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INVESTIGATIVE REPORT

Skin Barrier Integrity and Natural Moisturising Factor Levels After Cumulative Dermal Exposure to Alkaline Agents in Atopic Dermatitis

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Dermal exposure to alkaline agents may lead to skin barrier damage and irritant contact dermatitis. The objective of this study was to investigate the effects of cumulative exposure to 0.5% sodium lauryl sulphate (SLS) and 0.15% NaOH on the barrier function and natural moisturising factor (NMF) levels in atopic dermatitis and healthy volunteers with known filaggrin genotype. The skin response was monitored by measurement of erythema and transepidermal water loss. The stratum corneum NMF levels were determined by high-performance liquid chromatography. Repeated exposure to 0.5% SLS and/or 0.15% NaOH in atopic dermatitis resulted in more severe impairment of the skin barrier function. Cumulative exposure to the irritants reduced significantly NMF in both the atopic and healthy controls group. The pronounced decrease of NMF after repeated single and sequential irritant exposure may be a pathogenetically relevant factor for development of chronic irritant contact dermatitis in both healthy and atopic individuals. ***Key words: alkaline agents; atopic dermatitis; filaggrin mutations; natural moisturising factor; transepidermal water loss; irritant contact dermatitis.***

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The maintenance of water balance in the stratum corneum (SC) is regulated through the intercellular lipids that provide a barrier to the transport of water and a complex mixture of low molecular weight water-soluble compounds such as amino acids, organic acids, urea and inorganic ions, collectively referred to as natural moisturising factor (NMF). Studies performed in the 1980s identified the epidermal differentiation protein filaggrin as the predominant source of free amino acids and their derivatives, which together contribute to more than 50% of the total NMF content (1). Filaggrin expression in the skin and, consequently, the levels of NMF in the SC were further found to be modified by both genetic and environmental factors (1). Independent studies have shown that atopic dermatitis (AD) carriers of filaggrin gene (*FLG*) loss-of-function mutations have reduced NMF levels compared to non-carriers (2–4). Furthermore, the levels of NMF assessed by Raman spectroscopy have been shown to correlate with *FLG* genotype and distinguish between homozygous and heterozygous mutation carrier state (2).

Earlier and recent studies provide evidence that individuals with a history of AD and/or *FLG* mutations have an increased risk to develop chronic irritant contact dermatitis (ICD) (5–7) as a result of repeated occupational exposure to chemicals. As AD and filaggrin deficiency are associated with reduced NMF levels while conferring susceptibility for occupational skin disease, we studied the effects of cumulative exposure to alkaline agents on the barrier function and NMF levels in AD volunteers with known filaggrin genotype. We next sought to investigate whether differences in the effects exerted by repeated single and concurrent exposure to the irritants may contribute to the enhanced risk of manifest chronic ICD in individuals with atopic skin disposition.

MATERIALS AND METHODS

Study population

Twenty volunteers with AD according to the UK Working Party Criteria (18 women and 2 men; median age 28.9 years, range 20–65 years) (8) who had been free of inflammatory lesions for at least 4 weeks prior to recruitment and 20 healthy age-matched controls (15 women and 5 men; median age 31.1 years, range 20–62 years) were included in the study. Atopic individuals with inflammatory lesions or who received any topical or systemic treatment, except for emollients, within the preceding 4 weeks were not considered eligible for participation. All AD as well as healthy volunteers had been previously genotyped for the prevalent European R501X, 2282del4, R2447X and S3247X *FLG* mutations as described (9, 10). Based on the genotype the atopic volunteers were divided into AD *FLG* mutation carriers ($n = 8$; 3 heterozygous carriers of R501X, 1 homozygous and 4 heterozygous carriers of 2282del4 alleles) or non-carriers (wt; $n = 12$). There were no mutation carriers in the healthy controls group.

The study was performed in agreement with the Declaration of Helsinki principles. The protocol was approved by the Ethics

Committee of the University of Lübeck (Nr. 07-207; 09-044) and all participants gave written informed consent beforehand.

