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Reactivity to Nickel Sulfate at Sodium Lauryl Sulfate Pretreated Skin Sites Is Higher in Atopics: An Echographic Evaluation by Means of Image Analysis Performed on 20 MHz B-scan Recordings

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The aim of this study was to establish an objectively assessable procedure simulating simultaneous exposure to irritants and allergens in domestic and occupational environments, in order to evaluate differences in the reactivity to the combination of these substances in atopic and non-atopic nickel-sensitized subjects. Thirty-four nickel-sensitive patients, 20 of whom were affected by atopic dermatitis, underwent four patch tests with $NiSO_4$ 0.05% aq. on two adjacent sites of both volar forearms, with a 24-h application time. Two of the test sites were treated with sodium lauryl sulfate (SLS) 5% for 30 min, before application of the nickel sulfate preparation. Echographic recordings were performed by a 20-MHz B-scanner and processed by an image analysis program, providing a numerical representation of the picture data, based on the attribution of fictional values to the amplitudes of the echoes. The dermal inflammatory reaction was quantified by an amplitude band, marking the hypo-reflecting part of the dermis, whereas epidermal damage was assessed by a band highlighting the entrance echo. Pre-treatment with SLS of the skin area where nickel sulfate was subsequently applied greatly enhanced the allergic response at 24 and 72 h, both in subjects with atopic dermatitis and in subjects with allergic contact dermatitis. However, in atopics, the increase in the allergic reactivity after irritation of the skin was more pronounced both by clinical and by echographic evaluation. These observations stress the importance of the concurrent action of irritants and allergens in maintaining the dermatitis in atopics. ***Key words: nickel sensitization; irritation; atopic dermatitis; echography; regional variations.***

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Patch testing for the evaluation of patients suspected of having contact dermatitis is usually performed on normal skin. However, exposure to environmental allergens also occurs in association with substances, that can induce non-immune inflammation and enhance the penetration of sensitizing chemicals, thus evoking a response at sub-threshold concentrations. In well-known experimental procedures for screening and rating contact sensitizers, sodium lauryl sulfate (SLS) was introduced to increase the response to allergens (1). Post-application of SLS and anthralin has been shown to amplify skin reactions in patch tests performed with various allergens in contact sensitized patients, reducing the minimal eliciting concentrations (2). Moreover, skin damage as a result of arm immersion in SLS has been shown to greatly enhance the response to nickel on the forearm (3). In fact, the reactivity threshold to an allergen is a crucial concept for understanding the relevance of the concentration of a chemical in the environment and can be influenced by simultaneous irritation. Furthermore, impaired barrier function, with increased susceptibility to surfactants, and contact sensitization are common findings in atopics (4–11).

The aim of this study was to establish a test procedure acting as a model simulating simultaneous exposure to irritants and allergens in domestic and occupational environments, at the same time providing an objective assessment and a quantification of inflammation and skin barrier damage (12, 13), and to evaluate differences in the reactivity to a combination of irritants and allergens in atopic and non-atopic nickel-sensitized subjects. Differences in the reactivity to SLS and nickel sulfate related to site on the forearm were evaluated as well.

PATIENTS AND METHODS

Thirty-four nickel-sensitive patients, aged 14 to 58, selected on the basis of a previous ++ or +++ positive patch test reaction and a history of skin lesions at sites of metal contact, were studied, after informed consent. Twenty subjects were affected by allergic contact dermatitis (ACD) (mean age 29.25 ± 10.73), and 14 by atopic dermatitis (AD) (mean age 24.36 ± 7.60) according to the criteria of Hanifin & Rajka (14). No patients showed eczematous lesions on the forearms during the testing period. Patients underwent four patch tests with 40 µl $NiSO_4$ ($NiSO_4 \cdot 6H_2O$, 99.999%, Aldrich Chemical Co.), 0.05% aqueous (111.65 p.p.m. nickel), on adjacent sites of the volar surface of both forearms. In order to make a 24-h evaluation of the test sites possible, a 24-h application time was chosen. The concentration was established on the basis of preliminary experiments, performed with 0.01 to 0.05% nickel sulfate aq. and designed to identify a dilution of the allergen inducing weak clinical reactions in most subjects showing ++ or +++ responses to routine patch test examinations. Solutions were pipetted on filter paper disks, put into large Finn chambers (11 mm in diameter), and fixed to the skin by Scanpor tape. The upper test sites were put 2 cm below the elbow crease, the two lower test sites being lined up at 3 cm distance distally, on both forearms. Two of the test sites, on the right in proximal position (2 cm below the elbow crease), on the left in distal position (8 cm below the elbow crease), were treated with 40 µl SLS (99%, SIGMA Chemical Co.) 5% for 30 min, before application of the nickel solution (SLS + Ni areas). At control test sites, 8 cm below the elbow crease on the right, and 2 cm below the elbow crease on the left, 40 µl 5% SLS (SLS areas), with a 30-min exposure time, and an empty chamber, respectively, were applied. Untreated nickel areas were in an intermediate position on both forearms (5 cm below the elbow crease). After removal of nickel patches at 24 h, test sites were covered with an empty chamber.

