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The Impact of 10% α-Hydroxy Acid Emulsion on Skin pH

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Key Words
Fruit acid · α-Hydroxy acid · Topical application · Cutaneous application

Abstract
Background/Aims: The effects of a 10% α-hydroxy acid (AHA) oil/water (O/W) emulsion on the pH of human skin surface (pH_{ss}) and stratum corneum (SC; pH_{sc}) were evaluated in vivo. ***Methods:*** The AHA O/W emulsion was applied to an area on the volar forearm of male volunteers (n = 12), and then wiped off after 10 min. Prior to application and over the following 3 h, the pH_{ss} was measured. We used glass electrode measurements and time domain dual lifetime referencing (tdDLR) with luminescent sensor foils. In another experiment (n = 5), the impact of the AHA O/W emulsion on the pH_{sc} gradient was measured by tape stripping of the SC of the volar forearm after application of the AHA O/W emulsion. ***Results:*** Compared with pH_{ss} values prior to treatment [5.2 ± 1.7 (tdDLR) or 4.8 ± 0.5 (electrode)], the pH_{ss} was significantly reduced 10 min after application [4.0 ± 0.3 (tdDLR) or 4.1 ± 0.1 (electrode)] and the pH_{ss} remained significantly reduced over the measurement period of 3 h [after 3 h: 4.4 ± 0.2 (tdDLR) or 4.5 ± 0.3 (electrode)]. The AHA O/W emulsion significantly reduced the pH_{sc} even down to deep layers of the SC. ***Conclusion:*** After a 10-min application time, the 10% AHA O/W emulsion reduces the pH_{ss} (for at least 3 h) and pH_{sc} in deep layers of the SC.

Introduction

Topical fruit extract formulations are widely used for acne-prone skin, skin rejuvenation and regeneration purposes [1]. For example, glycolic acid (α-hydroxy acid, AHA) peelings have shown efficacy in the fight against signs of skin aging, melasma, hyperpigmentation disorders, acne and rosacea [2]. The pathomechanism is still unclear. It is known that AHA promotes epidermolysis, reduction of stratum corneum (SC) and collagen synthesis in the dermis [3]. The effects are mainly attributed to the pH of the final formulation. The SC pH gradient plays a crucial role in skin homeostasis and epidermal barrier. Abnormal pH gradients in the SC may be involved in the pathogenesis of several skin diseases such as atopic dermatitis, acne vulgaris and mycotic infections [4, 5].

Of clinical relevance, the changes in skin surface pH (pH_{ss}) may serve as one parameter to evaluate the intensity and duration of effects and may enable pH-based

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treatment [1]. In addition, the pH_{ss} is well known to affect bacterial growth and function, thus making antibacterial effects of acidic formulations conceivable [6, 7]. In terms of skin rejuvenation, previous studies have also investigated the impact of age on pH_{ss} [8].

To examine the effects of AHA on the pH_{ss} and on the pH of the SC (pH_{sc}), a 10% glycolic acid-containing oil/water (O/W) emulsion was applied to the volar forearm of healthy volunteers. The pH_{ss} was recorded prior to application and after removal of the cream. To obtain reliable data, the pH_{ss} was measured using two methods: the standard pH glass electrodes and luminescent pH sensor foils, a method previously described by our group [9]. In addition, the effect of AHA O/W emulsion on the pH_{sc} was studied to obtain data on the impact of AHA O/W emulsion on the pH in deeper skin layers by tape stripping according to a method used in a previous study by our group [9]. However, another protocol for standardized SC removal by tape stripping has recently been published [10].

Materials and Methods

Preparation of Microparticles and Sensor Foils

In short, fluorescein-isothiocyanate (FITC; Sigma-Aldrich Chemie GmbH, Talkirchen, Germany) was covalently conjugated to amino-cellulose particles (AC; Presens, Regensburg, Germany) to form FITC-AC pH indicator particles [9, 11]. Reference particles were synthesized by incorporating ruthenium(II)tris(4,7-diphenyl-1,10-phenanthroline) [$Ru(dpp)_3$; Sigma-Aldrich] in polyacrylonitrile (PAN; Sigma-Aldrich) to form $Ru(dpp)_3$-PAN particles [9, 12]. FITC-AC and $Ru(dpp)_3$-PAN (3:1) were mixed with 20 ml of a solution consisting of polyurethane hydrogel (Cardiotech International Inc., Wilmington, Mass., USA) in ethanol/water (90:10 v/v) [9, 13]. This mixture was spread on a transparent poly(vinylidene chloride) foil (Saran plastic wrap; Dow Chemicals, Midland, Mich., USA). For a detailed description of microparticle and sensor foil preparation, we refer to our methodology paper [9].

