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INVESTIGATIVE REPORT

Fruit Acids do not Enhance Sodium Lauryl Sulphate-induced Cumulative Irritant Contact Dermatitis *In vivo*

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Combined exposure to different irritants in the workplace may lead to irritant contact dermatitis, which is the main type of occupational dermatitis among bakers and confectioners. Following previous work on 'tandem irritation', a panel of healthy volunteers was exposed twice daily for 4 days to the organic fruit acids: citric, malic, and lactic acid, either alone or in tandem application with 0.5% sodium lauryl sulphate (SLS) in a repetitive irritation test. Irritant cutaneous reactions were quantified by visual scoring and non-invasive measurement of transepidermal water loss and skin colour reflectance. Twice daily application of either citric or malic acid alone did not induce a significant irritant reaction. Combined exposure to one of the fruit acids and SLS caused marked barrier disturbance, but the latter irritant effect was smaller than that obtained by combined exposure to SLS and water. Thus, combined exposure to the above-mentioned fruit acids and SLS did not enhance cumulative skin irritation. ***Key words: irritant contact dermatitis; foodstuffs industry; bioengineering; transepidermal water loss; sodium lauryl sulphate; citric acid; malic acid; lactic acid.***

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Employees in the food industry, particularly bakers and confectioners, are at high risk of developing occupational skin disease. Bakers were found to be the profession ranking at second highest risk for occupational skin disease in a population-based study (1). Irritant contact dermatitis (ICD) of the hands was identified to be the main type of occupational skin disease (2, 3). Exposure to detergents, fresh fruit and vegetables, preservatives and numerous additives together with wet work represents the common workplace situation in these professions. Particularly organic fruit acids may be suspected to induce epidermal barrier dysfunction and are sometimes blamed by employees suffering from chronic ICD. Originating from fresh fruits and vegetables as well as from sourdough or use in salad dressings, citric, malic and lactic acid are the main organic acids, with natural concentrations up to 5% for citric acid in citrus fruit and up to 2% for malic acid. However, to date the impact of those food components and their interaction with other irritants in the pathogenesis of ICD remains unclear, despite the widespread use of fruit acids in cosmetic dermatology for anti-ageing therapy (4, 5). Many studies have focused on methodological aspects and the effects of single irritant exposure (6–9), particularly sodium lauryl sulphate (SLS) (10), which has been studied as a common model irritant. Recently, our group has demonstrated that concurrent ('tandem') application of different irritants may modify the cutaneous response in contrast to repeated exposure to each irritant alone, thus indicating an aggravating effect of the combination of irritants (11–13).

Using non-invasive bioengineering methods, we therefore quantified the effects of combined exposure to organic fruit acids, such as citric acid, malic acid or lactic acid, and SLS *in vivo* that were repeatedly applied either alone or alternating with SLS in a panel of healthy volunteers.

MATERIALS AND METHODS

Study design

The study was performed in a single-blinded, randomized manner under standardized laboratory conditions between January and March 2002. Using air conditioning, room temperature was kept between 20 and 22℃, and relative humidity ranged between 30 and 40%.

Study population

Twenty healthy, non-preselected Caucasian volunteers (14 women and 6 men; aged 20–35 years, median age 28.5 years) without any skin diseases were included after informed consent. The study was approved by the Ethical Committee of the Friedrich-Schiller-University of Jena. During the study period subjects were allowed to shower as usual, but they were instructed to avoid application of detergents, emollients and moisturizers on their backs, as well as sun or UV exposure.

Procedure

The application areas were located on the clinically normal skin of the paravertebral mid back. According to the number of different treatment options (see Table 1) 15 test areas with a space of 3 cm in-between were marked with a stencil, resulting

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Table 1. *Test areas and different treatment options*

Area	Code	Morning	Afternoon
1	M2/M2	Malic acid; pH 2	Malic acid; pH 2
2	M4/M4	Malic acid; pH 4	Malic acid; pH 4
3	C2/C2	Citric acid; pH 2	Citric acid; pH 2
4	C4/C4	Citric acid; pH 4	Citric acid; pH 4
5	L2.5/L2.5	Lactic acid; pH 2.5	Lactic acid; pH 2.5
6	L4/L4	Lactic acid; pH 4	Lactic acid; pH 4
7	M2/SLS	Malic acid; pH 2	SLS
8	M4/SLS	Malic acid; pH 4	SLS
9	C2/SLS	Citric acid; pH 2	SLS
10	C4/SLS	Citric acid; pH 4	SLS
11	L2.5/SLS	Lactic acid; pH 2.5	SLS
12	L4/SLS	Lactic acid; pH 4	SLS
13	Aqua/SLS	Aqua dest.	SLS
14	Aqua/Aqua	Aqua dest.	Aqua dest. (negative control)
15	SLS/SLS	SLS	SLS (positive control)

SLS: sodium lauryl sulphate.

in four vertical rows. The test areas were randomized among the volunteers to avoid anatomical selection bias.

