

Barrier function and natural moisturizing factor levels after cumulative exposure to a fruit-derived organic acid and a detergent: different outcomes in atopic and healthy skin and relevance for occupational contact dermatitis in the food industry

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Summary

Background. Fruit-derived organic compounds and detergents are relevant exposure factors for occupational contact dermatitis in the food industry. Although individuals with atopic dermatitis (AD) are at risk for development of occupational contact dermatitis, there have been no controlled studies on the effects of repeated exposure to multiple irritants, relevant for the food industry, in atopic skin.

Objectives. The aim of the study was to investigate the outcomes of repeated exposure to a fruit-derived organic acid and a detergent in AD compared to healthy volunteers.

Methods. The volunteers were exposed to 2.0% acetic acid (AcA) and/or 0.5% sodium lauryl sulfate (SLS) in controlled tandem repeated irritation test. The outcomes were assessed by measurements of erythema, transepidermal water loss (TEWL) and natural moisturizing factor (NMF) levels.

Results. In the AD volunteers, repeated AcA exposure led to barrier disruption and significant TEWL increase; no significant differences after the same exposure in the healthy controls were found. Repeated exposure to SLS and the irritant tandems enhanced the reactions and resulted in a significantly higher increase in TEWL in the AD compared to the control group. Cumulative irritant exposure reduced the NMF levels in both groups.

Conclusions. Differences in the severity of irritant-induced barrier impairment in atopic individuals contribute to the risk for occupational contact dermatitis in result of multiple exposures to food-derived irritants and detergents.

Key words: atopic dermatitis; fruit-derived organic acids; irritant hand eczema; natural moisturizing factor; occupational contact dermatitis; skin barrier function; sodium lauryl sulfate.

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Chronic irritant hand eczema is a prevalent cause of work-related skin disease and occupational disability in the food-processing, manufacturing and catering industries (1, 2). Data derived from large-scale epidemiological studies and disease registries show consistently that kitchen workers, cooks, bakers and confectioners are at particular risk for development of hand eczema that may become manifest as early as within the first 6 months of occupational training (3–6).

Repeated contact with water, detergents and organic compounds derived from the handling and processing of ingredients are common exposure factors contributing to the development of occupational irritant hand eczema in apprentices and employees in the food industry (7, 8). Although atopic dermatitis (AD) is a known individual risk factor for work-related hand eczema, the cumulative effects of repeated exposure to the above mentioned irritants in atopic skin under standardized conditions have not been investigated so far. Recently, we reported different outcomes following tandem repeated irritation with alkaline agents in AD volunteers and healthy individuals (9). Based on these findings, in the present study we asked whether differences in the summation effects exerted by repeated single and concurrent exposure to model irritants relevant for the food industry may contribute to the increased risk for development of occupational irritant hand eczema in individuals with AD. For this purpose, we investigated the severity of barrier impairment and the changes in the levels of the stratum corneum (SC) natural moisturizing factors (NMF) after tandem repeated irritation with acetic acid (AcA) and sodium lauryl sulfate (SLS) in AD volunteers with known filaggrin genotype, and compared the outcomes with those of identical exposure in healthy volunteers.

Materials and Methods

Twenty adult female and male volunteers with AD according to the UK Working Party Criteria (10) (median age, 28.9 years; range, 20–65 years) and 20 non-atopic, age-matched controls (median age, 31.1 years; range, 20–62 years) were included in the study. AD volunteers with inflammatory lesions or who had received any topical or systemic medication, except for antihistamines and emollients, within 30 days prior to inclusion were not considered to be eligible for participation. The study participants had been genotyped for the prevalent European filaggrin gene (*FLG*) mutations, as previously described (11, 12). Based on the genotype, the AD group included 8 *FLG* mutation carriers (AD *FLG*) and 12 non-carriers (AD wt); there were no mutation carriers in the healthy control group.

The study was performed in agreement with the principles of the Declaration of Helsinki, after approval by the Ethics Committee of the University of Lübeck (No. 09-044; 07-207) and written informed consent from all participants had been obtained.

