott RJ, Yu R. Hyperkeratinization, corneocyte cohesion, and alpha hydroxy acids. J Am Acad Dermatol 1984; 11: 867–879.
3. Van Scott RJ, Yu R. Alpha hydroxy acids: procedures for use in clinical practice. Cutis 1989; 43: 222–228.
4. Ditre CM, Griffin TD, Murphy GF, Van Scott EJ. Improvement of photodamaged skin with alpha hydroxy acid (AHA): a clinical, histological, and ultra-structural study. Dermatology 2000 Congress. Book of abstracts. Vienna, 1993: 175.
5. Yu RJ, Van Scott EJ. Alpha hydroxy acids: science and therapeutic use. Cosmet Dermatol 1994, Suppl 1: 12–20.
6. Kligman AM, Grove GL, Hirose R, Leyden JJ. Topical tretinoin for photoaged skin. J Am Acad Dermatol 1986; 15: 836–859.
7. Olsen EA, Katz I, Levine N, Shupack J, Billys MM, Prawer S, et al. Tretinoin emollient cream: a new therapy for photodamaged skin. J Am Acad Dermatol 1992; 26: 215–224.
8. Griffiths CEM, Goldfarb MT, Finkel LJ, Roulia V, Bonawitz M, Hamilton TA, et al. Topical tretinoin (retinoic acid) treatment of hyperpigmented lesions associated with photoaging in Chinese and Japanese patients: a vehicle-controlled trial. J Am Acad Dermatol 1994; 30: 76–84.
9. Varani J, Astrom A, Griffiths CEM, Voorhees JJ. Induction of proliferation of growth-inhibited keratinocytes and fibroblasts in monolayer culture by sodium lauryl sulfate: comparison with all-trans retinoic acid. J Invest Dermatol 1991; 97: 917–921.
10. McKay IA, Winyard P, Leigh IM, Bustin SA. Nuclear transcription factors: potential targets for new modes of intervention in skin disease. Br J Dermatol 1994; 131: 591–597.
11. Fisher GJ, Esmann J, Griffiths CEM, Talwar HS, Duell EA, Hammerberg C, et al. Cellular, immunologic and biochemical characterisation of topical retinoic acid-treated human skin. J Invest Dermatol 1991; 96: 699–707.
12. Marks R, Hill S, Barton SP. The effects of an abrasive agent on normal skin and on photoaged skin in comparison with topical tretinoin. Br J Dermatol 1990; 123: 457–466.
13. Jansen LH, Hojyo-Tomoko MT, Kligman AM. Improved fluorescence staining technique for estimating turnover of the human stratum corneum. Br J Dermatol 1974; 90: 9–12.
14. Pinnagoda J, Tupker RA, Agner T, Serup J. Guidelines for transepidermal water loss (TEWL) measurement. Contact Dermatitis 1990: 22: 164–178.
15. Blichmann CW, Serup J. Assessment of skin moisture. Measurement of electrical conductance, capacitance and transepidermal water loss. Acta Derm Venereol (Stockh) 1988; 68: 284–290.
16. Frosch PJ, Kligman AM. The soap chamber test: a new method for assessing the irritancy of soaps. J Am Acad Dermatol 1979; 1: 35–41.
17. Leyden JJ, Shalita AR. Rational therapy for acne vulgaris: an update on topical treatment. J Am Acad Dermatol 1986; 15: 907–915.
18. Tagami H, Tadaki T, Obata M, Koyama J. Functional assessment of the stratum corneum under the influence of oral aromatic retinoid (etretinate) in guinea-pigs and humans. Comparison with topical retinoic acid treatment. Br J Dermatol 1992; 127: 470–475.
19. Wilhelm KP, Saunders JC, Maibach HI. Increased stratum corneum turnover induced by subclinical irritant dermatitis. Br J Dermatol 1990; 122: 793–798.
20. Effendy I, Weltfriend S, Maibach HI. Differential irritant skin responses to topical retinoic acid and sodium lauryl sulphate: alone and in crossover design. Br J Dermatol 1995; in press.
21. Smith WP. Hydroxy acids and skin aging. Cosmetic Toiletries 1994, 109: 41–48.