INVESTIGATIVE REPORT

Barrier Function and Natural Moisturizing Factor Levels After Cumulative Exposure to Short-chain Aliphatic Alcohols and Detergents: Results of Occlusion-modified Tandem Repeated Irritation Test

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Alcohol-based disinfectants and detergents are common workplace factors for irritant contact dermatitis (ICD). Though occlusion and water are relevant co-exposures, the tandem effects of occlusion and sequential exposure to alcohols and detergents have not been studied. We therefore investigated the combined effects of occlusion with water and repeated exposure to n-propanol and/or sodium lauryl sulphate (SLS) in an occlusion-modified tandem irritation test. The outcomes included visual scoring, measurement of erythema, transepidermal water loss, capacitance and natural moisturizing factor (NMF) levels. Occlusion abrogated the skin barrier function and significantly enhanced the irritant-induced barrier damaging effects. The NMF levels of all irritant-exposed fields decreased significantly compared with the non-exposed fields; occlusion enhanced the decrease in NMF. Although SLS exerted more pronounced effects on the measured parameters, the barrier function impairment and NMF decrease after exposure to n-propanol in workplace-relevant concentrations, found in the study, confirm the significance of short-chain aliphatic alcohols for occupational ICD. *Key words: barrier function; natural moisturizing factor; detergents; short-chain aliphatic alcohols; occlusion; irritant contact dermatitis.*

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Alcohol-based hand disinfectants, soaps and detergents are common workplace factors contributing to the risk for development of occupational irritant contact dermatitis (ICD) and hand eczema (1–4). Though water and occlusion are known relevant co-exposures, their combined effect with repeated exposure to alcohols and detergents have not been investigated so far. The aim of this study was to investigate the effects of occlusion on the outcomes of repeated single and concurrent exposure to n-propanol and sodium lauryl sulphate (SLS) and validate a workplace relevant model to assess differences related to each exposure factor. Based on recent publi-cations for decreased natural moisturizing factor (NMF) levels after sequential exposure to multiple irritants and the frequently reported skin dryness associated with the use of hand disinfectants and detergents (5–8), we further investigated the cumulative effects of n-propanol and SLS on NMF and assessed whether they were enhanced by occlusion.

MATERIALS AND METHODS

Study population

Twenty-five healthy adult volunteers aged 20–65 years (16 females and 9 males; mean age 26.8 years) with no history of skin or systemic diseases were included in the study. Intensive UV-exposure in the test area within the last 6 weeks prior to inclusion as well as throughout the study, pregnancy and lactation were defined as exclusion criteria. The protocol was approved by the ethics committee of the University of Lübeck (14-111; 09-044) and all volunteers gave written informed consent prior to the study.

Irritants and mode of exposure (Table SI[1])

Fifty µl of 60.0% aqueous solution of n-propanol (1-propanol, Merck KGaA, Darmstadt, Germany) and/or 0.5% aqueous solution of sodium lauryl sulphate (SLS; 99.0% purity; Sigma-Aldrich, Steinheim, Germany) were applied on 8 previously marked test fields on the upper-mid back on 4 consecutive days (D1–D4) twice daily for 30 min using large Finn chambers (12 mm diameter, SmartPractice, Reinbek, Germany) as described previously (5, 6, 9–11). The irritants were applied to the respective test fields at the same time of the day (± 1 hour). On 4 of the test fields, each irritant exposure was preceded by occlusion with distilled water for 30 min and on 4 fields the irritants were applied without preceding occlusion; 2 adjacent fields, respectively, exposed to distilled water only (occlusion) or left untreated (normal skin) served as controls. To minimize the possible mechanical irritation caused by the frequent placement and removal of the chambers, the area of tape around the chambers was reduced. The irritant exposures resulting from the described application mode are shown in Table SI[1]. The volunteers were allowed to take shower as usual; the use of skin care products or UV exposure in the test area was not allowed for the duration of the study (5 days).