Irritant exposure

Fifty microlitres of 0.5% sodium lauryl sulphate (SLS; 99% purity; Sigma-Aldrich, Steinheim, Germany) and/or 0.15% sodium hydroxide solution (NaOH; Mallinckrodt Baker, Deventer, The Netherlands) were applied over 4 consecutive days twice daily for 30 min under occlusion (12 mm Finn Chamber, Smart Practice, Barsbüttel, Germany) on the skin of the mid-back according to a validated and previously published protocol; an adjacent non-exposed field (normal skin) served as control (11–13).

In result of the application mode, the skin was repeatedly exposed from day 1 (D1) to day 4 (D4) to the following single irritants and irritant tandems: NaOH/NaOH, NaOH/SLS, SLS/NaOH and SLS/SLS, applied to the respective field at the same time of the day (± 1 h).

The volunteers were allowed to take showers as usual; the application of skin care products on the test area was not allowed for the entire duration of the study (5 days).

Monitoring of the skin irritant response

Monitoring of the skin irritant response was performed by non-invasive measurements of erythema and transepidermal water loss (TEWL) of the test and control fields repeatedly on D1–D4 before the first application of the irritants as well as on D5 before tape stripping (24 h after last irritant exposure). Erythema was measured using Chromameter CR200 (Minolta, Japan) and expressed in the L*a*b* three dimensional coordinate 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, 2 consecutive measurements per field were performed by the same observer under controlled environmental conditions (room temperature 21 ± 1°C; mean relative humidity 40–45%) and according to the published guidelines (14, 15).

Tape stripping and natural moisturising factor analysis

The levels of the NMF components (histidine, 2-pyrrolidone-5-carboxylic acid, trans-urocanic acid and cis-urocanic acid) were determined using a previously published method (16). Briefly, on D5, 24 h after the last exposure the SC layers were sequentially removed from the irritant-exposed and control fields with circular adhesive tapes (14 mm D-Squame® discs, CuDerm Corp., Dallas, TX, USA) as previously described (10, 17). For the analysis of NMF the 3rd and 4th tape strips were collected and stored in sterile 1.5 ml Eppendorf tubes (Eppendorf, Hamburg, Germany) at –80ºC until analysis. To assess the amount of harvested SC, the optical density (OD) of each tape was measured using the D-Squame Scan 850A (Monaderm, Monaco, France). The amount of SC was calculated from the OD and expressed as the mass of protein/cm^2 using a standardised procedure described elsewhere (18). The NMF components in the SC on the tape were extracted with 400 µl of 25% (w/w) ammonia solution, evaporated to dryness and reconstituted in 200 µl of pure water. Water extracts from 2 tapes were pooled together prior to high-performance liquid chromatography (HPLC-UV) analysis. NMF levels were corrected for the amount of protein and expressed as mmol NMF/g protein.

Statistical analysis

Statistical analysis was performed using GraphPrism Version 4 (GraphPad Software Inc, San Diego, CA, USA). The differences in erythema and TEWL between the fields in each the AD and healthy controls group at baseline were evaluated by analysis of variance (ANOVA). The differences were considered statistically significant if $p < 0.05$. The differences in the a*-value and TEWL over time were analysed by repeated measures ANOVA or Friedmann test for the respective field and parameter; for *p*-values less than 0.05, a *post hoc* test was performed. The differences in the respective parameters (Δ values) between the groups at each assessment point were analysed by unpaired *t*-test, Mann-Whitney test or ANOVA. The values are presented as mean and standard error of the mean (SEM) in the respective tables and figures.

The differences in the baseline NMF levels between the groups subdivided by AD and *FLG* mutations carrier state were tested by Kruskal-Wallis test followed by Dunn's multiple comparison test, while the differences in NMF levels after repeated single or tandem exposure were tested by Friedman test followed by Dunn's multiple comparison test. The differences in NMF decrease (% of control) between controls and AD and AD-wt vs AD-*FLG* groups were tested for each exposure by two-tailed Student's *t*-test or Mann-Whitney test (in case of non-Gaussian distribution). In the respective figures, the data are presented as median with interquartile ranges.

