Impact of a pH 5 Oil-in-Water Emulsion on Skin Surface pH

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Keywords

pH value · Human skin · Epidermal barrier · Acidification · Topical application

Abstract

Background: Human skin surface has a physiologically acidic pH (pH_{ss}). In cases of increased pH_{ss}, the acidity of the skin can be restored by topical formulations. We tested a pH 5 oil-in-water (O/W) emulsion for pH_{ss} regeneration and stabilization. **Methods:** We performed 2 experiments with 10 female study subjects in each. In both experiments, 2D imaging with luminescent sensor foils was used to determine pH_{ss}. Alkalization was reached by washing the volar forearm with a soap bar and warm running tap water for 20 min. Experiment 1: after defining the baseline pH_{ss}, we alkalized the respective area and measured pH_{ss} over a duration of 5 h, while applying emulsion every hour. Experiment 2: study subjects used the emulsion twice daily for 1 week. Then, pH_{ss} was measured before and after 5 min of washing a treated and an untreated area on the volar forearm. **Results:** (1) 5 h after alkalization, the treated arm showed a significantly lower pH_{ss} than the untreated one (5.87 ± 0.03 vs. 6.05 ± 0.03); (2) after washing, the treated area had a significantly lower pH_{ss} than controls (6.13 ± 0.03 vs. 6.27 ± 0.05). **Conclusions:** The tested pH 5 O/W emulsion seems to improve regeneration and stabilization of pH_{ss}.

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Introduction

It is known that the human skin surface has an acidic pH (pH_{ss}) and that there is a pH gradient within the stratum corneum (SC) reaching about neutral pH values in the deeper layers of the SC [1–4]. Different factors, such as the generation of free fatty acids [5], the expression of sodium/hydrogen exchanger isoform 1 (NHE1) in the epidermis [4, 6], and the production of urocanic acid from histidine in the SC [7], are known to contribute to the acidic pH_{ss} value of the human skin [8–10]. Acidic skin pH (pH_{ss} and pH_{sc}) is important for antimicrobial defense as well as for maintaining epidermal barrier homeostasis and SC integrity [5, 11–14].

pH_{ss} can, however, be altered by multiple factors. For example, alkalization of the skin surface can be caused by endogenous and exogenous factors, such as sweat, sebum, occlusive dressings, cleansers, particular soap bars, and alkaline skin care products [14, 15]. Fluhr et al. [5] and Ananthapadmanabhan et al. [16] showed that increased pH_{ss} affects epidermal barrier function and SC integrity/cohesion. Therefore, it is not surprising that many cutaneous diseases (e.g., atopic dermatitis, acne, ichthyosis, and mycotic infections) seem to be associated with an increased pH_{ss} [14, 17, 18]. These findings have led to the development of acidic skin care products. Several target groups that may profit from these products in particular

have been defined in other studies (e.g., elderly, patients with atopic dermatitis, diabetics) [14, 15, 19–22]. Prior research of our study group examined the effect of a pH 4 water-in-oil (W/O) emulsion as well as a 10% α -hydroxy acid O/W emulsion on pH_{ss} and pH_{sc} in healthy subjects and diabetics [19, 23].

In everyday life, the skin is regularly stressed by exterior influences, such as showering, cleaning, or sports (sweat), leading to increased pH_{ss} values that only slowly decrease to physiological levels. Faster regeneration as well as a higher resistance of the skin pH to external influences may be advantageous for epidermal barrier function. Therefore, the current study examined the effectiveness of an acidic oil-in-water emulsion (pH 5 O/W emulsion).

Materials and Methods

pH Imaging

Luminescent 2D imaging of pH_{ss} was done via RGB imaging [24]. pH sensor foils (SF-HP5R; PreSens GmbH, Regensburg, Germany) were preconditioned in Ringer solution (Merck, Germany) for at least 5 min prior to use. Sensors were gently applied to the skin surfaces and allowed to slowly adapt by adhesion forces. Signals were recorded with a luminescence pH imaging system (VisiSens; PreSens GmbH). The system records luminescence signals in the RGB color space. We developed this method in previous works [24]. The pH sensors were calibrated using 6 different PBS buffers (Merck, Germany) ranging from pH 5 to pH 7.3. Buffers were put into the wells of the calibration helper plate (CP-HP5R; PreSens GmbH). The imaging software (VisiSens Analytical 2; PreSens GmbH) was used to acquire the images of the pH optodes and to compute the quantitative maps from the raw sensor response images. Recorded data results in pseudocolor images of the 2D pH distribution (Fig. 1). Regions of interest (ROI; 100 × 100 pixels) in the images were chosen and used for calculating mean pH values.

