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Applicability of an exaggerated forearm wash test for efficacy testing of two corticosteroids, tacrolimus and glycerol, in topical formulations against skin irritation induced by two different irritants

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Background/Purpose: Alternatives to corticosteroids in the treatment of irritant contact dermatitis (ICD) are needed and may include glycerol and topical immunomodulators like tacrolimus. Because the efficacy of different treatments in experimentally induced ICD may vary depending on the irritant applied, we tested the efficacy of four anti-irritant compounds using the two different irritants sodium lauryl sulfate (SLS) and nonanoic acid (NON).

Methods: In a randomized, double-blind, controlled trial, healthy volunteers were exposed to 5% SLS and 50% NON (the right and the left forearm, respectively) in a cumulative wash test. Induction of ICD was obtained by three daily washings for 7 days, followed by a maintenance phase with two daily washings for 12 days. Treatment (triamcinolone acetonide, clobetasol propionate, tacrolimus and glycerol ointment) was started at day 7 and applied immediately after washing. Vehicle and no treatment served as the control. Reactions were evaluated clinically and instrumentally.

Results: No treatments were significantly better than the other treatments and controls. There was a tendency toward a dose-dependent response to corticoid treatment, and a trend toward worsened irritancy by tacrolimus on SLS-irritated skin. Explained variance in the experiment by ANOVA revealed a very small effect of treatments compared with an immense and significant subject effect.

Conclusion: No claims of effective anti-irritant properties for any of the ointments can be maintained. Application of the present wash test as a tool for anti-irritant efficacy testing may be complicated by the small observed variance explained by treatment.

Key words: irritant contact dermatitis – treatment – sodium lauryl sulfate – nonanoic acid – corticosteroid – tacrolimus – glycerol

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IRRITANT CONTACT DERMATITIS (ICD) is a major problem in occupational dermatology, and in its most frequent form, i.e. chronic ICD, it results from repeated exposure to low-grade irritants (1). Treatment trials using ICD patients are hampered by the large heterogeneity of ICD patients, the need for very large sample sizes and the fact that topical emollients cannot be withdrawn during the study due to ethical reasons. On the other hand, experimental induction of ICD in healthy volunteers has been widely used to evaluate treatment responses, but a comparison of the test results is complicated by differences in the exposure mode and the choice of irritants.

The cornerstone in the treatment of ICD is avoidance of irritant exposure and the use of emollients. While topical corticosteroids are still routinely used by dermatologists as the treatment of ICD, their effectiveness in ICD has been questioned in both experimentally induced ICD (2) and in patient treatment programs (3) due to their recognized side effects and detrimental effects on the skin barrier (4–5). The topical immunomodulators, tacrolimus and pimecrolimus are used against atopic dermatitis for their anti-inflammatory effect, and although induction of barrier damage has not been reported for these compounds, they are potential mild irritants. However, some reports suggest that they may have a role in the treatment of chronic hand eczema (6–7), while conflicting results in experimentally induced ICD have been reported (8–9). The humectant glycerol has a range of recognized beneficial effects on the skin (10) and has been reported to have an anti-irritant effect in several studies (11–13).

The irritant skin reaction varies between different irritants (14–15), and likewise, skin irritated by different chemicals may show different treatment responses. The aim of the present study was to repeat a study where two different irritants, sodium lauryl sulfate (SLS) and nonanoic acid (NON), were applied to the skin in a 3-week exaggerated wash test model. The study concluded that glycerol exerted an effect against SLS and NON-induced irritation, whereas the corticosteroid triamcinolone acetonide was efficient in NON-irritated skin only, and appeared to have a detrimental effect on SLS-induced irritation (11). In the present study, two strengths of corticosteroids were included to test whether the suggested corticosteroid effects – in the case of reproducibility – were dose-dependent, and furthermore, tacrolimus ointment was included for comparison.

Materials and Methods

The study protocol was approved by the local ethics committee (S-20080052) and was conducted in accordance with the IHC-guidelines for good clinical practice (EudraCT 2008-001678-34). Written informed consent was obtained from all participants.

Design

The study was a double-blind, controlled trial with two parallel studies in each subject, i.e. treatment response to SLS on right arms and to NON on left arms. Four active treatments were evaluated on each arm. Untreated and vehicle-treated sites served as controls. The position of the six treatment sites on each arm was randomized according to a latin square design.