Bioengineering assessment of the skin irritant response

The skin irritant response was monitored by visual scoring and non-invasive assessment of erythema, transepidermal water loss

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(TEWL) and skin hydration (capacitance). The assessments were performed every day before the first application of the irritants on D1-D4 and before tape stripping on day 5 (D5). Visual scoring was performed according to Frosch & Kligman (12), based on assessment of erythema, scaling and fissuring on a 0–4, respectively 0–3 point scale. Erythema was measured with the Colorimeter CL400 (Courage and Khazaka Electronics, Cologne, Germany) and expressed in the L*a*b* system; the a*-value on the green-red axis was used for assessment of erythema (13). TEWL was measured with the open chamber system (Tewameter TM300; Courage and Khazaka Electronics, Cologne, Germany) and skin hydration was assessed by measuring capacitance (Corneometer CM825; Courage and Khazaka Electronics, Cologne, Germany). For each parameter, 2 consecutive measurements per field were performed by the same investigator under controlled ambient conditions (temperature $21 \pm 1°C$; mean relative humidity 38–40%) and according to the published guidelines (14–16).

Natural moisturizing factor analysis

The samples for stratum corneum NMF analysis were collected by standardized tape stripping using commercially available 14-mm D-Squame® discs (CuDerm Corp., Dallas, TX, USA). Tape stripping was performed on D5, 24 h after the last application of the irritants as previously described (5, 6, 17). Six tapes per field per volunteer were obtained and stored in sterile 1.5 ml Eppendorf tubes (Eppendorf, Hamburg, Germany) at −80°C until analysis. The stratum corneum NMF components (histidine, 2-pyrrolidone-5-carboxylic acid, trans- and cis-urocanic acid) on the tape were extracted with 400 µl of 25% (w/w) ammonia solution, evaporated to dryness and reconstituted in 200 µl pure water. The extracts from 2 D-Squame® discs were pooled prior to high-performance liquid chromatography (HPLC-UV) analysis. The amount of stratum corneum on the tape has been estimated from the protein levels determined in the aqueous extract after ammonia extraction, as previously described (18). The 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 5 (GraphPad Software Inc., San Diego, CA, USA). The level of significance was $p < 0.05$. The differences in the a*-value, TEWL and capacitance between the fields at baseline were evaluated by analysis of variance (ANOVA). The changes over time were analysed by repeated measures ANOVA or Friedmann test for the respective field; for *p*-values less than 0.05, a *post hoc* test was performed. The differences in the visual score and

non-invasive measurement parameters between the previously occluded and non-occluded fields on D5 (Δ-values compared with baseline) were analysed by Wilcoxon signed-rank test. The differences in the NMF levels between the fields were evaluated by Kruskal–Wallis test followed by Dunn's multiple comparison test. In the respective tables and figures, the data are presented as mean and standard error (SEM), except for the visual score and NMF levels presented as median.

RESULTS

Occlusion preceding irritant exposure enhances the irritant-induced barrier damaging effects

At baseline there were no significant differences in the visual irritation score and barrier function parameters between the test and control fields.

On D5, the visual irritation score of all irritant-exposed fields was significantly increased compared with baseline (Table I). The comparison of the changes in the visual irritation score of the test fields exposed repeatedly to the same irritants and irritant tandems, with and without occlusion, at the end of the study are shown in Fig. 1a. Previous occlusion with water enhanced the skin irritant response and, the increase in the visual score of all irritant-exposed fields on D5, assessed as Δ-value compared with baseline, was significantly higher if the irritant exposure was preceded by occlusion.

No significant differences in the a*-values following occlusion alone as well as repeated exposure to n-propanol as a single irritant, with or without previous occlusion, were found. In contrast, repeated exposure to SLS as a single irritant (SLS/SLS) or in tandem with n-propanol (n-propanol/SLS; SLS/n-propanol), resulted in significantly higher a*-values on the previously occluded as well as non-occluded fields on D5 compared with baseline (Table I). The comparison of the a*-value increase of the test fields exposed to the same irritants and tandems with and without previous occlusion showed significantly higher Δa*-values ($\Delta a^* = a^*D5 - a^*D1$) only for the field exposed to SLS alone ($p < 0.01$).

