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Sodium Hydroxide-induced Irritant Dermatitis as Assessed by Computerized Elaboration of 20 MHz B-scan Images and by TEWL Measurement: A Method for Investigating Skin Barrier Function

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Sodium hydroxide-induced irritation was studied in 34 volunteers, by means of 24-h patch testing at different concentrations, and by a 10-min testing procedure employing 0.1 mol/l NaOH. As a supplement to subjective evaluation of skin changes, assessments of test areas by TEWL measurement and sonography were performed at 24, 48 and 72 h. After 24-h patch testing, instrumental evaluations showed an increase in the extension of the hypo-echogenic dermal area and in TEWL, whereas a 10-min NaOH application induced a decrease of the dermal and epidermal reflectivity and an increase in TEWL. Twenty-four hour patch testing with 4% NaOH allowed a classification of subjects into two categories: subjects who reacted normally and hyper-reactors. Hyper-reactors showed an enhanced inflammatory response and a more pronounced barrier function damage, as assessed clinically and instrumentally by decreased dermal reflectivity, and by higher postexposure TEWL. Subjects with a more marked inflammatory response to 4% NaOH also showed greater TEWL increases during the short-term testing procedure employing 0.1 mol/l NaOH. Moreover, these subjects were characterized by higher baseline TEWL values, indicating that cutaneous reactivity to NaOH is at least partly correlated to impaired stratum corneum function, which is inadequate to effectively prevent compounds from penetrating the skin. ***Key words: irritation; sonography; transepidermal water loss.***

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The study of irritant contact dermatitis utilizes test models reproducing skin irritation in a standardized manner. Alkalis have been extensively used for studying skin sensitivity (1–12). Measurement of resistance to alkalis and of blister formation time following ammonium hydroxide application has been proposed as a procedure aiming at identifying subjects with hyper-irritable skin (1, 4, 6). NaOH-induced irritation has been assessed on human and animal skin by 24–48 h patch tests, which were evaluated by impedance measurements (2), transepidermal water loss (3, 11), laser Doppler flowmetry (9), skin fold thickness measurements (12) and polysulfide rubber replicas (8).

As a supplement to subjective evaluation of skin changes, B-scanning measurements have been newly introduced in order to objectively assess and quantify the different aspects of experimental irritant reactions (13–16).

Recently, Wilhelm et al. (11) reported a short non-invasive testing procedure for evaluating sensitivity to irritants. Applications of NaOH (0.005 mol/l – 2.0 mol/l) on human skin resulted in greater skin surface water loss 1–10 min after exposure, which subsided 15 min after removal of the alkali.

The purpose of our study was to investigate the alterations induced by the application of NaOH onto the skin, to compare a 24-h and a 10-min exposure time, and to elaborate a testing procedure for studying skin barrier function. Skin reactions were evaluated by visual scoring, measurement of transepidermal water loss (TEWL) and computerized elaboration of 20 MHz B-scan images.

MATERIAL AND METHODS

Study population

Thirty-four volunteers (33 women and one man, aged 18 to 45 years) participated in the study after informed consent. All the subjects had been affected by eczematous dermatitis of limited extent and were contact-sensitized to one or more allergens. No patient was atopic. All the volunteers had been lesion-free for at least 3 months prior to the study. The study was performed from September 93 to February 94.

Test substances and study procedure

1) 24-h test: NaOH was applied on the volar side of the forearms as a 1% (0.25 mol/l), 2% (0.5 mol/l) and 4% (1 mol/l) aqueous solution. Forty microlitre were employed for each patch test and applied on filter paper discs put into Finn chambers. Distilled water served as vehicle control. The NaOH 1% and 2% patch tests were attached to the right forearm; the NaOH 4% and the distilled water patches were put on the left. Test chambers were positioned on the median line running from the antecubital fossa to the wrist at 2 and 4.5 cm, respectively, from the former, secured to the skin by Scanpor tape and removed after 24 h. Following removal of the chambers, the test areas were rinsed with distilled water and gently dried with filter paper, in order to remove the residual test substance. Clinical and instrumental evaluations were carried out 30 min later and at 48 and 72 h. After patch test removal and between assessments, patch test sites were covered by a slight gauze dressing.