Volunteers

Thirty-six healthy human volunteers (19 females and 17 males; mean age 25.6 years, range 19–36 years) were recruited. Volunteers were excluded if they had active skin disease, received immunosuppressive treatment, had endocrine or immune system diseases, were pregnant or breast-feeding. Topical treatment (including emollients) other than the study treatments was not allowed on the test sites during and 2 weeks before the study. UV exposure and washing at test sites was to be avoided during the study.

Irritant exposure

Irritants

All volunteers were exposed to 5% SLS (purity >99.9%; Sigma Aldrich, Brondby, Denmark) aq. on their right arms and to 50% NON (purity 99.3%; ABCR GmbH & Co., Karlsruhe, Germany) in *n*-propanol on their left arms. Concentrations were chosen based on dose–response studies (16) to obtain uniform moderate-strength skin reactions for both irritants.

Induction of dermatitis

The protocol for skin irritation was as described (11). Briefly, a pre-demarcated area of 18 × 4 cm on the volar aspect of both forearms was washed for 1 min with a soft sponge soaked in the irritant solution, and the arm was then rinsed under running lukewarm water and dried with a soft paper tissue. This was repeated three times daily for 7 days. The target level of irritation was a visual sum score of 2–4 corresponding to a weak to moderate reaction following European Society of Contact Dermatitis (ESCD) guidelines on the clinical scoring of subacute/cumulative SLS irritant reactions (17).

Maintenance of dermatitis

From day 7 to the end of the study (at day 17), the number of daily washes (procedure unchanged) was reduced to two (morning and evening). However, too strong reactions instigated a further reduction in the number of daily washes to one or zero. Hence, the design allowed the investigator to decrease extreme reactions. When reactions returned to a more moderate and desirable level of irritation, the number of daily washes was increased again (to a maximum of two according to the original protocol).

Treatment

At day 7, coinciding with the reduction of washes, treatment was started. Six circular treatment sites ($\varnothing = 20$ mm) were marked within the irritated area according to a latin square design. An amount of approximately 5 mg/m^2 (corresponding to a total of approximately 16 mg) was applied twice daily immediately after washing with the irritants and continued until day 17 (end of treatment). Careful instructions allowed self-administration of ointments by the participants.

Ointments

All ointments were blinded at the Central Pharmacy, Odense University Hospital. Glycerol oint-

ment and vehicle ointment were manufactured by the Central Pharmacy (Odense University Hospital). Attempts to purchase Dermovate® (clobetasol propionate) vehicle from GlaxoSmithKline (Brøndby, Denmark) were unsuccessful and instead, a strategy of one vehicle serving as a control for all the other treatments was applied. Thus, the vehicle constituted an approximated vehicle for the three commercially purchased ointments and an exact vehicle for glycerol ointment.

One site was left untreated and the other five sites were treated with (i) vehicle [80% soft white paraffin (Ph. Eur.) and 20% paraffin oil (Ph. Eur.)]; (ii) triamcinolone acetonide 1 mg/g (Kenalog® ointment; Bristol Myers Squibb, Lyngby, Denmark); (iii) clobetasol propionate 0.5 mg/g (Dermovate® ointment; GlaxoSmithKline); (iv) tacrolimus 1mg/g (Protopic® 0.1% ointment; Astellas Pharma, Munich, Germany) or (v) 20% glycerol (Ph. Eur.) ointment in a soft white paraffin/paraffin oil base (same composition as vehicle).

Evaluation of skin irritancy

All visits were carried out in the bioengineering laboratory at days 0, 7, 9, 11, 13, 15 and 17, and sites were evaluated clinically and biometrically. Measurements were undertaken before irritant exposure and treatment. Baseline values for all sites were obtained at day 0. At all visits, participants were acclimatized in the lab for 15 min with their forearms exposed before skin reactions were assessed.

Clinical assessment

Visual scoring of skin erythema, roughness, edema, scaling and fissuring was performed in accordance with the ESCD guideline on cumulative/subacute SLS irritation (17). With a possible score from 0 to 3 for each category, the total scoring range is 0–15, with a score >9 being very strong or caustic. The target level of irritation in our studies was a score of at least 2 but preferably not higher than 4, corresponding to a moderate reaction.

Transepidermal waterloss (TEWL)

For the assessment of epidermal barrier integrity, TEWL was determined using a DermaLab (Cortex, Hadsund, Denmark) TEWL probe.

