Sodium Hydroxide-induced Subclinical Irritation

*A test for Evaluating Stratum Corneum Barrier Function**

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This report concerns the development of a short, simple, non-invasive test for assessing sensitivity to irritant dermatitis. Application of NaOH (0.005–2.0 mol/l) to human skin resulted in significantly greater skin surface water loss directly after exposure (1–15 min) than of control (water). The increase in skin surface water loss after NaOH application was dose-dependent (0.005–0.1 mol/l) and application time-dependent (1–10 min). Application times exceeding 10 min did not further increase skin surface water loss and doses higher than 0.1 mol/l reversed the effect on skin surface water loss. 15 min after removal of the alkali, skin surface water loss baseline values were almost regained. This procedure did not cause visible reactions or discomfort for the volunteers. In a subsequent experiment, volunteers were exposed to 0.2 mol/l NaOH for 5 min on one forearm and to 1% sodium lauryl sulfate for 24 h contralaterally. Skin surface water loss after 5 min of NaOH application was significantly correlated with transepidermal water loss measurements after 24 h of sodium lauryl sulfate patch application. This is, to our knowledge, the first description of a procedure for quantifying interindividual differences in stratum corneum barrier function without inducing visible changes or causing volunteers discomfort. Use of this model should help to further investigate skin barrier function as well as to test protective devices and barrier creams. *Key words: Alkali resistance; transepidermal water loss; Sodium lauryl sulfate.*

This report concerns the development of a short simple, non-invasive test with which to assess sensitivity to irritant dermatitis. The alkali resistance test was proposed (1) to identify subjects with increased sensitivity to irritants. Although it had been the only assay available for testing an individual's sensitivity to chemical irritants, it has not attracted general attention in the United States and its clinical value has been debated in Europe (2–7). A disadvantage of this test, besides its low sensitivity and specificity, is that development of at least 10 erosions is required in order to obtain useful results; sodium hydroxide may have to be applied to skin with several erosions, an uncomfortable procedure. The risk of inducing severe alkali necrosis by this procedure, even with modifications (5), is about 1% (8). Recently, Frosch et al. showed that both the cutaneous whealing response to dimethyl sulfoxide (9) and the blister formation following ammonium hydroxide (10) application are correlated with skin barrier function. Both approaches, however, involve some volunteer discomfort.

In the present study the action of NaOH on skin was quantified by skin surface water loss (SSWL) as an indirect measure of stratum corneum water content. The severity of sodium lauryl sulfate induced irritant dermatitis was compared both with the magnitude of NaOH-induced SSWL increase and with the traditional alkali resistance. A young female study population was chosen because it shows high sensitivity to irritant dermatitis (11).

MATERIAL AND METHODS

Study population

Three panels of 10 female Caucasians (21 to 49 years) volunteered to participate after signing a consent form. Volunteers were deemed to be free of active skin disease by one of the authors. The study was approved by the University of California San Francisco Committee for Human Research.
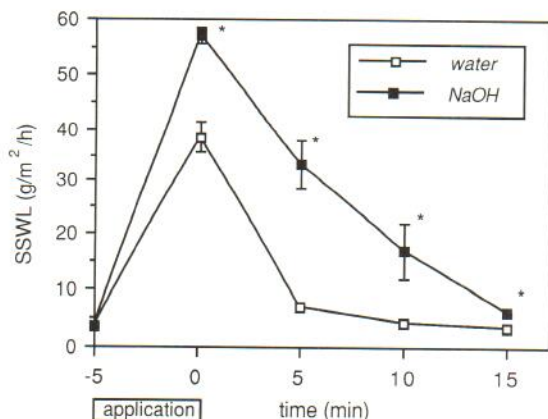
Fig. 1. SSWL after a 5-min application of 0.2 mol/l NaOH (mean ± SE). Directly after removal, SSWL is significantly higher than for controls, with the greatest difference 5 min after removal of the solution. *$p \leq 0.05$ (compared with controls, paired *t*-test). Note that SEM for control values are for certain time points too small to be illustrated ($n = 10$ volunteers).

Applications

0.1 ml of sodium lauryl sulfate (1.0%, in dist. water) (Sigma, St. Louis, Mo.) was applied to the mid-volar forearm using occlusive polypropylene chambers (Hilltop Laboratory, Cincinnati, Ohio) for 24 h. The patches were fixed with non-occlusive paper tape (Scanpore, Norgeplaster, Oslo, Norway).