2) Short-term test: In 30 subjects a patch test with 40 µl of 0.1 mol/l (0.4%) NaOH was placed on the right forearm, at 7.5 cm from the antecubital fossa. The test substance was applied on filter paper discs, put into Finn chambers and left at the site for 10 min. A patch test with saline solution, positioned symmetrically on the left forearm, served as control. After exposure, the skin was flooded with water to remove residual test substance, then dried with filter paper. Instrumental evaluations were carried out immediately after drying.

Clinical scoring

Since responses to NaOH varied from apparently dry skin to abrasion and crusting, the degree of inflammation was graded as follows: 0 = no visible reaction; 1 = dry skin; 2 = faint, patchy erythema with or without dry skin; 3 = erythema and oedema; 4 = erythema and oedema with spotty erosions or crusting; 5 = erythema, oedema and more extensive erosions or crusting.

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Table I. *Clinical scoring: mean score per area at NaOH patch test sites*

NR = normal-reacting; HR = hyper-reactive.
Number of subjects is in brackets.

	24 h		48 h		72 h	
	NR (25)	HR (9)	NR (25)	HR (9)	NR (25)	HR (9)
NaOH 1%	0.96	1.78	0.76	1.33	0.48	1.33
NaOH 2%	1.64	2.67	1.52	2.55	1.24	2.55
NaOH 4%	1.96	3.89	1.84	4.44	1.64	4.44
Distilled water	0.36	0.66	0.08	0	0.08	0

Table II. *Baseline values in normal-reacting and hyper-reactive subjects*

	0–30 band (number of pixels)	201–255 band (number of pixels)	TEWL (g/m^2 h)
Normal-reacting subjects (100 test areas)	1235±621	270±131	3.52±1.93
Hyper-reactive subjects (36 test areas)	1067±518	257±134	4.97±2.22
All subjects (136 test areas)	1190±598	267±131	3.90±2.10

Instrumental evaluations

Ultrasound equipment and software for image analysis. Echographic evaluations were performed using a 20-MHz B-scanner (Dermascan C, Cortex Technology, Denmark), which produces images representing a cross section of the skin. Equipment and calibration methods have already been described in detail elsewhere (17). Evaluations were performed by employing the zoom function in the axial direction at the first magnification, which enables exploration of the tissue to a depth of 6.71 mm. During recordings the distance between the probe membrane and the skin was kept at 1.7 ± 0.2 mm.

The echographic images were processed by a program (Dermavision 2D, Cortex Technology), enabling a numerical representation of the picture data, based on the attribution of fictional values to the amplitudes of the echoes, the possibility of selecting amplitudes of interest, the segmentation of the image, and the calculation of the extension of areas reflecting within the chosen amplitude range (in number of pixels). These procedures allow the enhancement of areas of interest within an image by marking parts of it, and by removing pixels with uninteresting values, in order to improve recognition of features corresponding to tissue structures or evolutive phases of processes to be studied. The sonographic recordings were evaluated by an amplitude band (0–30 interval) marking the hypo-reflecting parts of the dermis, corresponding to oedema and inflammatory infiltration. The superficial hyper-reflecting part of the skin, corresponding to epidermis, was evaluated by means of a 201–255 band.

TEWL measurements. TEWL was measured using an evaporimeter (Servomed EP1, Stockholm, Sweden) and operated according to the guidelines of the Standardization group of the European Society of Contact Dermatitis (18). The instrument measures the vapour-pressure gradient in the air above the skin expressed in g/m^2h. Measurements were performed when the readings were stabilised after a period of 30–45 s.