Fifty μl of the freshly prepared aqueous irritants were applied to each test area by an occlusive epicutaneous patch test system (Large Finn Chambers® on Scanpor®, 12 mm diameter, Epitest Ltd, Hyrlä, Finland, with filter discs). Patches were removed after 30 min and carefully dried with a paper tissue without rubbing, after rinsing the test areas with approximately 10 ml of tap water. After a 3-h interval, a second exposure with one of the irritants, according to the different treatment options, was performed. Using this scheme of application, each test site was repeatedly treated for 4 days, from Monday to Thursday (in each case at the same time of day ± 1 h), altogether resulting in eight single applications (9).

Test materials

The test areas were treated with 2% aqueous malic acid, pH 2 and pH 4; 5% aqueous citric acid, pH 2 and pH 4 (Fluka Chemie GmbH, Deisenhofen, Germany); 20% aqueous lactic acid, pH 2.5 and pH 4 (Merck KgaA, Darmstadt, Germany), and 0.5% aqueous SLS of highest purity (99%, Serva Feinbiochemie, Heidelberg, Germany), and aqua dest. pH 4 solutions were buffered to the target pH by using sodium bicarbonate (Merck KgaA). Test concentrations of malic and citric acid were chosen in accordance with realistic natural concentrations, e.g. in citrus fruit (citric acid) and plums (malic acid). However, the concentration of lactic acid was chosen according to our own previous experimental experiences with this irritant (8).

Two test areas, treated with aqua dest. both in the morning as well as in the afternoon, and with SLS both in the morning as well as in the afternoon, respectively, served as negative and as positive controls.

Measurements and instrumentation

Visual scoring, transepidermal water loss (TEWL) and skin colour reflectance measurements were used to assess skin irritation. Visual scoring and bioengineering measurements were performed each day in the morning, at the same time (± 1 h) by the same trained investigator, from day 1 to 4 prior to application of the patches. Final readings and measurements were performed on day 5. Before the measurements subjects had to acclimatize for at least 20 min to the standardized laboratory conditions.

Visual scoring was performed according to the scale of Frosch & Kligman (14), based on three main types of skin lesions: erythema, scaling and fissuring (erythema: 1+ slight redness (spotty or diffuse), 2+ moderate and uniform redness, 3+ intense redness, 4+ fiery redness; scaling: 1+ fine, 2+ moderate, 3+ severe with large flakes; fissures: 1+ fine cracks, 2+ single or multiple broader fissures, 3+ wide cracks with haemorrhage or weeping). Whenever the visual score developed to a value of $\geqslant 5$ in a single test field (cut-off criterion) the exposure to the irritant was prematurely discontinued in the respective field. In this case scores and values of the bioengineering measurements obtained at the time of discontinuation were carried forward and used for the final calculations.

TEWL (expressed in g/m^{2}h) as an indicator of the integrity of the epidermal barrier was measured using an evaporation meter (Tewameter TM 210®, Courage & Khazaka, Cologne, Germany) in accordance with the Guidelines of the Standardisation Group of the European Society of Contact Dermatitis (ESCD) (15). Measurements of erythema (a*) were conducted using a Minolta Chromameter CR-300® (Minolta, Osaka, Japan) following the guidelines of the ESCD (16) and recommendations of Elsner (17). For TEWL, each value used for the final calculations represented the arithmetic mean of two single measurements, in chromametry that of three.

Statistics

Statistical analysis was carried out using SPSS for Windows (Version 10.0, SPSS, Chicago, IL, USA). Data for visual scoring, chromametry and TEWL are presented as means and standard errors of means ($\pm$SEM). Differences between baseline (day 1, prior to irritation) and end (day 5, after the final irritation) were determined for TEWL and chromameter values (delta-values). Differences between different sites were checked for significance using the Wilcoxon test for paired data.

RESULTS

Results of measurements of visual scoring, TEWL and skin colour reflectance are given in Figs 1–3. There were no drop-outs or adverse reactions, except for the expected irritant skin reactions that occurred restricted to the test areas. However, in cases where the visual score exceeded a value of 5, no further irritation was conducted in the respective field (cut-off criterion), which was the case in only seven fields altogether.