Irritants and exposure

Fifty microlitres aqueous solution of 2.0% AcA (pH 2.6; Roth, Karlsruhe, Germany) and/or 0.5% SLS (99% purity;

Sigma-Aldrich, Steinheim, Germany) were applied over four consecutive days twice daily for 30 min under occlusion with large Finn Chambers® (12 mm diameter; SmartPractice, Barsbüttel, Germany) on previously marked test fields on the skin of the upper back, following a validated and previously published protocol; an adjacent non-exposed field (normal skin) served as a control (7, 9, 13, 14). As a result of the test procedure, the skin was repeatedly exposed to the following single irritants and irritant tandems: AcA/AcA, AcA/SLS, SLS/AcA and SLS/SLS, applied to the respective test field at the same time of the day (± 1 hour).

The volunteers were allowed to shower as usual. Application of skin care products in the test area was not allowed for the entire duration of the study (5 days).

Assessment of the skin irritant response

The irritant response was assessed by repeated non-invasive measurements of erythema and transepidermal water loss (TEWL) of the test and control fields performed every 24 hr, before the first application of the irritants on D1–D4 and before tape stripping on D5. Erythema was measured with a Chromameter CR200 (Minolta, Japan) and expressed in the $L^*a^*b^*$ system; the a^* -value reflecting the changes in the skin colour along the red-green axis was registered in the database. TEWL was measured with the open chamber system (Tewameter TM300; Courage and Khazaka Electronics, Cologne, Germany). For each parameter, two consecutive measurements per field were performed by the same observer under controlled environmental conditions (room temperature, $21 \pm 1^\circ\text{C}$; average relative humidity, 40–45%) and according to the published guidelines (15–17).

NMF sampling and analysis

The levels of the NMF components, including histidine, 2-pyrrolidone-5-carboxylic acid, *trans*-urocanic acid, and *cis*-urocanic acid, were determined with an established protocol that has previously been published in detail (9, 18). The samples were collected on D5, 24 hr after the last irritant exposure, by sequential tape stripping of the irritant-exposed and control fields with 14-mm standard sampling D-Squame® discs (CuDerm Corp., Dallas, TX, USA), as previously described (9, 19). For each volunteer, six tape strips per field were taken and stored in sterile 1.5 ml Eppendorf tubes (Eppendorf, Hamburg, Germany) at -80°C until analysis; the third and fourth D-Squame® discs were used for measurement of NMF.

The amount of SC collected by the tapes was assessed by measurement of the optical density (OD) of each

Table 1. Erythema (a^* -value) and transepidermal water loss (TEWL) in the atopic dermatitis (AD) and healthy controls (Ctrl) group at baseline (D1) and after 96-hour (D5) repeated single and tandem exposure to acetic acid (AcA) and sodium lauryl sulfate (SLS). Absolute values, mean $\pm$ SEM (n = 20 per group); level of significance < 0.05; *** p < 0.001; ** p < 0.01, * p < 0.05, compared to baseline. Control – non-exposed field (normal skin); AU – arbitrary units

		Control	AcA/AcA	AcA/SLS	SLS/AcA	SLS/SLS
a^* -value (AU)						
AD	D1	5.65 $\pm$ 0.34	6.38 $\pm$ 0.35	6.04 $\pm$ 0.34	6.03 $\pm$ 0.37	5.51 $\pm$ 0.32
	D5	4.95 $\pm$ 0.39	6.07 $\pm$ 0.58	7.33 $\pm$ 0.64***	7.43 $\pm$ 0.63**	9.54 $\pm$ 0.70***
Ctrl	D1	5.54 $\pm$ 0.42	6.48 $\pm$ 0.50	6.32 $\pm$ 0.49	6.27 $\pm$ 0.53	5.63 $\pm$ 0.43
	D5	5.00 $\pm$ 0.40*	6.25 $\pm$ 0.43	7.18 $\pm$ 0.59**	7.28 $\pm$ 0.58***	9.13 $\pm$ 0.91***
TEWL (g/m ² /hr)						
AD	D1	7.04 $\pm$ 0.39	6.88 $\pm$ 0.49	6.14 $\pm$ 0.35	7.03 $\pm$ 0.43	7.76 $\pm$ 0.42
	D5	6.72 $\pm$ 0.35	8.76 $\pm$ 0.59**	20.63 $\pm$ 2.05***	23.75 $\pm$ 2.18***	38.59 $\pm$ 2.35***
Ctrl	D1	6.36 $\pm$ 0.27	7.17 $\pm$ 0.43	6.28 $\pm$ 0.38	6.01 $\pm$ 0.34	6.45 $\pm$ 0.41
	D5	6.69 $\pm$ 0.29	7.65 $\pm$ 0.43	13.48 $\pm$ 1.29***	16.64 $\pm$ 1.61***	27.89 $\pm$ 2.87***