Statistics

Student's *t*-test for paired samples was employed for evaluating differences between baseline and 10-min values, whereas the ANOVA test for repeated values was performed to check differences between baseline values and 24, 48 and 72 h values.

Differences between NaOH and control treatment and between values referring to different patient groups (normal-reacting and hyper-reactive patients) were tested for significance using Student's *t*-test for unpaired observations. A p value ≤ 0.05 was considered statistically significant.

For evaluating the relationship between the different instrumental parameters, the correlation coefficient according to Pearson was calculated.

RESULTS

Clinical evaluation

1) 24-h application procedure. The intensity of skin responses at 24 h increased according to NaOH concentration, varying from apparently dry skin associated to faint or patchy erythema to erythema and oedema with severe erosions and crusting.

Skin reactions to the 1% concentration were quite uniform in all subjects, whereas for the higher concentrations great inter-individual differences were observed. According to the clinical appearance of the test areas, subjects were divided into two groups: normal-reactive and hyper-reactive. Results of clinical assessment and of instrumental measurements were evaluated for all patients together and also considered separately, according to the reactivity of the subjects. At the 4% concentration, the normal reacting 25 subjects showed skin responses with erythema and oedema, sometimes associated to spotty or follicular erosions and crusting, whereas the 9 hyper-reactive patients presented erosions and crusting covering 20 to 80% of the test area, sometimes accompanied by a burning sensation during the first 24 h. The mean scores per test area are reported in Table I.

2) Short-term test. No visible signs of inflammation were observed at 10 min on the test areas.

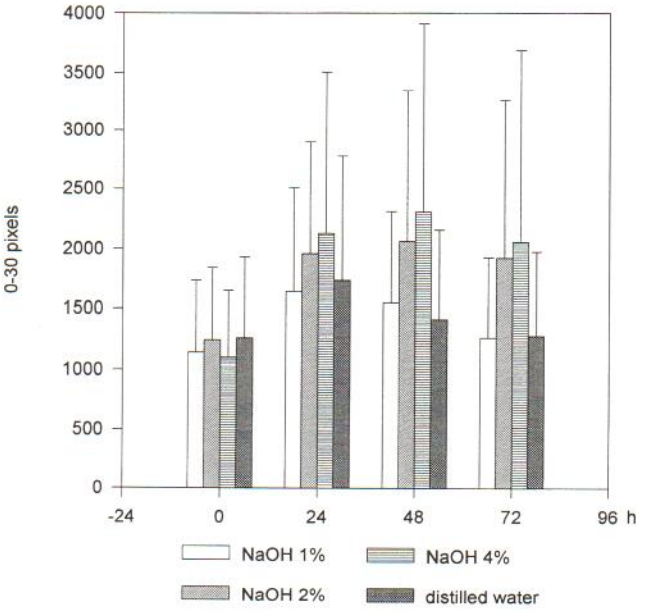

Fig. 1. Echographic evaluation of NaOH-induced reactions during a 24-h test: 0–30 band elaboration. The extension of 0–30 dermal areas is expressed in number of pixels.

Table III. *0–30 areas (expressed in number of pixels) at NaOH patch test sites in normal-reacting and hyper-reactive subjects*
NR = normal-reacting; HR = hyper-reactive. Number of subjects is in brackets.

	Baseline		24 h		48 h		72 h	
	NR (25)	HR (9)	NR (25)	HR (9)	NR (25)	HR (9)	NR (25)	HR (9)
NaOH 1%	1194±591	1017±596	1628±978	1671±504	1570±791	1490±724	1307±637	1152±757
NaOH 2%	1274±573	1166±689	1841±1010	2283±680 *	1949±1202	2377±1502 *	1804±1238	2255±1603 *
NaOH 4%	1166±581	936±391	1680±947	3383±1658	1768±1024	3810±2000	1580±1115	3388±2140

* statistically significant

Instrumental evaluation

Baseline values in normal-reacting and hyper-reactive subjects (Table II).