Clinical evaluation was performed at 72 h. Scoring was calculated as follows: erythema = 1; erythema, edema and vesiculation = 2–3; erythema, vesicles and abrasion = 4. Echographic evaluations were carried out by a 20-MHz B-scanner (Dermascan C, Cortex Technology, Denmark) at the beginning of the experiment, at 1 h, 30 min after removal of the SLS patch tests, at 24 h, 30 min after removal of the nickel patch tests, and at 72 h.

Ultrasound equipment

Echographic evaluations were performed using a 20-MHz B-scanner (Dermascan C, Cortex Technology, Denmark), which produces images

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Table I. *Clinical evaluation*

Number of subjects = number of subjects showing a positive reaction on the right or on the left; number of responses = number of responses per test site.

Test sites	AD patients			ACD patients		
	Number of subjects	Right proximal (number of responses)	Left distal (number of responses)	Number of subjects	Right proximal (number of responses)	Left distal (number of responses)
$NiSO_4$ 0.05%	8	8	6	16	9	10
SLS 5% × 30 min + $NiSO_4$ 0.05%	13	13	13	19	17	15

representing a cross section of the skin. Equipment and calibration methods have already been described in detail elsewhere (12, 15). Evaluations were performed by employing the zoom function in the axial direction at the first magnification (at factor 2), which makes it possible to explore the tissue to a depth of 6.71 mm. During recordings, the distance between probe membrane and the skin was kept at 1.7 ± 0.2 mm. Skin thickness was assessed in B mode, by determining the extension of the whole skin block appearing on the screen and by dividing its value by 22.4. In this way, a mean thickness value calculated from 224 A-scan lines can be obtained.

The echographic images were processed by a program (Dermavision 2D, Cortex Technology), providing 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). This software has already been described (16). The sonographic recordings were evaluated by an amplitude band (0–30 interval), marking the hyporeflecting parts of the dermis, corresponding to edema and inflammatory infiltration. The superficial hyper-reflecting part of the skin, corresponding to epidermis, was evaluated by means of a 201–255 band.

Statistics

For each test site, values referring to baseline skin and to 1, 24 and 72 h assessments were compared using the ANOVA test for repeated values and the Student-Newman-Keuls test. The same tests were used for evaluating differences between 72 h values referring to different patch test sites in the same group of subjects (AD or ACD). Comparisons between values belonging to different patient groups were performed by the Student *t*-test.

RESULTS

Clinical evaluation

Among AD subjects, 8 reacted to $NiSO_4$ 0.05% at untreated and 13 at SLS pre-treated areas. Sixteen subjects affected by ACD gave positive responses to nickel at untreated and 19 at SLS exposed skin sites, at least on one forearm (Table I). Mean scores per area at untreated and at SLS pretreated sites were 1.99 and 5.14, respectively, for AD patients, and 1.55 and 3.9 for ACD patients. In AD patients the SLS + Ni patch test in the right proximal position scored 2.43, the one on the left distal 2.71 (Table II).