pH Measurement

pH was recorded using glass electrodes and the luminescent sensor foils. For luminescence imaging (distance from camera to skin: 8 cm; focus controlled), we used data from standard-sized squares (100 × 100 pixels for time course experiments; triplicate samples of 50 × 50 pixels for tape stripping experiments). For time course experiments, data were obtained from the exact spot where the glass electrode had been placed to measure the pH.

In short, luminescence intensity ratios R were calculated for each pixel according to the time domain dual lifetime referencing (tdDLR) method we previously described [9, 14]. Means of R were then computed for the respective area. Foils were calibrated, and a 5-parametric sigmoidal fit was performed. The resulting equation was then solved for pH, thus enabling us to calculate the pH and the respective H^+ concentration based on R [9].

The camera was combined with a quickly pulsating, light-emitting 460-nm LED array (Luxeon V Star LXHL-LB5C; Lumileds Lighting Company, San Jose, Calif., USA). To image 2D pH, tdDLR detection [14] was performed using an ImageX time-gated imaging system (TGI; Photonic Research Systems, Salford, UK) with an integrated 12-bit CCD (charge-coupled device) chip (640 × 480 pixels). For details, we refer to our methodology paper [9]. Calculations were performed with ImageX software (Microsoft Corporation, Redmond, Wash., USA).

Pharmaceutical Formulation

The O/W emulsion (pH 4; 7.5% fat) contained: H_2O, glycolic acid, isohexadecane, PPG (polypropylene glycol)-15 stearyl ether, propylene glycol, steareth-2, steareth-21, ammonium hydroxide, cetearyl alcohol, dimethicone, phenoxyethanol, stearic acid, palmitic acid and xanthan gum. A special emulsifier/emollient system produces an O/W emulsion with evidence of liquid crystalline structures known as oleosomes. Oleosomes consist of oil droplets surrounded by multiple lamellar layers of water, emollient and emulsifier. These extra layers of water and oil help to give long-lasting moisturization. Oleosome formation also gives the O/W emulsion enhanced stability. This is because the multiple layers around the oil droplet form a rheological barrier to coalescence, lowering the van der Waals attraction forces and increasing the time for droplet coalescence to occur (product data sheet, Arlamol PS15E; Croda Europe Ltd., Snaith, UK). Due to the intrinsic antimicrobial and antifungal action of glycolic acid, the preservative efficacy of phenoxyethanol alone is sufficient.

Study Subjects

Male volunteers (n = 12; 25.4 ± 3.6 years) were included for experiments on the duration of effects of 10% glycolic acid-containing O/W emulsion on the pH_{ss}. Swabs were taken from the treatment site before and after the study to detect possible changes in bacterial colonization. Male volunteers (n = 5; 26.2 ± 1.3 years) were included for tape stripping experiments to assess the effect on the pH_{sc}. 10% glycolic acid-containing O/W emulsion was applied to the volar forearm (4 cm^2) for 10 min, and then gently wiped off. None of the volunteers had any history of skin disorders, suffered from a skin condition or had been subject to dermatological treatment of the volar forearm in the past or were so at the time of measurement. The volunteers had not exercised, washed or applied topical formulations to the volar forearm for 24 h prior to the measurements. All participants were provided with verbal as well as written information on the study, and signed informed consent was obtained from each participant. All experiments were conducted in full accordance with the current revision (Seoul, Korea, 2008) of the Declaration of Helsinki (1964).