Table I. *Visual score, erythema (a*-value), transepidermal water loss (TEWL) and capacitance after repeated single and tandem exposure to n-propanol and sodium lauryl sulphate (SLS) with and without prior occlusion with distilled water (Aq)* (n = 25)

	Visual score Median (IQR)		a*-value (AU) Mean ± SEM		TEWL (g/m²/h) Mean ± SEM		Capacitance (AU) Mean ± SEM	
	D1	D5	D1	D5	D1	D5	D1	D5
Occl.Aq/Occl.Aq	0 (0–0)	0.5 (0.5–0.5)***	11.85 ± 0.40	12.51 ± 0.77	6.86 ± 0.26	9.12 ± 0.42***	56.04 ± 1.91	53.68 ± 1.39
Occl.Aq/n-propanol/n-propanol	0 (0–0)	1.0 (0.5–1.0)***	12.94 ± 0.46	13.65 ± 0.53	5.90 ± 0.27	9.46 ± 0.46***	56.32 ± 2.48	36.86 ± 1.39***
Occl.Aq/n-propanol/SLS	0 (0–0)	1.0 (1.0–2.0)***	12.98 ± 0.63	14.31 ± 0.82*	5.83 ± 0.36	16.18 ± 2.54***	56.25 ± 2.27	36.82 ± 1.58***
Occl.Aq/SLS/n-propanol	0 (0–0)	2.0 (1.0–2.0)***	12.23 ± 0.40	14.69 ± 0.70**	6.00 ± 0.29	21.18 ± 2.60***	55.43 ± 1.94	36.28 ± 1.12***
Occl.Aq/SLS/SLS	0 (0–0)	3.0 (2.0–3.0)***	12.25 ± 0.43	17.80 ± 0.66***	6.72 ± 0.32	32.14 ± 2.64***	56.07 ± 2.17	33.91 ± 1.90***
n-propanol/n-propanol	0 (0–0)	0.5 (0.5–1.0)***	11.93 ± 0.39	12.43 ± 0.43	6.35 ± 0.20	8.23 ± 0.33***	51.65 ± 2.10	36.64 ± 1.04***
n-propanol/SLS	0 (0–0)	0.5 (0.5–1.0)***	11.55 ± 0.39	13.14 ± 0.60**	6.20 ± 0.24	12.61 ± 1.40***	51.67 ± 1.71	37.99 ± 1.22***
SLS/n-propanol	0 (0–0)	1.0 (0.5–1.5)***	11.00 ± 0.40	13.58 ± 0.67***	6.38 ± 0.19	15.54 ± 2.01***	51.03 ± 1.67	37.54 ± 0.90***
SLS/SLS	0 (0–0)	2.0 (1.0–2.5)***	11.28 ± 0.40	15.82 ± 0.67***	6.65 ± 0.25	24.08 ± 2.13***	52.28 ± 1.91	36.31 ± 1.67***
Control (normal skin)	0 (0–0)	0.0 (0.0–0.0)	11.27 ± 0.41	10.97 ± 0.43	6.86 ± 0.23	6.65 ± 0.30	50.74 ± 1.82	53.22 ± 1.33

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with baseline (D1); D1: baseline (0 h); D5: end of the study (96 h); control: non-exposed field (normal skin). IQR: interquartile range; SEM: standard error of the mean; AU: arbitrary units.
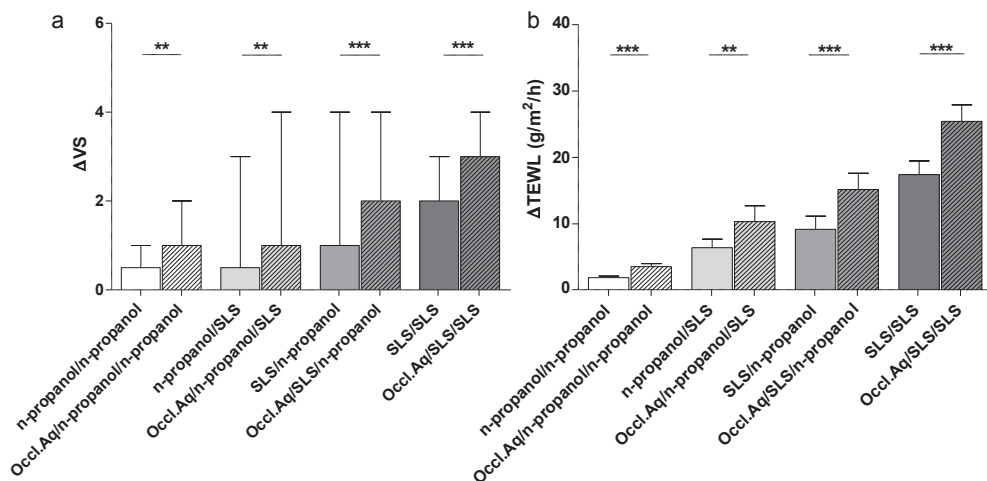
Fig. 1. Occlusion-modified tandem repeated irritation test with 60.0% n-propanol and 0.5% sodium lauryl sulphate (SLS): comparison of the visual score (VS) and transepidermal water loss (TEWL) increase between the test fields exposed to the same irritants and irritant tandems with and without previous occlusion. The results are shown as Δ-value compared with baseline: (a) ΔVS (median and range); (b) ΔTEWL (mean ± standard error of the mean (SEM)); level of significance < 0.05, **p < 0.01, ***p < 0.001; Occl.Aq: occlusion with distilled water.