Twice-daily application of either malic or citric acid alone as well as of aqua (negative control) did not induce any significant irritant reactions. There were no statistically significant differences between the fields treated twice daily with malic or citric acid and the negative control or between the different pH values (pH 2 vs pH 4) for those fruit acids. Twice-daily exposure to lactic acid pH 2.5 caused marked clinical irritant reaction (visual score) as well as marked erythema (chromametry), and distinct impairment of the epidermal barrier function as expressed by an increase of TEWL. This was not the case for lactic acid pH 4.

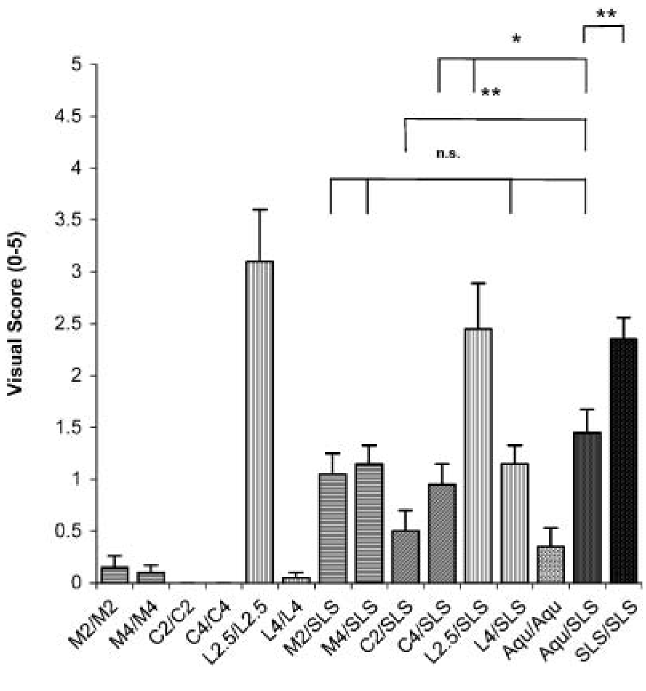

Fig. 1. Mean values and standard errors of the mean (SEM) of the visual score (differences to baseline) at day 5 of the tandem repeated irritation test (n.s., not significant; *$p \leqslant 0.05$; **$p \leqslant 0.01$; *** $p \leqslant 0.001$).

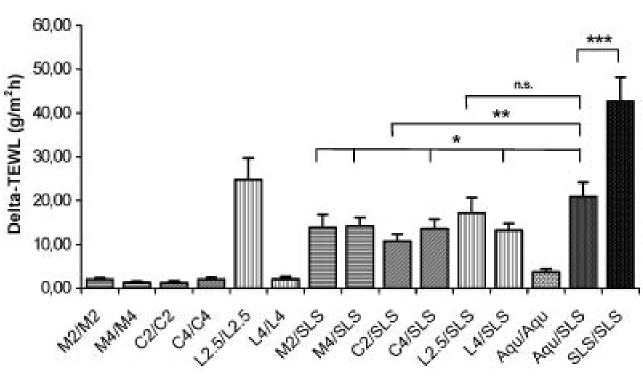

Fig. 2. Mean values and standard errors of the mean (SEM) of the transepidermal water loss (differences to baseline) at day 5 of the tandem repeated irritation test (n.s., not significant; *$p \leqslant 0.05$;

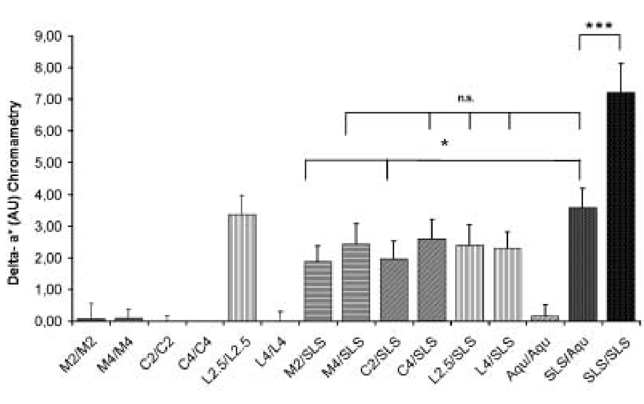

Fig. 3. Mean values and standard errors of the mean (SEM) of *a obtained by chromametry (differences to baseline) at day 5 of the tandem repeated irritation test (n.s., not significant; *$p \leqslant 0.05$; **$p \leqslant 0.01$; *** $p \leqslant 0.001$).