Colorimetry

Skin color reflectance was determined using a colorimeter (Minolta CR300, Osaka, Japan). The *a**-parameter quantifies erythema as a measure of inflammation. Means from triplicate measurements were used in further data analysis.

Stratum corneum hydration

Conductance was measured using the DermaLab (Cortex) moisture pin probe. As for colorimetry, triplicate measurements were performed.

Data analysis

Where triplicate measurements were used (colorimetry and skin hydration), the means were calculated and used in further analyses. An analysis of variance (ANOVA) was performed to describe the amount of variation explicable by treatment differences in relation to the between-subject variation. The results are summarized in Table 3. Both the descriptive statistics in Tables 1 and 2 and the ANOVA were performed using the R package (http://www.r-project.org/). To test whether irritant reactions outside the target level of irritancy (i.e. hypo- and hyperresponders) affected the applicability of treatments in our model, we subjected

TABLE 1. Effect of treatments on sodium lauryl sulfate (SLS)-irritated skin evaluated by four parameters

	No treatment	Tacrolimus	Triamcinolone acetonide	Clobetasol propionate	Glycerol 20%	Vehicle
Visual score						
Mean (SD)	−1.67 (3.35)	−1.11 (3.86)	−1.9 (3.42)	−2.44 (3.73)	−2.01 (3.45)	−1.72 (3.46)
Range	(−9, 5)	(−11.5, 7)	(−10, 5)	(−11.5, 5)	(−9, 6)	(−9.5, 6)
Colorimetry						
Mean (SD)	−2.78 (2.95)	−1.94 (3.36)	−2.55 (3.08)	−2.27 (3.66)	−2.33 (3.48)	−2.29 (3.13)
Range	(−9.4, 1.42)	(−8.62, 6.19)	(−10.3, 2.94)	(−9.29, 6.7)	(−11.9, 3.63)	(−11.6, 6.27)
Transepidermal waterloss (TEWL)						
Mean (SD)	−8.66 (11.7)	−5.38 (10.9)	−8.06 (11.2)	−7.03 (13.2)	−6.59 (11.9)	−7.57 (12.2)
Range	(−40.2, 6.2)	(−36.7, 15.8)	(−35.5, 9)	(−33.6, 26.6)	(−40.4, 21.3)	(−39.4, 10.4)
Hydration						
Mean (SD)	−1.11 (37.6)	3.24 (39.6)	11.2 (48.8)	13.9 (49)	17.1 (52.1)	4.35 (46.2)
Range	(−107, 86.7)	(−76.7, 86.7)	(−83.3, 163)	(−60, 147)	(−63.3, 187)	(−96.7, 143)

Values are end of treatment (day 17) changes from treatment baseline (day 7). Thus, for visual score, colorimetry and TEWL the highest negative values represent the best effect, whereas for hydration the best effect is positively correlated.

the dataset to a subanalysis. Hypothetically, ranking of the treatment response may be problematic in hyper- and hyporeactions because the skin would be either too injured or too unaffected to allow for ranking between treatments. Therefore, reactions (as determined by the visual score at day 7) were divided into three groups for the two arms separately: (1) the lower quartile; (2) the second and third quartile and (3) the upper quartile. Differential reactions induced by the two irritants within a subject implied that one subject did not necessarily belong to the same quartile in SLS- and NON-elicited reactions.

Results

Untreated reactions

The irritation level at day 7 was comparable for the two irritants, whereas healing was faster for NON reactions

The goal from a previous optimization study was to obtain as uniform reactions as possible by the two irritants. The untreated natural course of reactions obtained by the present concentrations is shown in Fig. 1. The day 7 response was comparable for the two irritants, especially by visual scoring and hydration, whereas NON reactions induced less increase in redness and TEWL compared with SLS. The healing was faster for NON reactions as determined by all the parameters. Consequently, the gap in the mean visual scoring between the two irritants had progressed to 1.3 by day 17. During the induction phase (day 0–7), irritant exposure due to exaggerated reactions was discontinued for SLS and NON in three and 11 subjects, respectively. One subject reported stinging/pain due to SLS during the induction phase, whereas 11 subjects reported stinging due to NON.