Alkali resistance was determined using Locher's modifications (5). Briefly, 0.1 ml 0.5 mol/l NaOH was pipetted onto volar forearm skin. Uniform distribution was achieved by fixing a clear plastic block ($3.5 \times 2 \times 1.5$ cm) with slight pressure to the surface using non-occlusive paper tape. After 5 min the solution was wiped off. The procedure was repeated in the absence of damage until a positive reaction occurred, i.e. small erosions. Counting erosions was facilitated by staining them with 0.1% nitrazine yellow and by using a magnifying glass. The alkali resistance was denoted by the number of applications required to induce a minimum of 10 erosions. However, to avoid unacceptable injury the procedure was stopped if there was extensive erythema around the exposure site, if any erosion exceeded 2 mm in diameter, or when a total of six applications was reached (8). The next period was then designated the alkali resistance.

To evaluate quantitatively the influence of NaOH on stratum corneum barrier function, 0.4 ml NaOH was applied to the volar forearm with occlusive plastic chambers for 1, 5, 10 or 20 min at the following concentrations: 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 mol/l. Distilled water served as vehicle control. At the end of exposure, the skin was wiped with a soft paper towel to remove remaining solution (Kimwipes, Kimberly-Clark, Roswell, Ga, U.S.A.), rinsed with distilled water and gently dried with a soft paper towel.

Measurements

Transepidermal water loss (TEWL) allows the estimation of water flux through the stratum corneum and provides an assessment of stratum corneum barrier function (12, 13). SSWL in the special case of occlusion or direct topical application of water or of a solution, correlates positively with stratum corneum hydration, once the occlusive material (or the solution) is removed (14). In essence, SSWL equals excess water loss plus TEWL (14). TEWL and SSWL were measured with an Evaporimeter (EP1, Servo med, Stockholm, Sweden). This instrument uses the method of vapor pressure gradient calculation described in detail by Nilsson (12). The probe was held in place for each measurement until a stable value had been established (approximately 30 s). Measurements were performed 1 to 20 min after application of NaOH and 24 h after removal of the sodium lauryl sulfate patch, respectively. Sodium lauryl sulfate induced irritant dermatitis was also visually graded according to a 5-point scale (15).

Statistics

Differences in SSWL between NaOH and control treatment were tested for significance using Students *t*-test for paired samples (16). A *p*-value ≤ 0.05 was considered statistically significant. Correlation coefficients were calculated according to Pearson (16).

RESULTS

The time-course of SSWL after a 5-min application of NaOH is shown in Fig. 1. Since the greatest difference between control and NaOH was observed 5 min after removing the alkaline solution, the effects of concentration and exposure time were determined at that time. A log dose-response relationship
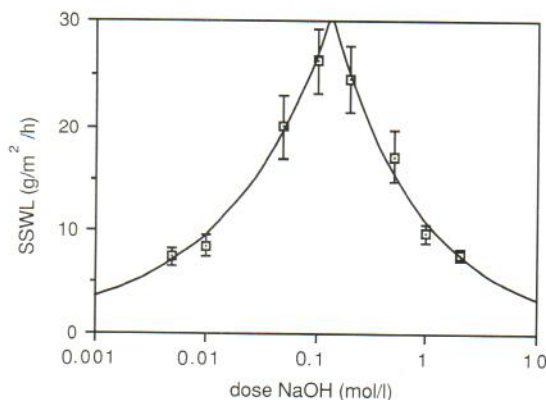