Table I. *Erythema (a* -value) and transepidermal water loss (TEWL) in the atopic dermatitis (AD) and healthy controls (Ctrl) group at baseline (D1) and after 96 h (D5) repeated single and tandem exposure to sodium lauryl sulphate (SLS) and sodium hydroxide (NaOH). Mean ± SEM (n = 20 per group); level of significance < 0.05; ***p < 0.001; **p < 0.01, *p < 0.05, control – non-exposed field (normal skin)*

		Control Mean ± SEM	NaOH/NaOH Mean ± SEM	SLS/NaOH Mean ± SEM	NaOH/SLS Mean ± SEM	SLS/SLS Mean ± SEM
a* -value (AU)						
AD	D1	5.65 ± 0.34	6.45 ± 0.35	6.14 ± 0.31	5.73 ± 0.35	5.51 ± 0.32
	D5	4.95 ± 0.39	6.97 ± 0.49	8.24 ± 0.67***	6.17 ± 0.42	9.54 ± 0.70***
Ctrl	D1	5.54 ± 0.42	6.94 ± 0.50	6.43 ± 0.45	5.95 ± 0.47	5.63 ± 0.43
	D5	5.00 ± 0.40*	7.44 ± 0.58	8.47 ± 0.70***	7.10 ± 0.63**	9.13 ± 0.91***
TEWL (g/m^2/h)						
AD	D1	7.04 ± 0.39	8.50 ± 0.53	7.39 ± 0.36	7.61 ± 0.46	7.76 ± 0.42
	D5	6.72 ± 0.35	15.28 ± 1.26***	26.24 ± 2.19***	17.38 ± 1.27***	38.59 ± 2.35***
Ctrl	D1	6.35 ± 0.27	7.48 ± 0.36	6.68 ± 0.26	6.62 ± 0.33	6.45 ± 0.41
	D5	6.69 ± 0.29	11.56 ± 0.83***	18.10 ± 1.62***	15.68 ± 1.66***	27.89 ± 2.87***

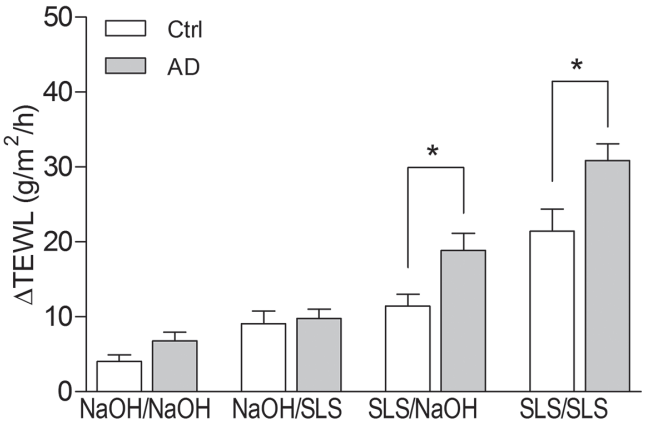

Fig. 1. Transepidermal water loss (TEWL) increase after repeated single and tandem exposure to 0.5% sodium lauryl sulphate (SLS) and 0.15% sodium hydroxide (NaOH) in volunteers with atopic dermatitis (AD) and healthy controls (Ctrl). The data are presented as Δ value compared to baseline (mean ± SEM; $n = 20$ for each group); *$p < 0.05$.

RESULTS

More severe impairment of the skin barrier function after cumulative exposure to alkaline agents in atopic dermatitis

The mean a*-values and TEWL of the irritant-exposed and control fields in the AD and healthy controls group on D1 (baseline) and D5 (end of the study) are presented in Table I.

At baseline there were no significant differences in the measured parameters of the test and control fields within as well as between the groups. On D5, the mean a*-values of the irritant-exposed fields in the atopic and healthy controls group were increased compared to D1. The comparison of the respective Δa* -values on D5 (Δa* = a*(D5)−a*(D1)) showed no significant differences between the AD and control group (data not shown).