Table II. *Clinical evaluation (scores)*

	AD (14 p.)		ACD (20 p.)	
	Ni	SLS + Ni	Ni	SLS + Ni
Total score right + left	28	72	31	78
Total score right	16	34	15	38
Total score left	12	38	16	40
Mean score right + left	1.99	5.14	1.55	3.9
Mean score right (prox)	1.14	2.43	0.75	1.99
Mean score left (dist)	0.85	2.71	0.8	2

Skin thickness measurements

Skin thickness values are shown in Tables III and IV. Both in AD and in ACD patients, skin thickness values increased progressively in time. Values at 72 h were significantly different in respect of baseline for all test areas except empty chamber test sites. In AD patients, skin thickness values were significantly higher at SLS in respect of nickel patch test sites, whereas there was no significant difference between SLS + Ni and SLS test areas. In both patient groups, thickening of the skin at SLS pre-treated nickel test sites was significant in respect of untreated nickel test areas only regarding SLS + Ni areas in distal position. At SLS patch test areas, skin thickness increased in respect of baseline at 72 h approximately by 20% in ACD patients and by 30% in AD patients. However, differences between the two groups, as evaluated by the *t*-test, were not statistically significant.

0–30 band evaluation

The results of the 0–30 band elaboration of echographic images are shown in Tables V and VI. Both in AD and in ACD patients, at Ni, SLS + Ni, and SLS patch test areas a clear increase in the extension of the hypo-echogenic area, which was at its maximum at 72 h, was observed. Statistical significance was reached at 72 h for all areas in atopics and for SLS + Ni and SLS areas in ACD patients. Exposure to SLS before nickel patch testing induced an enlargement of the hypo-echogenic dermis area, which was greater than that at untreated nickel patch test sites. In both patient groups, the response at distal SLS + Ni sites was more intense than at proximal ones. Moreover, the increase in the extension of the hypo-echogenic area of the dermis of SLS pre-treated Ni-sites in respect of untreated nickel areas was higher in atopics. In the AD group, 72 h pixel values of SLS + Ni test areas (both proximal and distal) were significantly higher in respect of Ni and SLS areas, whereas in ACD patients only differences between distal SLS + Ni areas and Ni areas reached statistical relevance.

201–255 band evaluation

In both patient groups, evaluation of echographic images by the 201–255 band showed no significant modifications at nickel sulfate test sites, whereas at skin sites to which SLS had been applied a decrease of the reflectivity of the epidermis (extension

Table III. *Skin thickness values in AD patients*

Mean values ± sd are expressed in mm.

	Baseline	1 h	24 h	72 h
SLS + Ni, proximal	0.95 ± 0.13	0.99 ± 0.11	1.16 ± 0.24	1.25 ± 0.22
Ni, right	1.01 ± 0.09		1.10 ± 0.15	1.16 ± 0.21
SLS	1.04 ± 0.08	1.09 ± 0.09	1.32 ± 0.20	1.36 ± 0.16
Empty chamber	0.96 ± 0.09		0.99 ± 0.29	1.05 ± 0.13
Ni left	0.97 ± 0.07		1.08 ± 0.17	1.15 ± 0.20
SLS + Ni, distal	0.99 ± 0.08	1.06 ± 0.08	1.24 ± 0.25	1.38 ± 0.21

Table IV. *Skin thickness values in ACD patients*

Mean values ± sd are expressed in mm.

	Baseline	1 h	24 h	72 h
SLS + Ni, proximal	0.98 ± 0.01	0.99 ± 0.11	1.14 ± 0.20	1.24 ± 0.23
Ni, right	1.05 ± 0.01		1.13 ± 0.17	1.14 ± 0.18
SLS	1.07 ± 0.11	1.05 ± 0.11	1.26 ± 0.21	1.24 ± 0.22
E.C.	0.97 ± 0.11		1.01 ± 0.12	1.03 ± 0.12
Ni left	0.97 ± 0.23		1.11 ± 0.13	1.15 ± 0.16
SLS + Ni, distal	1.05 ± 0.12	1.04 ± 0.13	1.02 ± 0.20	1.34 ± 0.34

of the 201–255 area) could be observed at 1 h, both in AD and in ACD patients (Tables VII and VIII). Statistical significance was reached in AD patients at all SLS-treated areas and in ACD patients at SLS + Ni areas both proximal and distal. At 24 h a significant decrease was still present where no nickel sulfate had been applied in AD patients.