Statistics

We used Sigma Plot 11.0 (Systat Software Inc., Chicago, Ill., USA) for all analyses. Data are given as means ± SD of pH unless otherwise denoted. Means were calculated from the respective H^+ concentration values, which were obtained for each study subject, time point and number of strippings, respectively. Subsequently, mean pH values were calculated from the mean H^+ concentrations. Paired t tests were performed to analyze differences between baseline pH_{ss} values and during the time after cream application. Analogous testing was performed to analyze differ-

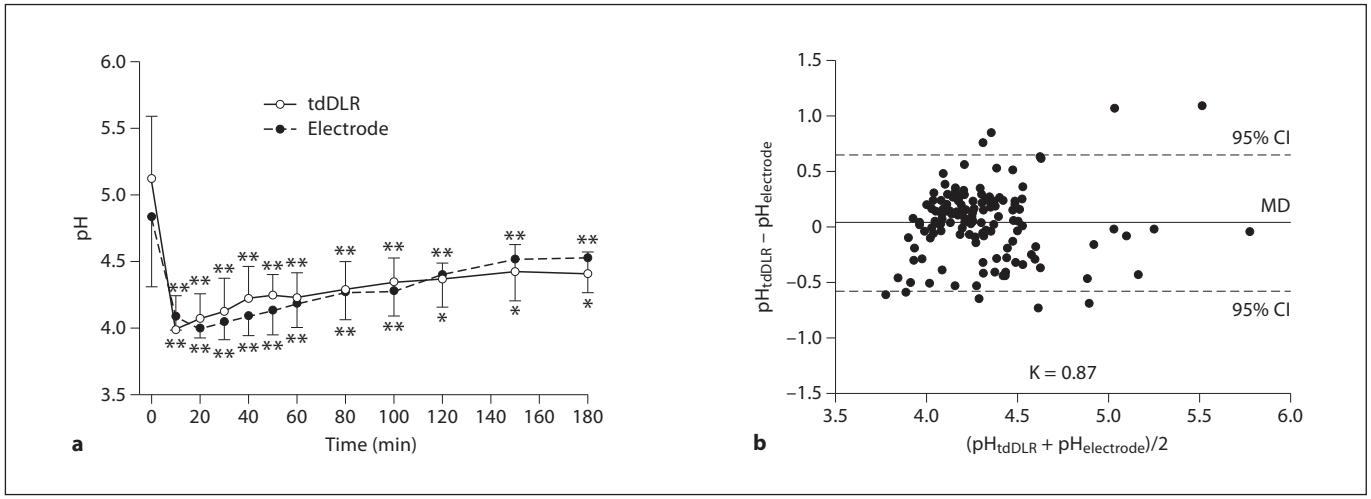

Fig. 1. pH_{ss} after topical application of 10% AHA O/W emulsion. **a** pH_{ss} before and during a time span of 3 h after application of 10% AHA O/W emulsion to the volar forearm. The pH_{ss} was significantly reduced 10 min after cream application and throughout the entire study time. n = 12. Means ± SD. * $p < 0.05$, ** $p \leq 0.01$ vs. respective baseline values. **b** Bland-Altman MD plot for method comparison (glass electrode vs. luminescence imaging using tdDLR). 94.5% of measurements were within the 95% CI, thus showing the reliability of luminescence imaging for evaluating the effect of topical pharmaceuticals on the pH_{ss}.

ences between baseline pH_{ss} and pH_{sc} after tape stripping experiments. In case normality testing failed, Wilcoxon signed-rank tests were used. We performed t tests to check for differences between the measurements obtained by luminescence imaging and glass electrodes. Mann-Whitney rank sum tests were used in case normality testing failed. We considered $p < 0.05$ to be significant, and $p \leq 0.01$ to be highly significant. Results were marked with one or two asterisks within the graph. To assess the precision of the methods, the relative standard deviation (RSD) of the measurements was calculated as (SD $mean^{-1}$) × 100%. For comparisons between methods, a Bland-Altman mean-difference (MD) plot [15] for pH measurements was created and the respective Krippendorff coefficient K calculated.

Results

Time Course of Effects on pH_{ss}

Prior to application of the 10% glycolic acid-containing O/W emulsion, the pH_{ss} amounted to 5.2 ± 1.7 (tdDLR) or 4.8 ± 0.5 (electrode) (fig. 1a). Three swabs (16.7%) taken before application of the 10% glycolic acid-containing O/W emulsion were positive, one for *Acinetobacter lwoffii,* one for *Micrococcus* spp., and one for coagulase-negative staphylococci. None of these was of clinical relevance. Ten minutes after application, the pH_{ss} was significantly reduced to 4.0 ± 0.3 (tdDLR) or 4.1 ± 0.1 (electrode; both methods: $p \leq 0.01$ compared with baseline). Throughout the entire study time of 3 h, the pH_{ss} remained significantly lower than before application of the 10% glycolic acid-containing O/W emulsion. Three hours after cream application, the pH amounted to 4.4 ± 0.2 (tdDLR) or 4.5 ± 0.3 (electrode). Swabs taken after 3 h were all negative.