No treatments were significantly better than the other treatments and controls

The effect of treatments is summarized for all the evaluation parameters in Table 1 (SLS-irritated

TABLE 2. Effect of treatments on nonanoic acid (NON)-irritated skin evaluated by four parameters

	No treatment	Tacrolimus	Triamcinolone acetonide	Clobetasol propionate	Glycerol 20%	Vehicle
Visual score						
Mean (SD)	−3.32 (3.18)	−2.82 (3.51)	−2.67 (3.84)	−3.28 (3.2)	−2.57 (3.33)	−2.82 (3.48)
Range	(−11, 3)	(−8.5, 6.5)	(−11.5, 5.5)	(−10, 5)	(−9, 3.5)	(−11.5, 3)
Colorimetry						
Mean (SD)	−3.21 (2.44)	−2.62 (2.53)	−2.85 (3.33)	−3.12 (3.3)	−2.78 (2.77)	−2.32 (2.48)
Range	(−9.52, 1.59)	(−8.25, 4.74)	(−11.1, 4.4)	(−10.7, 4.11)	(−8.84, 3.8)	(−7.47, 2.26)
Transepidermal waterloss (TEWL)						
Mean (SD)	−6.49 (7.95)	−4.36 (7.32)	−5.86 (10.1)	−7.14 (9.32)	−5.82 (8.58)	−5.83 (7.14)
Range	(−32.2, 3.7)	(−20.2, 19.3)	(−38.5, 9.9)	(−44.2, 10.1)	(−29.8, 7.5)	(−29.5, 7.3)
Hydration						
Mean (SD)	23 (32.9)	21 (32.3)	19.2 (45.6)	26.4 (34.9)	17.7 (46.1)	18.7 (42.4)
Range	(−46.7, 93.3)	(−33.3, 103)	(−63.3, 133)	(−40, 103)	(−113, 167)	(−66.7, 127)

Values are end of treatment (day 17) changes from treatment baseline (day 7). Thus, for visual score, colorimetry and TEWL the highest negative values represent the best effect, whereas for hydration the best effect is positively correlated.

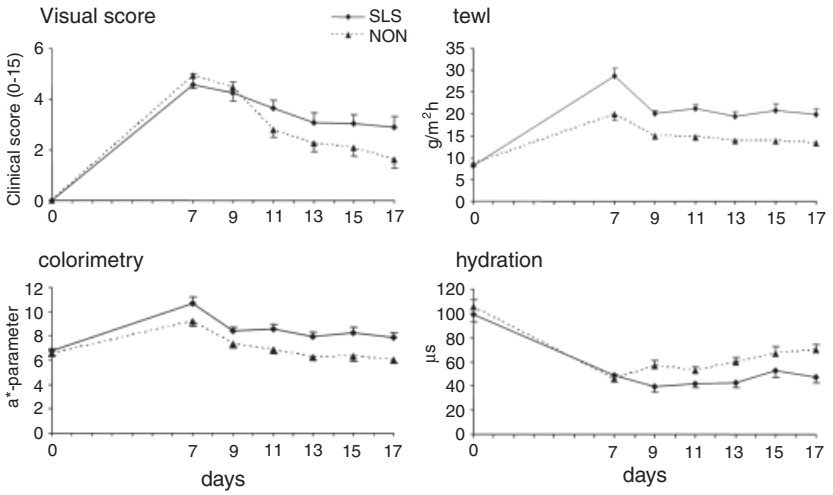

Fig. 1. Untreated course of reactions induced by nonanoic acid and sodium lauryl sulfate. Data are mean ± SEM.

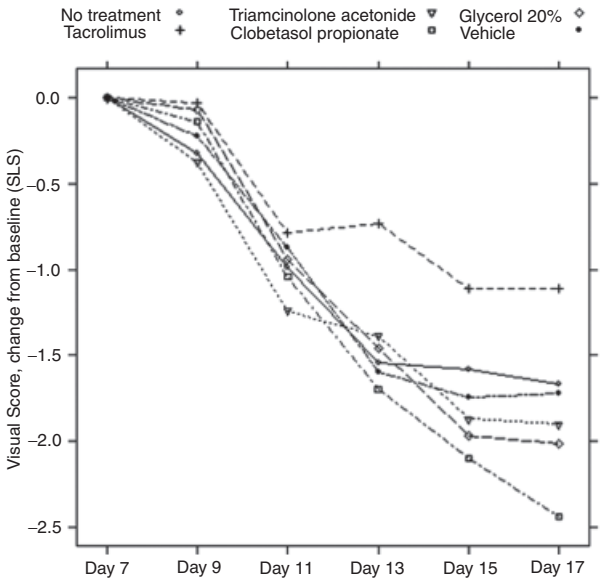

Fig. 2. Mean change in the visual score induced by six treatments on sodium lauryl sulfate-irritated skin. Error bars are omitted for clarity.