D-Squame[®] disc with the D-Squame Scan 850A (Monaderm, Monaco, France). The amount of SC was calculated from the OD, and expressed as the mass of protein/cm² according to a standardized procedure described elsewhere (20). The NMF components in the SC on the tape were extracted with 400 μ l of 25% (w/w) ammonia solution, evaporated to dryness, and reconstituted in 200 μ l of pure water. The extracts from two tapes were pooled together prior to high-performance liquid chromatography (HPLC-UV) analysis. The NMF levels were corrected for the amount of protein, and expressed as mmol NMF/g protein.

Statistical analysis

Statistical analysis was performed with GraphPrism Version 4 (GraphPad Software Inc., San Diego, CA, USA). The differences in the TEWL and chromametry a^* -values between the fields within the AD and healthy controls group at baseline were evaluated by ANOVA. The differences were considered to be statistically significant when the p -value was < 0.05. The differences in TEWL and the a^* -value over time were analysed by repeated measures ANOVA or Friedmann test for the respective field and parameter; for p -values < 0.05, a *post hoc* test was performed. The differences in the respective parameters (Δ -values compared to baseline) between the groups at each assessment point were analysed by unpaired t -test, Mann–Whitney test, or ANOVA. In the tables and figures, the values are presented as mean and standard error of the mean, unless otherwise indicated.

The differences in the baseline NMF levels between the groups subdivided by AD and *FLG* mutation carrier state were tested with the Kruskal–Wallis test followed by Dunn's multiple comparison test, and the differences in

the NMF levels after repeated single or tandem exposure were tested with the Friedman test followed by Dunn's multiple comparison test. The differences in the NMF decrease (% of control) between the control and AD groups, and between the AD wt and AD *FLG* groups, were tested for each exposure by a two-tailed Student's t -test or Mann–Whitney test (in case of non-Gaussian distribution). In the text and figures, the data are presented as median with interquartile range (IQR).

Results

At baseline, there were no significant differences in the a^* -values and TEWL of the test and control fields within the AD and the healthy control groups. In addition, there were no significant differences in the measured baseline parameters between the groups (Table 1).

The a^* -values before and after cumulative exposure to the single irritants and the irritants applied in tandem are shown in Table 1. Repeated exposure to AcA as a single irritant (AcA/AcA) induced no significant changes in the a^* -values in the AD and healthy controls group by the end of the study (D5); in contrast, the a^* -values of the test fields exposed to AcA/SLS, SLS/AcA and SLS/SLS increased significantly as compared with baseline. On D5, there were no significant differences in the a^* -value increase (Δa^*) between the AD and the control group or between the AD *FLG* mutation carriers and non-carriers.

Impairment of the skin barrier integrity after repeated exposure to AcA in atopic individuals

In the AD group, repeated exposure to AcA as a single irritant (AcA/AcA) led to impairment of the skin barrier function and significant increase in TEWL (TEWL

on D1 and D5, 6.88 ± 0.49 and 8.76 ± 0.59 g/m²/hr, respectively, $p < 0.01$; Table 1); the changes in TEWL after the same exposure in the healthy control group were not significant (7.17 ± 0.43 and 7.65 ± 0.43 g/m²/hr on D1 and D5, respectively, $p > 0.05$).

In both groups, the mean TEWL values after repeated exposure to SLS/SLS were significantly higher than at baseline (AD group – on D1 and D5, 7.76 ± 0.42 and 38.59 ± 2.35 g/m²/hr, respectively; control group – on D1 and D5, 6.45 ± 0.41 and 27.89 ± 2.87 g/m²/hr, respectively; for both, $p < 0.001$). Compared to the healthy controls, the AD group showed a more pronounced TEWL increase at each measurement time point and at the end of the study, the difference between the groups, assessed as Δ -value compared to baseline, was significant (Δ TEWL in the AD and control group, 30.84 ± 2.26 and 21.45 ± 2.94 g/m²/h; $p < 0.05$). Within the AD group, no significant differences in Δ TEWL between the *FLG* mutation carriers and non-carriers were found.