Significant differences between mean values referring to baseline skin of the 4 test areas in the two patient groups were observed for TEWL, which was higher in hyper-reactive subjects, whereas baseline values of the echographic parameters showed no significant differences.

1) 24-h application test

Echographic evaluation: 0–30 band elaboration. Extension of areas formed by pixels reflecting within the 0–30 interval reached maximum values at 24 h for the 1% concentration and at 48 h for the 2 and 4% concentrations (Fig. 1). Increases were significant in respect to baseline values for the 1% test areas at 24 and 48 h, and for the 2% and the 4% test areas at 24, 48 and 72 h. 0–30 values were significantly higher in hyper-reactive subjects in respect to normal-reacting ones at all times of assessment for 4% NaOH (Table III).

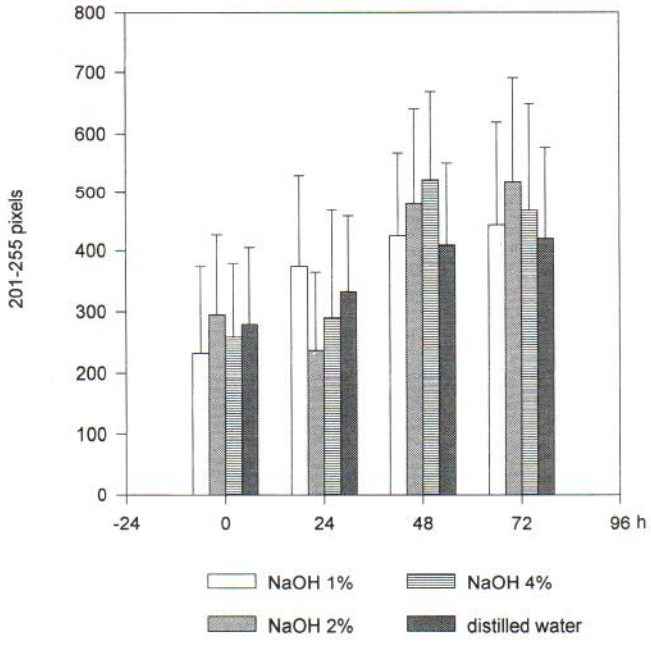

Fig. 2. Echographic evaluation of NaOH-induced reactions during a 24-h test: 201–255 band elaboration. The extension of 201–255 epidermal areas is expressed in number of pixels.

Echographic evaluation: 201–255 band elaboration. A significant increase in 201–255 pixel values was observed at 48 and 72 h for the 2 and 4% concentrations (Fig. 2), whereas at 24 h a significant decrease was observable for the 2% concentration. No significant differences between normal-reacting and hyper-reactive subjects were observed for any concentration (data not shown).

TEWL measurements. TEWL reached its maximum value 24 h after exposure (Table IV). Increases were significant in respect to baseline for the 2% and 4% concentrations at all times of assessment. In hyper-reactive subjects TEWL values were higher than in normal reacting ones, especially for the 4% concentration (Table V). Statistical significance is reported in the table.

Correlations. For the 4% concentration, the correlation coefficient between baseline and 24-h TEWL values in hyper-reactive subjects was 0.7118. Considering the whole study population, the correlation coefficient between 24-h 0–30 values and TEWL values was 0.5472 (NaOH 4%).

2) Short-term test

After a 10-min 0.1% mol/l NaOH exposure, a significant increase in the extension of the dermal hypo-echogenic area and a significant decrease of the epidermal reflectivity were observed (Fig. 3). A more than tenfold increase in TEWL was noticeable (Table VI). On the basis of the clinical results of the 24-h application test, subjects were divided into two groups, and data referring to these groups were separately considered. According to this subdivision, no differences in the echographic parameters at 10 min were observed, whereas the TEWL increase was significantly higher in the 8 hyper-reactive subjects compared to the 22 normal-reacting ones (Table VI).