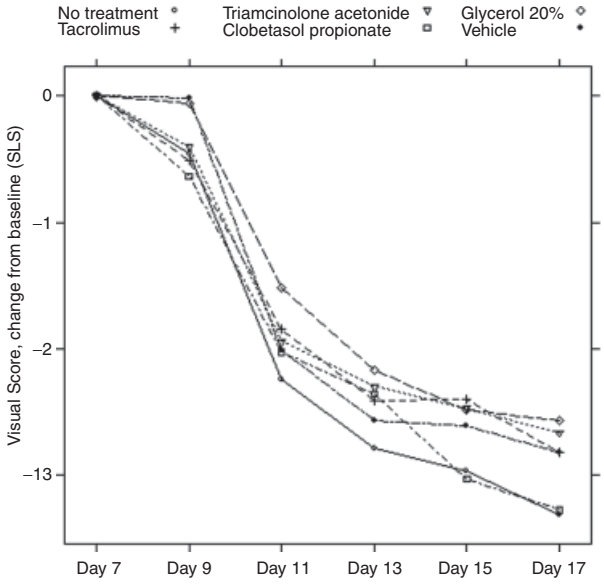

Fig. 3. Mean change in the visual score induced by six treatments on nonanoic acid-irritated skin. Error bars are omitted for clarity.

skin) and Table 2 (NON-irritated skin) as the end of treatment (i.e. at day 17) changes from treatment baseline (i.e. day 7). Additionally, the visual scorings are plotted for SLS and NON in Figs 2 and 3, respectively. No treatment was significantly better than all treatments and controls (vehicle and untreated site). However, some trends were apparent. There was a tendency toward a dose-dependent treatment response for the corticosteroids (i.e. clobetasol propionate better than triamcinolone acetonide) on both SLS- and NON-irritated skin, and for both irritants, clobetasol propionate ranked the best (on NON-irritated skin as good as no treatment). Tacrolimus ranked worse than the other treatments and the controls in SLS- but not NON-irritated skin. By biometric evaluation, in SLS-irritated skin, tacrolimus consistently ranked poorly in SLS irritation, whereas the other ointments ranked inconsistently between the evaluation methods (Table 1). For NON-irritated skin (Table 2), clobetasol propionate and no treatment consistently ranked better than the other ointments. Glycerol seemed to rank poorly regardless of the evaluation parameter.

The subanalysis on the upper, lower and second/third quartiles of day 7 reactions also showed no statistical differentiation between treatments.

Variance in the experiment explained by treatment is minute and insignificant

The pronounced interindividual variation in skin irritancy studies (18) was confirmed. In Table 3, the variance explained by subject effect is presented in relation to the variance explained by treatment. It is striking that the subject effect accounts for 57.3–83.9%, while only 0.6–1.5% of the variance can be explained by treatments. This was true regardless of the summary measure used (change from baseline at EOT vs. AUC). Three *P* values related to the treatment of SLS-irritated skin were actually below 0.05. However, given the multiple number of tried-out end points, this may well be explained by multiplicity.

Discussion

The experiments yielded largely negative results, and we could not confirm the previous results, and there was no significant difference between the treatments.

The hypothesis that corticosteroids could enhance SLS-induced irritation dose-dependently is unsubstantiated. In contrast, the strongest corticosteroid (clobetasol propionate) ranked the best among all the treatments in both SLS- and NON-treated skin (although for NON irritation as good as no treatment). It is important to note, however, that our data do not support any claims that clobetasol propionate is an efficient anti-irritant either. Ramsing and Agner (19) were able to demonstrate a small but significant effect of betamethasone-17-valerate (a corticosteroid with potency somewhere between that of triamcinolone and clobetasol propionate) 7 days after the

TABLE 3. Percentage of explained variance by subjects and treatments, respectively