DISCUSSION

Contact eczema due to nickel sensitization is one of the most important causes of contact dermatitis, both in a domestic context and in occupational environments, and is often linked to wet work and exposure to surfactants. However, routine patch testing modalities do not take into account the simultaneous action of water, irritants and tensides, as agents damaging the skin and predisposing it to allergen absorption.

This study shows that the combination of irritants and subthreshold doses of allergens can induce a dermatitis in nickel-sensitive patients, which may explain the development of contact eczema deriving from products containing far lower concentrations of allergens than patients react to when undergoing routine patch testing. After a 30-min exposure to SLS, 8 subjects out of 34 (5 atopic patients and 3 non-atopic patients) showed clinically visible reactions to nickel concentrations, which did not elicit any reactions in the case of undamaged skin. Moreover, mean clinical scores at SLS pre-treated nickel areas were higher in respect of untreated nickel sites both in AD and in ACD patients. Echographically, exposure of the skin to SLS prior to the application of nickel sulfate determined a more marked thickening of the skin and a more pronounced increase of the extension in the hypo-echogenic area in respect of untreated nickel test sites. These parameters have been proved to correlate well with the intensity of the allergic response, increasing in time according to nickel concentrations (12).

Enhancement of allergic reactions both in the induction and the elicitation phase by repeated exposure to an irritant, prior to the application of an allergen, represents a fundamental principle of the maximization testing procedure (1). Garioch et al. were able to show that 0.2% nickel sulfate in the presence of SLS 1% produced a greater reaction than 1% nickel sulfate alone and suggested that this was due to an enhancement of the absorption of nickel (17). In order to minimize this mechanism, Mc Lelland et al. applied SLS and anthralin at various concentrations at sites treated 24 h previously with various allergens (2). The authors observed that the response to both allergen and irritant was greater than to either alone and concluded that the

Table V. *0–30 band evaluation in AD patients*

Mean values ± sd are expressed in number of pixels.

	Baseline	1 h	24 h	72 h
SLS + Ni, proximal	1029.57 ± 581.18	1282.50 ± 719.30	2185.57 ± 2037.88	3224.92 ± 2807.35
Ni right	904.50 ± 598.08		1277.35 ± 1044.79	1586.78 ± 1141.93
SLS	942.57 ± 382.28	1092.42 ± 440.73	2497.92 ± 1863.32	2143.21 ± 1031.62
Empty chamber	915.71 ± 423.94		1425.92 ± 776.67	1531.64 ± 996.04
Ni left	1048.92 ± 433.36		1636.35 ± 949.10	1957.71 ± 1368.90
SLS + Ni, distal	969.64 ± 348.58	1248.28 ± 584.05	2809.50 ± 2011.21	3659.42 ± 2186.84

Table VI. *0–30 band evaluation in ACD patients*

Mean values ± sd are expressed in number of pixels.

	Baseline	1 h	24 h	72 h
SLS + Ni, proximal	993.05 ± 345.76	1171.85 ± 662.35	1912.25 ± 1496.04	2358.20 ± 2698.06
Ni right	954.15 ± 468.90		1467.60 ± 1210.12	1621.85 ± 1563.90
SLS	1027.35 ± 385.89	1133.85 ± 511.73	1609.05 ± 1338.82	2018.10 ± 1422.44
Empty chamber	908.05 ± 445.41		1082.40 ± 799.59	1260.85 ± 824.89
Ni left	932.45 ± 532.57		1673.90 ± 1063.43	1303.05 ± 797.42
SLS + Ni, distal	1057.90 ± 448.69	1332.25 ± 691.03	1753.70 ± 1090.71	2902.70 ± 2480.27

Table VII. *201–255 band evaluation in AD patients*

Mean values ± sd are expressed in number of pixels.