Repeated exposure to the single irritants (NaOH/NaOH; SLS/SLS) and irritant tandems (NaOH/SLS; SLS/NaOH) resulted in impairment of the skin barrier function and significant TEWL increase in both groups (Table I). Monitoring of the barrier function every 24 h showed consistently higher ΔTEWL values in the AD group for each measurement time point and irritant-exposed field; on D5 the differences in ΔTEWL after exposure to SLS/SLS and SLS/NaOH between the groups were significant (ΔTEWL after repeated exposure to SLS/SLS in the AD and control group, 30.84 ± 2.26 and 21.45 ± 2.94 g/m²/h; $p < 0.05$; after SLS/NaOH exposure, respectively 18.85 ± 2.30 and 11.43 ± 1.61 g/m²/h; $p < 0.05$; Fig. 1).

The comparison of the Δ values for each measured parameter and test field on D5 with regard to filaggrin genotype showed no significant differences between the AD *FLG* mutation carriers and non-carriers (data not shown).

Exposure to alkaline agents leads to pronounced decrease of natural moisturising factor

The baseline NMF levels of the AD and healthy controls group are shown in Fig. 2a. Repeated exposure to the single irritants (SLS/SLS; NaOH/NaOH) and to the irritant tandems (NaOH/SLS; SLS/NaOH) lead to significant decrease of the NMF levels in the SC in both AD and the healthy controls groups (Fig. 2b). In contrast to the severity of barrier function impairment (TEWL increase), there were no significant differences in the relative changes of the NMF levels between the AD and healthy volunteers. Cumulative exposure to NaOH/NaOH and SLS/NaOH resulted in significant differences in the relative reduction of the NMF between AD *FLG* mutation carriers and non-carriers (for both exposures, $p < 0.05$); the differences between the AD *FLG* mutations carriers and the healthy controls however were not significant.

DISCUSSION

Chronic ICD results from cumulative exposure to weak irritant stimuli. Though AD is a significant risk factor for development of chronic ICD, the effects of repeated exposure to multiple irritants in atopic skin under controlled experimental conditions have not been previously investigated. In the present study we have shown that in comparison to healthy volunteers,

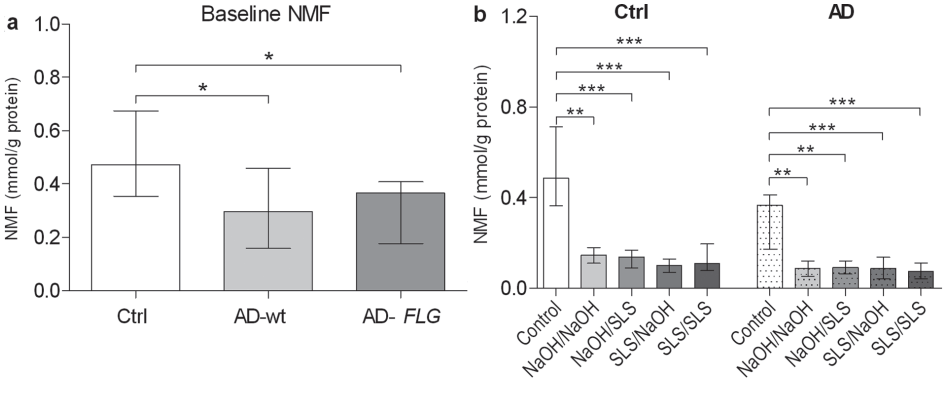

Fig 2. (a) Baseline levels of natural moisturising factor (NMF) in the stratum corneum of the healthy controls (Ctrl) and atopic dermatitis (AD) volunteers without (AD-wt) and with *FLG* mutations (AD-*FLG*); (b) Reduction of the NMF levels after repeated single and tandem exposure to 0.5% sodium lauryl sulphate (SLS) and 0.15% sodium hydroxide (NaOH) in the AD and Ctrl groups. The data are shown as median with interquartile; level of significance < 0.05, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

repeated irritation with SLS and/or NaOH in AD leads to a more pronounced impairment of the skin barrier function. Although based on a small number of volunteers, the results of the study suggest that within AD the severity of barrier impairment is not influenced by the filaggrin genotype. These findings are in agreement with the results of previous investigations of the cutaneous response to acute irritant challenge with 1% SLS with respect to the genotype and extend the published knowledge on the skin reactivity in AD *FLG* mutation carriers to other chemically unrelated primary irritants and mode of exposure relevant to real-life settings (10, 19).