Validity of Measurements

No significant differences between the values obtained by luminescence imaging (tdDLR) and glass electrodes were detected. The Bland-Altman MD plot shows 94.5% of measurements within the 95% CI (fig. 1b). The RSD was small for an in vivo setting (RSD_{tdDLR} = 5.0%; $RSD_{electrode}$ = 4.9%).

Effects of AHA O/W Emulsion on pH_{sc}

Prior to treatment, the pH_{sc} amounted to 5.2 ± 0.2 (fig. 2). Ten minutes after treatment with the 10% glycolic acid-containing O/W emulsion, the pH was significantly reduced to 4.4 ± 0.3 ($p \leq 0.01$ compared with baseline). Even after 60 tape strippings, the pH_{sc} was still significantly reduced at 4.9 ± 0.1 ($p < 0.05$) compared with baseline values prior to treatment. When compared with untreated pH_{sc} gradients which have been published in our methodology paper [9], the pH_{sc} after application of 10% glycolic acid-containing O/W emulsion was markedly reduced throughout the whole stripping experiment. The pH_{sc} was reduced even after complete removal of

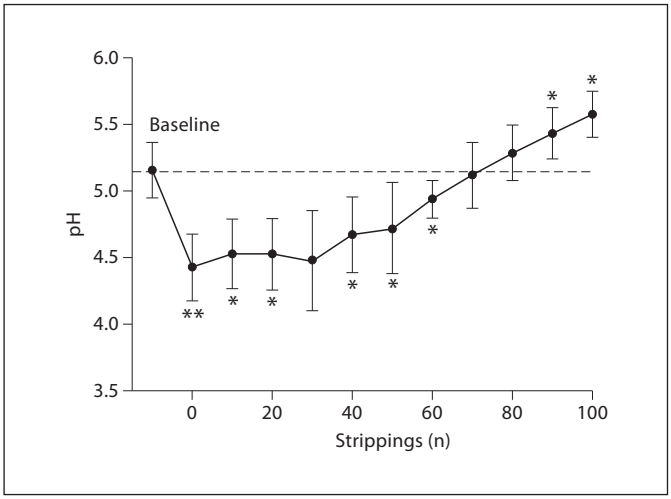

Fig. 2. pH_{sc} after topical application of 10% AHA O/W emulsion. pH_{sc} before (baseline) and after AHA O/W emulsion (0 strippings), and during tape stripping of the SC on the volar forearm. The pH_{sc} was significantly reduced after application of AHA O/W emulsion, even after 60 strippings (down to lower layers of the SC). After complete removal of the SC (80–100 strippings), the pH_{sc} was significantly higher than the baseline pH_{sc}. n = 5. Means ± SD. * $p < 0.05$, ** $p \leq 0.01$ vs. baseline.

the SC (80–100 strippings), thus indicating an effect of the 10% glycolic acid-containing O/W emulsion even on deep layers of the SC.

Discussion

In this study, we have shown that the application of 10% glycolic acid-containing O/W emulsion leads to a significant reduction in pH_{ss} for at least 3 h. Patients with a higher pH_{ss} may possibly benefit from such treatments, e.g. patients with bacterial colonization of the skin or epidermal barrier dysfunction. In addition, we have shown that it is possible to monitor the duration of effects of topically applied formulations on pH by continuous measurements with glass electrodes and luminescent sensor foils. However, it has to be mentioned that glass electrodes are not approved for clinical use. Therefore, luminescence imaging of pH using transparent sensor foils is an interesting alternative for clinical routine or investigational purposes. The foils are sterilized during the fabrication process and they are nontoxic.

The 10% glycolic acid-containing O/W emulsion leads to a significant reduction in pH_{sc} compared with the baseline pH_{sc} prior to treatment. Compared with the results for normal pH_{sc} values after tape stripping presented in our methodology paper [9], the pH_{sc} after application of 10% glycolic acid-containing O/W emulsion was markedly reduced. The pH_{sc} was significantly reduced even after complete removal of the SC, thus indicating an effect of the 10% glycolic acid-containing O/W emulsion on deep layers of the SC.