Cosmeceutical Formulation

The pH 5 O/W emulsion we used for the study (Eucerin® pH5 Lotion; Beiersdorf AG, Hamburg, Germany) contained the following: aqua, cetyl palmitate, glycerin, paraffinum liquidum, panthenol, cetyl alcohol, polybutene, sorbitan stearate, aluminum starch octenylsuccinate, tocopheryl acetate, sodium citrate, citric acid, carbomer, dimethicone, phenoxyethanol, pentyleneglycol, ethylhexylglycerin, parfum, benzyl alcohol, limonene, linalool, butylphenyl methylpropional, hexyl cinnamal, alpha-isomethyl ionone, and benzyl salicylate. To alkalize the skin in our clinical trials, we used a commercial soap (Balea® Savona Creme Seife Milch & Honig, Seifenstück, 150 g; DM-Drogerie Markt GmbH & Co. KG) containing the following: sodium talloate, sodium cocoate, aqua, glycerin, hydrogenated coconut acid, parfum, mel, whole dry milk, tetrasodium etidronate, tetrasodium EDTA, sodium chloride, benzyl alcohol, hexyl cinnamal, limonene, and CI 77891.

Study Subjects

Healthy female volunteers ($n = 20$, age 23.9 ± 2.5 years, mean $\pm$ SD) with Fitzpatrick phototype I ($n = 4$), II ($n = 9$), or III ($n = 7$) participated in the study. The volunteers, all aged between 20 and 30 years, did not have any skin disorders in the past or at the time of measurement. The first experiment was carried out between December 2015 and January 2016, and the second experiment between March 2016 and June 2016. We recruited 10 study subjects for the first experiment and looked for 10 new volunteers for the second one. Written informed consent was obtained prior to the study. The participants did not exercise, wash, or apply any lotions to their volar forearms within 24 h prior to measurements.

Experiments

Experiment 1: Impact of a pH5 O/W Emulsion on pH_{ss} after Alkalization

Subjects ($n = 10$, age 25.2 ± 2.7 years) washed both of their volar forearms with running tap water ($37\text{--}39^\circ\text{C}$) and a soap bar for 20 min to alkalize the skin. As a first step, they wetted their volar forearms for 15 s under the running tap water and then foamed the soap bar in their hands for another 15 s. Afterwards, they rubbed both arms alternately for 1 min with their hands before washing the soap off again. The described steps were repeated until 20 min were over. Fresh paper towels were used to dry off the arms after the washing procedure. The volunteers were briefed to gently pat and not rub their arms with the paper towels. Before and after this procedure, pH_{ss} was measured on 3 different spots per arm. Subsequently, pH 5 O/W emulsion was applied to the right volar forearm. One hour after application, pH_{ss} was measured again in the same way on both arms (treated and untreated side). This process of measuring, treatment, and measuring again was repeated 4 more times.

Experiment 2: Long-Term Impact of a pH5 O/W Emulsion on pH_{ss}

Subjects ($n = 10$, age 22.7 ± 1.3 years) applied the pH 5 O/W emulsion to their right volar forearm twice daily, in the morning and in the evening, over a period of 7 days. The left arm stayed untreated and served as control. The study subjects were instructed to apply the emulsion (amount approximately the size of a hazelnut) on the whole right volar forearm, from the elbow to the wrist. The experiment always started with the first application in the evening (day 1) and ended with the last application in the morning (day 8), approximately 8–10 h prior to measurements. At the beginning, baseline pH_{ss} was measured on 3 different spots of both volar forearms. Subsequently, the arms were washed for 5 min in the same way as described in the first experiment with running tap water and a soap bar. Afterwards, pH_{ss} was measured again.