Regarding the skin barrier function, occlusion with water alone as well as repeated exposure to the irritants and irritant tandems, with and without previous occlusion, led to abrogation of the barrier and significant TEWL increase on D5 compared with baseline ($p < 0.001$, for occlusion with water as well as all irritant-exposed test fields); no significant differences in the TEWL values of the non-exposed (control; normal skin) field were found between D1 and D5 (Table I). The comparison of the TEWL increase on D5 with baseline (ΔTEWL) of the test fields exposed to the same irritants and irritant tandems with and without previous damage to the skin by occlusion showed significantly higher ΔTEWL values, if exposure was preceded by occlusion (Fig. 1b).

Repeated exposure to the irritants and irritants tandems led to significant decrease in capacitance on all test fields on D5 compared with baseline ($p < 0.001$ for all irritant-exposed fields; Table I). The capacitance values of the control fields (occlusion with water and non-exposed skin), in contrast, did not change significantly throughout the study. As for TEWL, the Δ-values on D5 compared with baseline were significantly greater if exposure to the same irritants and tandems was preceded by occlusion (data not shown).

Repeated single and tandem exposure to n-propanol and sodium lauryl sulphate leads to decreased levels of the natural moisturizing factor in the stratum corneum

On D5 the NMF levels of all irritant-exposed fields, with and without previous occlusion, were significantly decreased compared with the control fields (non-exposed/ normal skin, respectively occlusion with water) (Fig. 2); there were no significant differences between the control fields. The NMF levels after cumulative exposure to n-propanol preceded by occlusion were significantly lower compared with occlusion with water alone ($p < 0.05$). Occlusion as co-exposure enhanced the irritant-induced effects on NMF, consequently the relative NMF decrease

of the test fields exposed to the same irritants and irritant tandems was more pronounced if the irritant exposure was preceded by occlusion. Compared with n-propanol, SLS exerted more pronounced effects on NMF (relative reduction after exposure to n-propanol/n-propanol, −55.4%; after exposure to SLS/SLS, −79.2%); the differences were significant when exposure to the same irritants was preceded by occlusion (relative reduction respectively, −60.8% and −87.4%; $p < 0.01$).

DISCUSSION

The high prevalence of work-related ICD in healthcare and wet work occupations has stimulated continuous
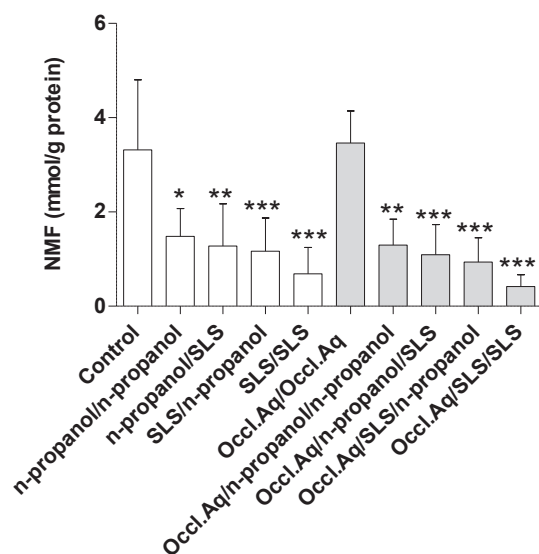