Significant differences in the severity of barrier impairment after tandem exposure to AcA and SLS in atopic and healthy individuals

Repeated sequential exposure to the irritant tandems AcA/SLS and SLS/AcA resulted in impairment of the skin barrier function; on D5, the TEWL values of the respective fields in both groups were significantly increased compared to baseline (Table 1). Monitoring of the TEWL increase every 24 hr showed consistently higher Δ TEWL values in the AD group at each measurement time point; at the end of the study, the differences in Δ TEWL between the AD group and the healthy control group were significant (Δ TEWL after tandem repeated exposure to AcA/SLS in the AD and control group, 14.49 ± 2.08 and 7.20 ± 1.43 g/m²/hr, respectively, $p < 0.01$; Δ TEWL after tandem repeated exposure to SLS/AcA, 16.73 ± 2.02 and 10.64 ± 1.67 g/m²/hr, respectively, $p < 0.05$; Fig. 1). As with repeated exposure to the single irritants, there were no significant differences in the severity of barrier function impairment (Δ TEWL) after tandem exposure to AcA/SLS or SLS/AcA between the AD *FLG* mutation carriers and non-carriers.

Repeated exposure to AcA and SLS leads to reduction of the NMF levels in the SC

The NMF levels (median and interquartile range) of the non-exposed (normal skin) control field in the AD volunteers and the healthy controls group were respectively, 0.35 (IQR 0.16–0.41) mmol/g protein and 0.48 (IQR 0.35–0.67) mmol/g protein; the differences

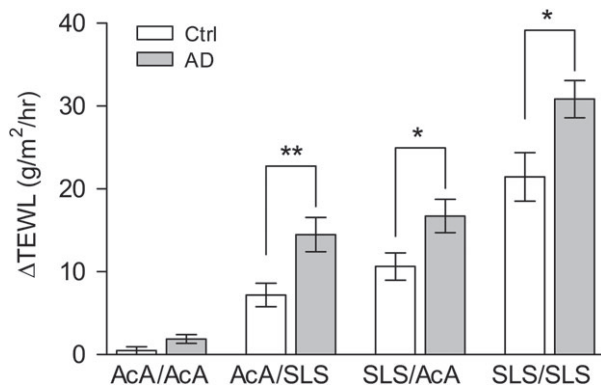


Fig. 1. Transepidermal water loss (TEWL) increase after repeated single and tandem exposure to 2.0% acetic acid (AcA) and 0.5% sodium lauryl sulfate (SLS) in volunteers with atopic dermatitis (AD) and healthy controls (Ctrl). The data are presented as Δ -value compared to baseline (mean $\pm$ standard error of the mean; $n = 20$ for each group); * $p < 0.05$, ** $p < 0.01$.

were significant ($p < 0.01$). Within the AD group, there was no significant difference between the carriers and non-carriers of *FLG* mutations [AD *FLG*, 0.16 mmol/g protein (IQR 0.15–0.41); AD wt, 0.37 mmol/g protein (IQR 0.24–0.41)].

Compared to the non-exposed skin, cumulative exposure led to reduced NMF levels of all irritant-exposed fields on D5. In both AD and healthy skin, the changes in the NMF levels were significant for the test fields exposed to SLS as a single irritant (SLS/SLS) and in tandem (AcA/SLS or SLS/AcA) (Fig. 2). Repeated exposure to SLS alone caused more pronounced changes in the NMF levels than repeated exposure to AcA alone, and the differences between the respective fields in both AD and healthy skin were significant (for both groups, $p < 0.001$). No significant differences in the relative changes of the NMF levels between the control and AD groups or between AD volunteers with or without *FLG* mutations were found.

Discussion

Irritant contact dermatitis, manifested as hand eczema, is the most common cause of occupational contact dermatitis in the food industry, and according to the data source, has been reported to account for up to 70% of all notified cases (2, 21). The more severe course, prolonged sick leave and more frequent job loss in food-related compared to other at-risk occupations determine a poor disease prognosis associated with notable impairment of life quality and substantial socio-economic costs (22–24).