Statistics

Impact of a pH5 O/W Emulsion on pH_{ss} after Alkalization

Mean values were calculated for every subject and each measurement, resulting in 14 values per subject (7 measurements on both arms). These values were entered into a repeated-measures 2-way ANOVA to determine changes over time; the 2 factors were condition (left vs. right arm, respectively, untreated vs. treated) and time (baseline, after alkalization, after 1–5 h). Furthermore, the mean values for each time of measurement were calculated and

Fig. 1. Impact of a pH 5 O/W emulsion on skin surface pH (pH_{ss}). Pseudocolor images (100×100 pixels) of pH_{ss} 2D luminescent sensor foil measurements recorded with the imaging system VisiSens (PreSens GmbH) and processed with the imaging software VisiSens AnalytiCal 2 (PreSens GmbH). **a** Untreated left volar forearm ($n = 10$, age 25.2 ± 2.7 years). **b** Treated right volar forearm ($n = 10$, age 25.2 ± 2.7 years).

entered into t tests to specifically analyze the difference between values on the treated versus the untreated arm. We considered $p < 0.05$ to be significant and $p \leq 0.001$ to be highly significant.

Long-Term Impact of a pH5 O/W Emulsion on pH_{ss}

Again, the mean values were calculated for every subject and measurement, resulting in 4 values per subject (measurements before and after washing on both arms). These values were entered into t tests to compare mean pH_{ss} on the treated versus the untreated arm before and after alkalization. We considered $p < 0.05$ to be significant and $p \leq 0.001$ to be highly significant.

Results

Impact of a pH 5 O/W Emulsion on pH_{ss} after Alkalization

Due to the washing procedure, pH_{ss} increased from 5.83 ± 0.21 to 6.43 ± 0.19 (left volar forearm, mean $\pm$ SEM) and from 5.85 ± 0.20 to 6.47 ± 0.21 (right volar

forearm). During the 5-h observational period, the 2 values (pH_{ss} on the treated right volar forearm vs. pH_{ss} on the untreated left volar forearm) constantly decreased (Fig. 2). The 2-way repeated-measures ANOVA shows that this change in pH_{ss} during the 5-h period is highly significant on both arms ($F = 59.97$, $p \leq 0.01$). It also shows that the condition (treated vs. untreated) had a significant effect on the regeneration of pH_{ss} ($F = 8.17$, $p = 0.02$), i.e., pH_{ss} on the treated arm decreased significantly more quickly than that on the untreated arm. The interaction of time and condition was also found to be highly significant ($F = 9.48$, $p \leq 0.001$). The t tests revealed significant differences for pH_{ss} values on the treated versus the untreated arm for the measurements after 3 h ($p = 0.030$), 4 h ($p = 0.007$), and 5 h ($p \leq 0.001$) (Fig. 1, 2). Thus, 3, 4 and 5 h after alkalization, pH_{ss} values on the treated arm were significantly lower than those on the untreated arm.

Fig. 2. Short-term effect of pH 5 O/W emulsion on skin surface pH (pH_{ss}). Comparison: pH_{ss} of the untreated left (continuous line) and the treated right (dashed line) volar forearm of 10 healthy female volunteers before and during 5 h hours after washing both arms for 20 min with water and soap and applying pH 5 O/W emulsion to the right volar forearm every hour after the measurement. On the treated right volar forearm, pH_{ss} was significantly lower at 3, 4, and 5 h compared to pH_{ss} values on the untreated left volar forearm ($n = 10$, age 25.2 ± 2.7 years, triplicates, mean $\pm$ SEM). * $p < 0.05$, ** $p \leq 0.001$, t test.

Long-Term Impact of a pH 5 O/W Emulsion on pH_{ss}

After 7 days of treatment, no significant difference in baseline pH_{ss} was found on the treated arm compared to the untreated one (5.63 ± 0.03 vs. 5.68 ± 0.05). However, after washing the skin with water and soap, a significantly lower mean pH_{ss} could be observed on the treated volar forearm (6.13 ± 0.03) in contrast to the untreated arm (6.27 ± 0.05) (Fig. 3). This difference was found to be significant ($p = 0.022$).