Fig. 2. Dose-response relationship beween SSWL and NaOH. Values illustrated are SSWL 5 min after a 5-min exposure to varying concentrations NaOH and a computer derived curve determined by least squares curve fitting. SSWL increased for concentrations from 0.005 to 0.1 mol/l. Further increasing doses led to a decrease in SSWL (mean ± SE; $n = 10$).
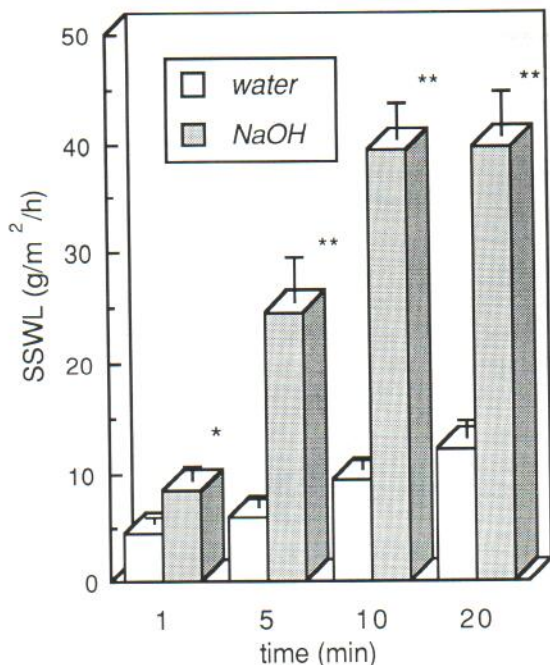
Fig. 3. Effect of exposure time on SSWL. SSWL, 5 min after application of a 0.2 mol/l NaOH solution for various intervals. Absolute SSWL was highest after 10 min of exposure, with no increase for 20 min exposure. Ratios of SSWL after NaOH / controls increased from 3 (1 min) to 4 (5 and 10 min) and decreased to 3 for 20 min exposure (mean ± SE; $n = 10$) *$p \leq 0.05$ (compared with controls, paired t-test.).

Table I. *Transepidermal water loss measurements (TEWL) 24 h after treatment with 1% sodium lauryl sulfate and skin surface water loss (SSWL) 5 min after a 5-min application of 0.5 mol/l NaOH in 10 volunteers with their corresponding alkali resistance (AR); controls were treated with water for 24 h*

Volunteer no.	TEWL (g/m²/h)		SSWL (g/m²/h)	
	Control	24 h SLS	5 min NaOH	AR
1	3.9	19.0	23.0	7[a]
2	4.9	6.0	14.5	6
3	3.5	22.0	26.9	6
4	3.8	11.6	19.3	6
5	5.5	9.2	22.5	5
6	4.7	22.2	42.1	7
7	4.2	16.7	30.9	5
8	2.9	13.3	13.3	7
9	5.5	10.5	21.5	5
10	2.8	9.1	18.3	7
Mean±SD	4.2±0.9	14.0±5.7	23.2±8.5	6.1±0.9

[a] Number of applications (5 min each) until a positive reaction occurred.

DISCUSSION

Alkali resistance determination had previously been the only clue for dermatologists in detecting sensitive skin. Since its introduction in 1947, its value for the practising dermatologist has been debated (1–7). Individuals with active extensive atopic or seborrheic dermatitis unequivocally showed a lower alkali resistance than healthy controls (1, 2, 6). Czernie-
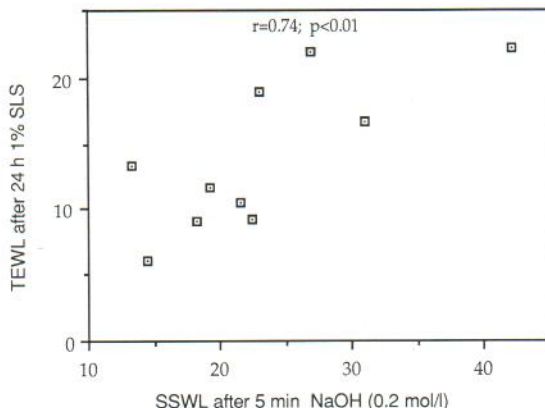

Fig. 4. Relationship between SSWL, 5 min after a 5-min NaOH (0.2 mol/l) application and TEWL after a 24-h SLS patch test. A significant linear correlation was observed between the two exposures ($n = 10$ volunteers).

between SSWL and concentration NaOH was seen in the range of 0.005 to 0.1 mol/l. Further increases in concentration reversed that effect (Fig. 2). A 10-minute exposure elicited maximum response with no further increase in SSWL thereafter (Fig. 3). This treatment did not cause erythema or discomfort to the volunteer.