	Baseline	1 h	24 h	72 h
SLS + Ni, proximal	344.43 ± 156.10	171.93 ± 109.41	345.14 ± 201.33	411.36 ± 221.03
Ni right	317.50 ± 144.06		388.86 ± 162.22	369.71 ± 154.59
SLS	344.71 ± 133.79	224.14 ± 157.44	138.64 ± 95.32	311.78 ± 129.24
E.C.	378.28 ± 177.05		417.36 ± 154.07	462.78 ± 132.90
Ni left	318.21 ± 166.40		351.43 ± 122.78	351.14 ± 110.02
SLS + Ni, distal	339.00 ± 122.81	229.14 ± 147.68	260.14 ± 173.99	259.50 ± 83.36

augmented response was due to the simultaneous presence of non-immune inflammation. By means of an arm immersion procedure, Allenby & Basketter demonstrated that skin damage caused by arm immersion in SLS greatly enhanced reactivity to nickel on the forearm, both in number of reactions at each concentration and in the minimal eliciting concentration (3). However, especially when evaluating responses to low nickel doses, appearing sometimes only with erythema and slight edema and without vesiculation, an objective way of assessment can be useful (18). In this study, instrumental evaluation was performed at well-defined skin sites, to which localized stimuli could be applied, allowing a comparison between pre- and post-treatment conditions. Assessment of skin thickness was not sufficiently accurate to provide statistical differences between atopics and non-atopics. Image analysis of 20 MHz B-scan recordings, enabling a quantification of allergic reactions and a differentiation between nickel and SLS induced responses, represents a reliable method for evaluating subclinical allergic and irritant reactions (12, 13, 18).

Concerning regional variations in the reactivity to nickel after pre-treatment with SLS, patch test reactions in the left distal position were more intense than those at right proximal sites. Results of the echographic evaluation confirmed the clinical observations. Both in AD and in ACD patients, thickening of the skin was significant in respect of nickel test areas only regarding SLS + Ni areas in distal position. Moreover, in ACD patients the increase in the extension of the hypo-echogenic dermis area at SLS + Ni sites was significant in respect of nickel areas only for left distal sites. These data validate previous observations regarding skin reactivity to nickel sulfate increasing from the ante-cubital fossa to the wrist (16).

Superficial echogenicity of the skin corresponding to epidermis has been shown to decrease according to SLS concentration at irritant patch test sites (13). Moreover, at SLS patch test sites, an inverse correlation between 201–255 pixel values and TEWL values has been observed (13). A decrease of the epidermal reflectivity can therefore be interpreted as an expression of barrier function damage. In this study, hypo-reflectivity of the epidermis was already observable at SLS treated skin sites at 1 h and was more evident in atopics. At 24 h a significant decrease of the 201–255 area was noticeable at SLS areas, to which no nickel sulfate had been applied, only in atopic subjects. These data indicate an increased reactivity to SLS in the AD group in respect of the ACD one and a specific susceptibility of atopic skin in respect of surfactants.

SLS treatment prior to the nickel sulfate application elicited

Table VIII. *201–255 band evaluation in ACD patients*

Mean values ± sd are expressed in number of pixels.

	Baseline	1 h	24 h	72 h
SLS + Ni, proximal	295.95 ± 171.98	179.95 ± 117.05	390.45 ± 172.54	415.10 ± 146.42
Ni right	344.45 ± 155.89		371.85 ± 134.89	421.95 ± 182.97
SLS	364.55 ± 149.89	282.90 ± 172.69	277.95 ± 179.34	357.25 ± 137.06
Empty chamber	349.85 ± 147.02		403.20 ± 161.88	464.40 ± 137.50
Ni left	332.20 ± 122.84		356.90 ± 159.28	469.70 ± 148.12
SLS + Ni, distal	386.40 ± 137.82	292.90 ± 150.83	336.50 ± 122.26	388.40 ± 171.72

an allergic reaction in 5 out of 6 AD and in 3 out of 4 ACD patients, who had shown no response to nickel application at untreated sites. However, at SLS pre-treated nickel sites, mean clinical scores and values referring to both echographic parameters of inflammation (skin thickening and increase of the extension of the 0–30 area) were higher in atopics. Thus, subsequent to a slight irritant stimulus, such as a 30-min exposure to SLS, an earlier inflammatory response and more pronounced skin damage are induced in atopics followed by a more marked allergic reaction, probably depending on enhanced nickel penetration and/or combination of immune and non-immune mechanisms. These observations indicate that nickel-allergic individuals who are more likely to develop allergic contact eczema, when simultaneously exposed to allergens and surfactants, are those with AD, and stress the importance of the concurrent action of irritants and allergens in maintaining the dermatitis in atopics.

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