Discussion

In the present study, we have shown that pH_{ss} regeneration after alkalization was accelerated by regular application of the pH 5 O/W emulsion. Thus, the tested lotion contributes to reducing the time span in which pH_{ss} is above its optimal range. This might be beneficial as the functionality of human skin (e.g., antimicrobial defense, permeability barrier homeostasis, and SC cohesion/integrity) seems to depend on an acidic skin pH [1, 5, 13, 14]. Bathing, showering, the use of alkaline cleansers (e.g., soap), or sweating lead to increased pH_{ss} [20, 25–27]. The

Fig. 3. Long-term effect of pH 5 O/W emulsion on skin surface pH (pH_{ss}). Comparison: pH_{ss} on the untreated left and the treated right volar forearm before and after 5 min of washing the skin with water and soap. Previously to the measurement, the volunteers had used pH 5 O/W emulsion twice a day for 1 week. Approximately 8–10 h passed between the last application (the same morning) and the measurements, depending on the daily routine of the study subjects. While there was no significant difference in the pH_{ss} baseline values, a significant difference could be observed after the washing procedure, when pH_{ss} was significantly lower on the treated right volar forearm than on the respectively untreated left volar forearm ($n = 10$, age 22.7 ± 1.3 years, triplicates, mean $\pm$ SEM). w/o, without pH5 O/W emulsion (untreated left volar forearm); w, with the application of pH 5 O/W emulsion twice a day for 1 week (treated right volar forearm); ns, no significant difference could be found. * $p < 0.05$, t test.

time needed for pH_{ss} regeneration after alkalization is between hours and days according to the respective literature [10, 13, 26, 28].

In our study, pH_{ss} on the treated arm recovered quicker than that on the untreated arm, indicating a beneficial effect of pH 5 O/W emulsion in terms of pH_{ss} recovery after alkalization. Furthermore, we found that pH_{ss} was significantly lower after the washing/alkalization process in an area which was regularly treated compared to an untreated control area. Thus, the used pH 5 O/W emulsion seems to have a stabilizing effect on the physiological human pH_{ss}. These findings highlight the benefits of the tested pH 5 O/W emulsion not only for short-term recovery after alkalization, but also in the long run as a method to reduce the effects of alkalization on the skin.

With regard to an influence of the pH 5 O/W emulsion on baseline pH_{ss}, we did not find a significant difference between the treated and untreated side. After regular ap-

plication of the pH 5 O/W emulsion, the baseline pH_{ss} on the treated forearm was only slightly more acid when compared to the untreated forearm. The values measured on the untreated forearm serve as general baseline values for the respective study subjects as we did not measure baseline values on both arms before the start of the application period. In the literature [29–32] as well as in our own first experiment, we found that there is no significant difference between the baseline pH_{ss} of different body sites, which is why we did not carry out further measurements.

The lacking significant difference between the baseline pH_{ss} on the treated and untreated arm after 7 days of application is not surprising as the pH value of the tested pH 5 O/W emulsion was only slightly below the general baseline pH_{ss} of the volunteers (5.0 vs. 5.68). A significant reduction of the baseline pH_{ss} could potentially be achieved by a longer application period or an O/W emulsion with a more acidic pH, like in previous studies [22, 23].

The results of this study could play a role in the prevention and/or therapy of cutaneous diseases, which have been linked to elevated pH_{ss} values [14, 17, 18]. Possibly, the use of acidic toiletries can prevent the development of pH_{ss}-associated skin irritations, e.g., in patients suffering from eczema. More research addressing this topic may help affected patients and promote changes in skin hygiene and skin care behavior. For the elderly, who are also known to have elevated pH_{ss} values and often suffer from

dry skin, acidic toiletries have already been recommended in the past [15, 19, 21, 22, 33].

In summary, the tested pH 5 O/W emulsion helps to stabilize pH_{ss}, which could be essential for the integrity and the barrier function of intact human skin.

In order to give a more precise recommendation about the general use of acidic skin care products, our findings need to be verified with further products and a larger sample size as well as an observation over a longer period of time. The question of which ingredient of the product is the crucial factor for its effect on pH_{ss} must be answered in further comparative studies with different emulsions of the same pH. Our test regimen with 2D luminescent sensor foils may also serve as an approach for other groups with similar questions.

Statement of Ethics

The experiments were conducted in accordance with the sixth revision (Seoul, Korea, 2008) of the Declaration of Helsinki (1964), and the local ethics committee gave approval (No. 06/171: 2007). Written informed consent was obtained from the participants prior to the study.

Disclosure Statement

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