Correlations. No correlation was found between baseline

Table IV. *TEWL (g/m^2 h) at NaOH patch test sites*

	Baseline	24 h	48 h	72 h
NaOH 1%	3.82±2.26	4.53±2.96	4.23±2.53	4.38±2.48
NaOH 2%	3.94±2.12	7±4.35	6.5±4.89	5.73±4.01
NaOH 4%	3.97±2.11	19.85±22.14	14.32±18.28	11.35±15.54
Distilled water	3.88±1.99	4.73±2.11	3.38±1.95	3.29±1.66

Table V. *TEWL (g/m² h) at NaOH patch test sites in normal-reacting and hyper-reactive subjects*
NR = normal-reacting; HR = hyper-reactive. Number of subjects is in brackets.

	Baseline		24 h		48 h		72 h	
	NR (25)	HR (9)	NR (25)	HR (9)	NR (25)	HR (9)	NR (25)	HR (9)
NaOH 1%	3.52±2.25	4.66±2.17	3.96±2.31	6.11±4.01	3.56±1.60	* 6.11±3.65	3.92±1.65	5.66±3.84
NaOH 2%	3.68±1.97	4.66±2.45	5.80±2.92	* 10.33±5.95	5.00±2.67	* 10.66±7.11	4.56±1.98	* 9.00±6.18
NaOH 4%	3.40±1.71	* 5.55±2.40	9.72±6.67	* 48.00±26.00	5.68±3.27	* 38.33±21.54	5.36±2.74	* 28.00±23.44

* statistically significant

TEWL and 10-min TEWL values. Moreover, correlation coefficients between 10-min and 24-h TEWL values were low (for example, considering the whole study population, r was 0.3237 for NaOH 4%).

DISCUSSION

Our data show that NaOH-induced inflammation following a 24-h application appears echographically with a marked hypo-echogenicity of the dermis. Variations of the epidermal component, as assessed echographically, were not univocal at 24 h, whereas at 48 and 72 h, an increase of the superficial hyper-reflecting band was observable. At the 10-min assessment, an increase in the extension of the hypo-echogenic area, accounting for approximately 20% of the initial value, and a significant decrease of the epidermal reflectivity were appreciated. Recently, the echographic aspect of irritant reactions induced by sodium lauryl sulfate (SLS), hydrochloric acid and nonanoic acid has been described (13–16). While the inflammatory component of these reactions has a fairly uniform echographic appearance, owing to the increase in the hypo-echogenic component of the dermis, epidermal aspects vary according to the test substance: at 24 h SLS causes a decrease of the superficial reflectivity of the skin, whereas nonanoic acid and HCl induce an enhancement of the entrance echo, which we also observed for NaOH at 48 and 72 h. Since no evaluations of the superficial reflectivity have been performed during short-term tests using other irritant substances, no comparison can be made regarding the decrease observed at 10 min for NaOH. However, one could presume that variations of epidermal and dermal reflectivity might be due to rapid fluctuations of tissue water, occurring via neural stimuli. Whereas at SLS-induced reactions an inverse correlation between 24-h 201–255 values and TEWL values has been described (13, 14), modifications of the epidermal reflectivity at NaOH patch test sites were not correlated to TEWL. However, TEWL augmentation was partly related to the inflammatory component of the reaction, as assessed by the 0–30 evaluation.