Alternating exposure to either of the three fruit acids and SLS caused a clear irritant reaction, indicated by an increase of the visual score, of the a*-value in chromametry, and a rise of the TEWL. These irritant reactions were less pronounced than that obtained by once-daily exposure to SLS (aqua/SLS), thus indicating a protective potential of the fruit acids against SLS-induced irritation. Regarding the results obtained by measurement of TEWL, those differences were significant for combined exposure to malic acid/SLS versus exposure to aqua/SLS, and for citric acid/SLS versus aqua/SLS, as well as for lactic acid pH 4/SLS versus aqua/SLS, respectively.

The most distinct irritant reaction was obtained by twice-daily exposure to SLS 0.5%, indicated by a moderate increase of the visual score, a marked increase in the TEWL values, and rise in the a*-values obtained by chromametry. Once-daily application of SLS (aqua/SLS) also induced a clear irritant reaction, but the increases of a*- and TEWL values were significantly lower than twice-daily SLS exposure.

DISCUSSION

Epidemiological data show a high incidence of chronic ICD for professionals in the food processing industry with bakers, confectioners, and cooks being particularly at risk (1, 3). Frequent skin contact with detergents together with a high load of wet work and insufficient skin protection is supposed to lead to ICD (1, 2). Besides these well-known factors, exposure to a variety of food ingredients and additives with suspected mild irritant potential has to be taken into consideration when looking closer at the mechanisms of ICD. Although organic fruit acids and their concentration-dependent effects on the epidermal barrier have been extensively studied due to their widespread use in cosmetic dermatology in concentrations from 8 to 70% (4, 18, 19), little is known about their role in the development of ICD in lower, natural concentrations and in combination with other irritants. Recent studies have directed awareness to the probability of synergistic or over-additive irritant effects induced by combined exposure to various irritants in different working environments (11). Combined exposure to detergents and organic solvents, which is relevant in many industrial workplaces, has been demonstrated to lead to an enhanced cumulative irritation in an experimental setting, by using the 'tandem repeated irritation test' (11, 13). In this regard, interaction of irritants relevant in the food industry is of great concern. However, our results do not indicate that exposure to low concentrations of organic fruit acids either alone or in combination with SLS significantly contributes to the development of ICD. This finding is not in accordance with some of the affected employees' perceptions, who sometimes blame acidic food ingredients for the worsening of their conditions. Sensory irritation on already impaired skin, which is well known for lactic acid, might in part be responsible for these stinging phenomena.

Of course, relevant irritation due to long-term exposure to fruit acids in higher concentrations cannot be excluded by the results of our study. Twice-daily exposure to lactic acid pH 2.5 that was used at a comparatively higher concentration of 20% did actually cause a significant irritant reaction, which might be attributed to the higher amount of free acid in the aqueous solution, compared with the buffered pH 4 solution. However, with the low concentrations chosen for citric and malic acid we did not observe relevant pH-dependent variablity of skin irritation.

We did not expect sequential application of fruit acids and SLS to result in a less pronounced irritant reaction than that obtained by once-daily exposure to SLS followed by aqua dest. Clinical trials have shown conflicting effects of alpha-hydroxy acids (AHA) on the stratum corneum barrier. On the one hand, desquamation and decrease of stratum corneum layers induced by AHAs (19) might enhance penetration of irritants into the barrier. On the other hand, low concentrations of fruit acids were reported to increase epidermal thickness and lamellar bodies as analysed by conventional histology and electron microscopy in humans as well as in animal models (4, 18). Repeated application of four different AHAs in 8% concentration was also found to reduce susceptibility to skin irritation with SLS as indicated by significantly lower TEWL compared with vehicle-treated controlled sites in a 4-week model (4). Our results are in accordance with these findings, although the short study period makes induction of epidermal changes questionable. Besides modulation of stratum corneum barrier function, interaction of fruit acids with the remaining SLS in the stratum corneum and vice versa, thus mitigating SLS-induced irritation, has to be taken into account.

In conclusion, fruit acids at the low concentrations encountered in workplace cannot be accused of significantly contributing to the development of ICD or increasing susceptibility to SLS-induced irritation. Indeed, experimental conditions and short-term exposure in this study have to be taken into account. The pathogenesis of ICD is complex and may be related to a combination of different types of irritants as well as to different types of irritation. Besides looking at different chemical irritants, further studies should also integrate the role of physical and mechanical irritation in the food industry (20, 21).

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