To evaluate the significance of the observed SSWL increase after NaOH application, another panel was exposed for 5 min to 0.2 mol/l NaOH on one arm and for 24 h to sodium lauryl sulfate on the corresponding contralateral site. The results together with the alkali resistance are summarized in Table I. No significant correlation was observed between alkali resistance and severity of irritant dermatitis after 24 h of sodium lauryl sulfate application ($r = 0.431$; data not shown). However, a significant linear correlation was demonstrated for the severity of irritant dermatitis induced by 24 h of sodium lauryl sulfate patch and SSWL after 5 min of NaOH exposure (Fig. 4).

lewski, on the other hand, also found the determination of alkali resistance valuable as a predictive test for occupational hand eczema (4).

Frosch and co-workers, elegantly showed that intra- and interindividual differences in skin barrier function can be correlated with the cutaneous response to both dimethyl sulfoxide (whealing) and ammonium hydroxide (blister formation) (9, 10). The dimethyl sulfoxide test, however, is subjective and both approaches involve some volunteer discomfort.

In our study, differences in alkali resistance were not pronounced, which was as expected, since we looked exclusively at healthy volunteers. However, the same panel showed marked differences in susceptibility to sodium lauryl sulfate-induced irritation, as demonstrated by almost 4-fold differences in TEWL.

In a quantitative, function-related approach, we measured the effect of sodium hydroxide on SSWL after varying exposure times and different NaOH concentrations. Only 1 min of exposure to 0.2 mol/l NaOH resulted in significantly greater SSWL than in controls. The increase in SSWL in controls reflects the evaporation of excess moisture following temporary stratum corneum hydration. Since excess solution was carefully removed after application, the higher and substantiated increase in SSWL 5 min after application of NaOH might be explained as increased hydration of stratum corneum. Indeed, the hygroscopic NaOH induces a marked swelling of keratinocytes, which was utilized by Christophers & Kligman to visualize stratum corneum structure (17). Another explanation for the increased SSWL might be continuous disruption of the secondary and tertiary structure of keratin proteins, resulting in exposure of new water-binding sites as also observed in anionic surfactant-induced stratum corneum swelling (18). Quick reversibility of the SSWL increase supports this view.

The effect of NaOH on SSWL was dose-dependent (0.005–0.1 mol/l) and application time-dependent (1–10 min). However, application times longer than 10 min did not further increase SSWL, suggesting that the maximum effect for a 0.2 mol/l NaOH solution is reached after 10 min of exposure. Use of concentrations higher than 0.1 mol/l NaOH resulted in lower SSWL than with 0.1 NaOH. This might be due to a denaturation of proteins thereby destroying water-binding sites. Higher NaOH concentrations, once they have penetrated into the stratum cor-

neum, might also yield lower SSWL because the hygroscopic force of the NaOH then prevents the dehydration of the swollen keratinocyte. Fifteen minutes after removal of the solution, SSWL almost regained baselines level, but was still significantly higher for NaOH than for controls. No visible changes were induced by this procedure, nor did the volunteer feel discomfort or pain.

The stratum corneum and especially its outermost layers are generally regarded as a major barrier preventing external compounds from penetrating through the skin and partially regulating water loss and retention. TEWL is a sensitive parameter for barrier function (12, 13). However, baseline TEWL as a predictor for sodium lauryl sulfate-induced irritation has been debated (19–22).

In the present study, we demonstrated that substantial interindividual differences in the stratum corneum barrier, quantified by SSWL measurements following a 5-min exposure to a strong base, were correlated with the severity of visible dermatitis after 24 h of exposure to the model irritant sodium lauryl sulfate. This correlation could be explained by two possible mechanisms:

1. The effectiveness of the cutaneous barrier determines how much NaOH can penetrate in a given time. The amount NaOH penetrating into the stratum corneum is then indirectly measured by the consequent dehydration. Thus the strength of the skin barrier can be estimated after no more than 5 min of NaOH exposure.
2. It has been shown *in vivo* that the irritation potential of anionic surfactants is correlated with their potential to hydrate stratum corneum (18). One might speculate that interindividual differences in stratum corneum hydration potential as demonstrated with NaOH application might account for differences in susceptibility to surfactant-induced dermatitis.

In summary, cutaneous sensitivity to irritation is at least partially correlated to a weak stratum corneum which is unable to effectively prevent irritant compounds from penetrating into skin. Future studies should elucidate the effect of more primary irritants on the stratum corneum barrier at a preclinical stage. Use of this model should further the investigation of skin barrier function as well as be of use in testing protective devices and barrier creams.

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