	SLS				NON			
	Subject		Treatment		Subject		Treatment	
	Explained variance (%)	*P* value	Explained variance (%)	*P* value	Explained variance (%)	*P* value	Explained variance (%)	*P* value
Visual score								
CBL	72.8	<0.001	1.3	0.125	57.3	<0.001	0.7	0.703
AUC	76	<0.001	0.6	0.458	58.3	<0.001	0.7	0.683
*a**-parameter								
CBL	78.5	<0.001	0.6	0.378	68.9	<0.001	1.1	0.254
AUC	83.8	<0.001	1.1	0.026	68.1	<0.001	0.7	0.522
Transepidermal waterloss (TEWL)								
CBL	74.2	<0.001	0.8	0.343	71.5	<0.001	1	0.273
AUC	83.9	<0.001	1.3	0.011	72.4	<0.001	1.3	0.126
Hydration								
CBL	73.9	<0.001	2	0.016	67.3	<0.001	0.6	0.694
AUC	72.8	<0.001	1.5	0.07	61.9	<0.001	0.7	0.644

Summary of results from analysis of variance (ANOVA). Four endpoints were tried in the two groups defined by the two irritants.
Visual score, *a**-parameter (colorimetry), transepidermal water loss (TEWL) and hydration. Change from baseline to end of treatment (CBL) and area under the curve (AUC) of change from baseline were computed and used as a response variable in a standard block model with subjects as blocks.
The percentage of variation explained by subjects and treatments, respectively, was computed together with *P* values testing for effect of subjects and treatments.
SLS, sodium lauryl sulfate; NON, nonanoic acid.

acute induction of irritancy by 24-h SLS patch tests. Moreover, the simultaneous application of topical corticosteroid with an irritant in patch tests resulted in reduced irritancy (20). However, in a cumulative model using SLS, van der Valk and Maibach (21) were unable to find any beneficial effects of different corticosteroids with varying potency, and they even suggested induction of irritancy by corticosteroids. In another repetitive SLS irritation study with concurrent topical treatment, erythema and TEWL were not significantly changed by triamcinolone (22).

In the present study, a trend toward enhanced irritancy by tacrolimus was seen in SLS-irritated skin. This is consistent with one earlier study, where repetitively induced SLS irritation was enhanced by tacrolimus as assessed by visual scoring, erythema and TEWL (8). In another study, erythema induced by a 24-h SLS patch test was significantly reduced by pimecrolimus – in parallel to 1% hydrocortisone – compared with vehicle and untreated sites, whereas no effect on TEWL was found (9). The conflicting results may reflect differences in compounds (tacrolimus vs. pimecrolimus) as well as exposure (single vs. repeated).

The anti-irritant effect of glycerol could not be confirmed in this study. In SLS-irritated skin, the hydration was improved to some extent by glycerol (Table 2), which is consistent with the well-known humectant properties of glycerol (23,24). In NON-irritated skin, glycerol consistently ranked poorly for all the evaluation parameters including hydration, where it, unexpectedly, ranked the worst. No explanation for this other than the general randomness of our results can be given.

Applicability of the wash test for the evaluation of and/or the treatment of ICD

The wash test model was designed to emulate everyday life, in which the patient develops contact dermatitis, then contacts a physician and is told to avoid/reduce irritant exposure, and is given treatment. Compared with occlusive/open (single as well as repetitive) application of irritants, the cumulative wash test is likely to mimic everyday exposure to irritants much more closely. This assumption is shared by others (25,26). The major drawback of the model, however, is that it is more difficult to standardize. The irritant insult is both chemical and frictional, and the degree of friction cannot be quantified under the present experimental conditions. The fact that many subjects had their number of washings reduced does not interfere with the ranking of ointments because they are compared within the same individual. Future modifications of the wash test model could imply (1) reduction of irritant concentration because the target mean level of irritation at day 7 was exceeded for both irritants

and (2) addition of visits in the induction phase in order to monitor reactions better to avoid extreme reactions.

The considerable variance between experimental results can be explained largely by the interindividual variation and less by variation between treatments (Table 3). This impedes the development of realistic human experimental skin irritation assays, which can be used to study treatment effects in a reproducible way. One possibility in future studies is to preselect a more homogeneous group of individuals for treatment studies. However, in the present study, the subanalysis of the more homogenously irritated second and third quartile subgroup did not change the response pattern.

In conclusion, we could not demonstrate any difference in the anti-irritant effect between any of the ointments. An immense variance between subjects along with minute changes explained by treatments may render the wash test design unsuitable for efficacy testing of anti-irritants.

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