The outcomes of occupational exposure to irritants show considerable variations, and understanding of

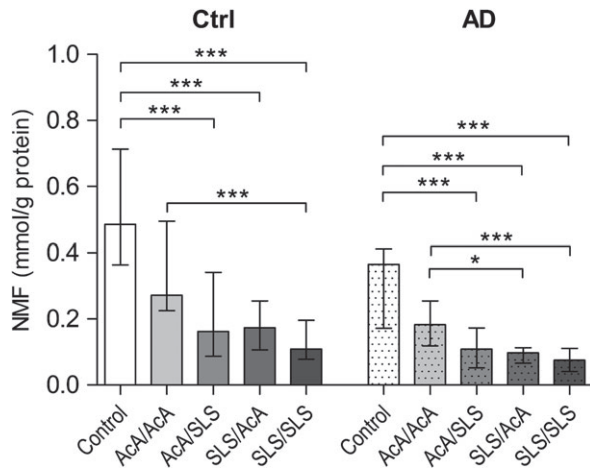


Fig. 2. Reduction of the natural moisturizing factor (NMF) levels in the stratum corneum after repeated single and tandem exposure to 2.0% acetic acid (AcA) and 0.5% sodium lauryl sulfate (SLS) in volunteers with atopic dermatitis (AD) and healthy controls (Ctrl). The data are shown as median with interquartile range; * $p < 0.05$, *** $p < 0.001$.

the factors that influence the endpoints is of primary importance for disease prevention. Our findings for differences in the severity of irritant-induced barrier impairment between AD and healthy individuals show that, under the same controlled exposure conditions, atopic skin is more susceptible to damage, even by mild workplace irritants that would not exert a significant negative impact on healthy skin. These results provide experimental background in support of the published epidemiological data and risk analysis in occupational groups related to the food industry (3, 25). Whereas there have been no investigations on the cumulative effects of combined exposure to organic acids and detergents in AD *FLG* mutation carriers so far, the findings of the study are consistent with earlier publications showing no genotype-dependent differences in the severity of irritant-induced barrier impairment (9, 26, 27). As in the previous publications, however, the conclusions are based on a small number of AD *FLG* mutation carriers.

Skin dryness and scaling are major clinical features of chronic irritant hand eczema (13, 14, 28, 29). Both symptoms may be early manifestations of irritant damage, and appear long before the visible signs of inflammation and the fully developed disease morphology. The skin dryness following irritant exposure has been commonly attributed to interaction with the intercellular lipids, resulting in changes in the skin lipid content and composition. Recent experimental studies however have shown that model irritants with alkaline pH consistently

reduce the NMF levels in the SC, and may thus possibly contribute to the dry skin phenotype found in patients with chronic irritant hand eczema (9, 19, 30).

In the present study, we extend these observations to irritants with pH within the acidic range, suggesting that the decrease of NMF may be a common endpoint that may not be related solely to the pH of the applied irritant. We used AcA, a naturally occurring weak organic acid found in low concentrations in fruit and frequently added to food as an acidity regulator or a preservative (E260 additive). AcA and other fruit acids are common relevant workplace irritants that have been frequently studied in the context of occupational irritant hand eczema in healthy individuals (7, 31, 32). Controlled and standardized exposure studies in at-risk populations, in contrast, have not been previously published.

The mechanisms by which the model irritants used in our study reduce NMF remain to be further explored. Several of the major NMF components are derived from filaggrin degradation, and irritants causing barrier disruption may impact multiple steps in this process related to alterations in the pH and ion gradients, changes in the activity of the enzymes involved in the generation of free amino acids, or the effects of occlusion and humidity, as previously reported (9, 30). Compared to AcA, in the present study, cumulative exposure to SLS had more pronounced effects on NMF and on D5 the differences in the NMF decrease of the respective fields, assessed as percentage of control, in both the AD and healthy controls group were significant. Whereas SLS is an alkaline agent that in addition to barrier abrogation may cause directly a shift in the skin pH and thus influence the levels of filaggrin breakdown products, the applied irritant concentration may be a further possible contributing factor for the observed differences.

Because of their mild effects on TEWL in healthy skin found in previous and also in the present tandem irritation study, AcA and other fruit-derived organic acids have been considered to be rather protective with regard to cumulative irritant-induced skin barrier damage. The different endpoints following the same exposure in AD volunteers were therefore unexpected but important outcomes of the study, providing a rationale for further assessment of the role of pre-existing barrier damage in the pathogenesis of occupational irritant hand eczema related to the food industry, using standardized repeated irritation models. Such an approach, together with research on the pathways underlying the irritant-induced NMF decrease, may prove valuable for development of targeted preventive measures to improve the unfavourable disease prognosis in both atopic and healthy individuals.

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