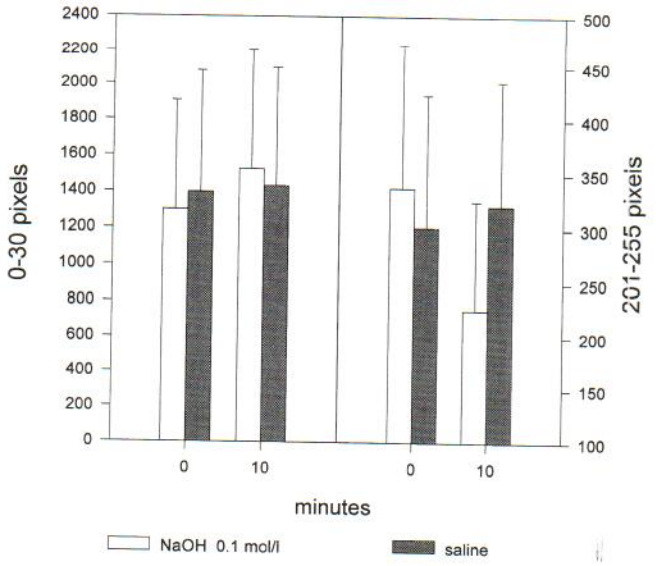

Fig. 3. Echographic evaluation of skin reactions to a 10-min application of 0.1 mol/l NaOH. The extension of 0–30 dermal areas and 201–255 epidermal areas is expressed in number of pixels.

This study also demonstrates that response to NaOH evidences great variations in skin reactivity in different subjects. Most subjects reacted with erythema and oedema, rapidly decreasing after patch test removal, whereas 9 subjects were afflicted by erosions and crusting of variable degrees, confirming other authors' observations on great inter-individual differences in the response to this irritant (8). Thus, testing with NaOH allowed a clinical distinction of subjects into two categories: normal reacting and hyper-reactive ones.

No clinical differences between our two patient groups regarding the course and the extent of the dermatitis or contact sensitization were identified. All the patients had been affected by eczema circumscribed to a limited area of the body and had been free of skin lesions during the last 3–12 months. On the basis of literature data (4, 19, 20), we can presume that in our study population skin reactivity was similar to that of healthy subjects. None of our hyper-reactive patients reported a history of severe irritant contact dermatitis; however, since none of them were particularly exposed to domestic or occupational irritants, no definite conclusion can be drawn regarding their skin reactivity to strong irritant environmental substances.

Baseline dermal echogenicity showed no significant variations in the two subject groups, whereas after a 24-h exposure, the intensity of the inflammatory response was greater in hyper-

Table VI. *TEWL (g/m² h) after a 10-min 0.1 mol/l NaOH application*

	Baseline			10 minutes		
	All subjects	Normal-reacting	Hyper-reactive	All subjects	Normal-reacting	Hyper-reactive
NaOH 0.1 mol/l	4.13±2.44	3.77±2.18	5.12±3	54.57±20.63	48.54±19.36	71.12±14.51
Saline	3.87±2.48	3.39±2.45	4.87±2.36	15.85±5.34	14.78±4.93	18.25±5.77

reactive subjects, as shown by a more marked dermal hyporeflectivity. As regards the 201–255 band evaluation, no differences between the two groups were observed in baseline, 10-min and 24-h post-exposure epidermal reflectivity.

Hyper-reactive subjects differed from normal-reacting ones by higher baseline TEWL values and by post-exposure TEWL values, which were significantly higher throughout the whole observation period at 2% and 4% NaOH patch test sites. When 24-h and 10-min TEWL values in the same subjects were compared, correlation coefficients were low. However, hyper-reactive subjects, with a higher average increase in 24-h TEWL in respect to normal-reacting ones, showed higher TEWL values at the 10-min evaluation, too. Moreover, in this same patient group, correlation coefficients between baseline and 24-h TEWL values were high.

In conclusion, 24-h testing with NaOH at a high concentration allows the identification of subjects reacting with greater intensity, i.e. showing an enhanced inflammatory response and a more pronounced barrier function damage, as assessed clinically and instrumentally by decreased dermal reflectivity, and by higher post-exposure TEWL. The subdivision of subjects according to the 24-h evaluation leads to the identification of a subpopulation reacting with greater TEWL increases during a short-term testing procedure, which also presents higher baseline TEWL values.

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