In conclusion, the 10% glycolic acid-containing O/W emulsion penetrates deep into the SC and exerts a long-lasting effect. The benefits of reducing or normalizing the skin pH with this emulsion have recently been clinically shown in patients suffering from mild acne [16].

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Disclosure Statement

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References

1 Scholz D, Brooks GJ, Parish DF, Burmeister F: Fruit acid extracts, a fresh approach to skin renewal. Int J Cosmet Sci 1994;16:265–272.

2 Knott A, Reuschlein K, Mielke H, Wensorra U, Mummert C, Koop U, Kausch M, Kolbe L, Peters N, Stab F, Wenck H, Gallinat S: Natural *Arctium lappa* fruit extract improves the clinical signs of aging skin. J Cosmet Dermatol 2008;7:281–289.

3 Yang EJ, Moon JY, Lee JS, Koh J, Lee NH, Hyun CG: *Acanthopanax koreanum* fruit waste inhibits lipopolysaccharide-induced production of nitric oxide and prostaglandin E_2 in RAW 264.7 macrophages. J Biomed Biotechnol 2010;2010:715739.

4 Cork MJ, Danby SG, Vasilopoulos Y, Hadgraft J, Lane ME, Moustafa M, Guy RH, Macgowan AL, Tazi-Ahnini R, Ward SJ: Epidermal barrier dysfunction in atopic dermatitis. J Invest Dermatol 2009;129:1892–1908.

5 Schreml S, Szeimies RM, Karrer S, Heinlin J, Landthaler M, Babilas P: The impact of the pH value on skin integrity and cutaneous wound healing. J Eur Acad Dermatol Venereol 2010;24:373–378.
6 Greenman J, Holland KT, Cunliffe WJ: Effects of pH on biomass, maximum specific growth rate and extracellular enzyme production by three species of cutaneous propionibacteria grown in continuous culture. J Gen Microbiol 1983;129:1301–1307.
7 Kjeldstad B, Johnsson A, Sandberg S: Influence of pH on porphyrin production in propionibacterium acnes. Arch Dermatol Res 1984;276:396–400.
8 Man MQ, Xin SJ, Song SP, Cho SY, Zhang XJ, Tu CX, Feingold KR, Elias PM: Variation of skin surface pH, sebum content and stratum corneum hydration with age and gender in a large Chinese population. Skin Pharmacol Physiol 2009;22:190–199.
9 Schreml S, Meier JR, Wolfbeis OS, Szeimies R-M, Landthaler M, Babilas P: 2D luminescence imaging of pH in vivo. Proc Natl Acad Sci USA 2011;108:2432–2437.
10 Dickel H, Goulioumis A, Gambichler T, Fluhr JW, Kamphowe J, Altmeyer P, Kuss O: Standardized tape stripping: a practical and reproducible protocol to uniformly reduce the stratum corneum. Skin Pharmacol Physiol 2010;23:259–265.
11 Posch HE, Leiner MJP, Wolfbeis OS: Towards a gastric pH-sensor: an optode for the pH 0–7 range. Anal Chem 1989;334:162–165.
12 Kürner JM, Klimant I, Krause C, Preu H, Wolfbeis OS: Inert phosphorescent nanospheres as markers for optical assays. Bioconjug Chem 2001;12:883–889.
13 Kocinkova AS, Nagl S, Arain S, Krause C, Borisov SM, Arnold M, Wolfbeis OS: Multiplex bacterial growth monitoring in 24-well microplates using a dual optical sensor for dissolved oxygen and pH. Biotechnol Bioeng 2008;100:430–438.
14 Liebsch G, Klimant I, Krause C, Wolfbeis OS: Fluorescent imaging of pH with optical sensors using time domain dual lifetime referencing. Anal Chem 2001;73:4354–4363.
15 Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986; 1:307–310.
16 Abels C, Kaszuba A, Michalak A, Werdier D, Knie U, Kaszuba A: A 10% glycolic acid containing oil-in-water emulsion improves mild acne: a randomized double-blind placebo controlled trial. J Cosmet Dermatol, in press.