The results of the study confirm that AD patients have reduced levels of NMF compared to healthy individuals (3, 4, 20). Repeated single as well as sequential exposure to SLS and NaOH exerted pronounced effects on NMF and the post-exposure NMF amount was approximately 30% of the baseline (non-exposed skin) value. The NMF was measured in tape strips 3 and 4 which correspond to a SC depth of about 5 μm (21); thus the effect of the applied irritants on NMF is not limited to the SC surface only. Whereas cumulative exposure to the irritants resulted ultimately in significant decrease of NMF, the differences in the relative NMF reduction between the healthy and AD volunteers groups were not significant.

Both increased TEWL and reduced levels of NMF will result in reduced SC hydration and xerosis. As AD and filaggrin deficiency are associated with dry skin, it is likely that exposure to alkaline agents will further worsen the barrier function, facilitate the penetration of irritants and allergens (22) and result in irritant dermatitis as shown by the findings of independent general population studies (23, 24). These observations have been supported by a recent publication showing an increased risk for development of occupational ICD in individuals with *FLG* mutations and/or AD referred to a tertiary clinic (7). The results of that study provide evidence that *FLG* mutations adjusted for AD increased the risk 1.6-fold, whereas the adjusted risk for AD was 2.9 fold. The individuals who carried a *FLG* mutation and had AD showed almost 5-fold risk for ICD. Based on these findings, in the present investigation, we expected that cumulative exposure to SLS or/and NaOH would exert more pronounced effects in the most susceptible sub-group i.e. AD *FLG* mutations carriers. Whereas we found no significant differences in the severity of barrier impairment (ΔTEWL) between the AD *FLG* mutation carriers and non-carriers that may explain the increased risk for occupational skin disease, the more pronounced relative changes in the levels of NMF as a result of cumulative exposure to NaOH/NaOH and SLS/NaOH in the AD *FLG* mutation carriers might possibly facilitate the development of manifest ICD.

The more pronounced effect of SLS on TEWL compared to NaOH might be explained by specific interactions with different structural components of the epidermal barrier that are not solely dependent on the alkaline nature of irritant. SLS may alter the SC lipid organisation, bind to SC proteins causing changes in their structure and after penetration into the living epidermis – exert direct toxic effects to the keratinocytes (25, 26). Furthermore, SLS accumulates in the epidermis and may thus, prolong exposure (27). The effects of NaOH in contrast, may be primarily attributed to the alkaline pH. Although the pH of the NaOH solution (pH ~12.6) used in the present study was even more basic than that of the applied SLS (pH ~9.2), the cumulative effect of SLS on the skin barrier function was more severe.

The more pronounced irritant-induced barrier function impairment in AD may involve different pathways; the increased skin permeability in the presence of atopic skin disposition (21, 28, 29) resulting in the penetration of larger amounts of SLS across the SC may contribute to the observed differences in the TEWL increase between the atopic and healthy volunteers group.

There are several possible mechanisms by which SLS and NaOH exposure might lead to a decrease in the NMF levels. Some of these effects might occur directly during exposure, e.g. absorption of water will result in corneocyte swelling. Due to the swelling, the osmotic pressure might change the permeability of the cornified envelope and facilitate the escape of NMF from the cells. Furthermore, both SLS and NaOH are known to interact with the cellular protein structures resulting in leakier corneocytes (30–32). Another possible mechanism might involve interference with the processing of filaggrin from its precursor profilaggrin or the breakdown of filaggrin into the NMF components. As SLS and NaOH are alkaline, exposure to any of them, or in tandem, will lead to increase of the skin pH that may in turn alter the activity of the enzymes involved in the degradation of filaggrin.

In conclusion, our results provide evidence that controlled repeated single and tandem exposure to SLS and NaOH in AD results in more severe damage to the skin barrier function that, at least in this study, was not influenced by filaggrin genotype. The irritant-induced decrease in the levels of NMF may be pathogenetically relevant for development of chronic ICD in both healthy and atopic individuals.

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The authors declare no conflict of interest.

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