Fig. 2. Natural moisturizing factor (NMF) levels after repeated single and tandem exposure to 60.0% n-propanol and 0.5% sodium lauryl sulphate (SLS) with and without previous occlusion with distilled water. The data are shown as median with interquartiles ($n = 25$); level of significance < 0.05, *p < 0.05, **p < 0.01, ***p < 0.001 compared with the non-exposed (normal skin) control field. Occl.Aq: occlusion with distilled water; control: non-exposed (normal skin) field.

interest in studying the effects of water and occlusion on the skin barrier function and their contribution to skin irritation (19–23). Although both factors have been considered to enhance irritancy, the findings of the so far published bioengineering studies show controversial results based on different exposure models and limited to the model irritant SLS (24–30). The effects of occlusion, water and combined exposure to multiple workplace relevant irritants, in contrast, have been poorly investigated.

The present study used an exposure protocol based on a previously validated model, the tandem repeated irritation test (TRIT), and aimed towards further development of the model, which, in addition to the effects of repeated single and combined irritant exposure, provides a standardized approach for investigating the role of occlusion in cumulative *in vivo* human skin irritation (9, 10, 30–32). The results of the study show that the occlusion-modified tandem repeated irritation test design (occlusion-modified TRIT) was sensitive to detecting differences in objective parameters of the skin barrier function between the previously occluded and non-occluded fields, exposed to the same irritants and irritant tandems applied in the same order. Based on the findings for significantly increased TEWL after occlusion with water alone as well as significant differences in ΔTEWL between the occluded and non-occluded fields exposed to the same irritants, our study provides new evidence that repeated low-grade trauma by occlusion/water exposure alone abrogates the skin barrier function and if, as in workplace situations, this is followed by repeated irritant exposure, enhances the summation effects exerted by the irritants. As in previously published studies, we observed a limited effect of occlusion/water exposure on skin hydration measured by capacitance (21, 26); the findings for significantly different Δ values between the previously occluded and non-occluded fields exposed to the same irritants, however, point further to the major role of occlusion, respectively repeated water exposure in the interplay of factors leading to cumulative ICD. These findings are important as, in contrast to the numerous studies that have focused on the role of occlusion for enhancement of the irritant responses on skin previously damaged by irritants, the effects of occlusion prior to irritant exposure have rarely been investigated (29).

Along with the further development of the test model, the results of the study provide important novel aspects regarding the irritant properties of the short-chain aliphatic alcohol, n-propanol. Short-chain aliphatic alcohols, including ethanol, n-propanol and isopropanol have widespread applications in topical drug and skin care formulations and have been shown to exert profound effects on the stratum corneum lipids while having only minor impact on TEWL, unless the skin barrier has not been previously damaged by po-

tent irritants, such as SLS (33–40). The results of our study, however, suggest that previously compromised barrier function is not an obligate factor for the barrier damaging effects of n-propanol, as shown by the significant increase in TEWL after repeated exposure to n-propanol as a single irritant. In addition to abrogation of the skin barrier function, our results provide evidence that cumulative exposure to n-propanol, applied in a workplace relevant concentration (60%), leads to a significant decrease in the levels of NMF in the stratum corneum. The significant differences in the NMF levels between the test fields exposed to water and n-propanol (i.e. occlusion with water compared with n-propanol/n-propanol) confirm that the observed changes cannot be attributed to the effects of water alone and suggest that short-chain aliphatic alcohols may cause skin dryness through interaction with both the skin lipids and reduction in NMF.

The results of this study confirm in addition our previous findings for significantly reduced NMF levels after cumulative exposure to SLS under the same exposure conditions and show further that previous occlusion aggravates the irritant-induced effects on NMF (6). Whereas, to the best of our knowledge, these are the first reported *in vivo* human skin data on the changes in the NMF levels following combined exposure to irritants and occlusion, these findings have important implications for the pathogenesis of work-related ICD in occupations with repeated sequential daily exposures to multiple irritants, occlusion and frequent hand washing.

In conclusion, the occlusion-modified TRIT model may be a useful tool for investigating the effects of cumulative exposure to workplace irritants and their modulation by relevant co-exposures at both functional and biochemical levels. Although as in earlier studies, compared with n-propanol, SLS exerted more pronounced effects on the measured parameters, our findings of compromised barrier function and NMF decrease after cumulative exposure to n-propanol independently of previous damage, provide evidence for the irritant potential and role of short-chain aliphatic alcohols in the development of occupational ICD.

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