

Pre-treatment of nickel test areas with sodium lauryl sulfate detects nickel sensitivity in subjects reacting negatively to routinely performed patch tests

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A fair % of patients with a clinical history of nickel allergy show negative patch test results. To improve the response rate to NiSO_4 5% pet. patch tests, a testing procedure utilizing pre-treatment of the test area by a 24-h application of sodium lauryl sulfate (SLS) was introduced. 46 women with a clinical history of nickel sensitivity, who exhibited negative reactions to nickel sulfate 5% pet. patch tests, were studied. Patients underwent 6 patch tests on adjacent sites on the volar surface of the forearms. 4 patch tests were performed with a 72-h application of 40 mg nickel sulfate 5% pet. While 1 of these patch tests served as control, 3 test areas underwent 24-h pre-treatment with 40 μl SLS, 1 with 0.1% and 2 with 0.5% solution. To evaluate differences in the reactivity to SLS plus nickel sulfate related to the site on the forearm, 0.5% SLS pre-treatment was performed both on a proximal and on a distal test site. At the 72-h evaluation, 19 subjects out of 46 showed positive reactions to nickel sulfate 5% pet. at skin sites pre-treated with 0.1% SLS, whereas 23 patients reacted positively at 0.5% SLS pre-treated areas. Echographic values of skin thickness and of hypo-echogenic dermal areas at positive pre-treated nickel test areas were higher than at control test areas, confirming the clinical evidence of an increased response to NiSO_4 after SLS pre-treatment. The inflammatory reaction, as evaluated clinically and echographically, was much higher at distal skin areas (0.1% SLS and distal 0.5% SLS) than at proximal 0.5% SLS ones.

Key words: nickel sensitivity; negative nickel patch tests; pre-treatment of patch test area; sodium lauryl sulfate; echography; patch testing technique. © Munksgaard, 1996.

Accepted for publication 18 May 1995

A practical problem in nickel patch testing is that in some subjects with a positive history of metal sensitivity, i.e., having repeated eczematous reactions on skin exposed to jewelry or clothing metal, negative test results can be obtained (1-5). Apart from errors in the performance and reading of the patch test, or sensitivity to other metals such as cobalt, this may be caused by insufficient bioavailability of the test substance (5).

It is well known that application to the skin of surfactants greatly enhances the penetration of various compounds (6). In experimental procedures for screening and rating contact sensitizers, sodium lauryl sulfate (SLS) was introduced to increase the response to allergens (7). Exposure of nickel test sites to SLS by arm immersion (8), or by topical application (9, 10), enables the detection of skin reactions to nickel solutions at a concentration that cannot elicit the allergic response

on undamaged skin. Thus, to devise a simple testing system with greater sensitivity than routinely employed patch testing methods, enabling the identification of false-negatively-responding nickel-sensitive subjects, an SLS pre-treatment procedure was introduced. Subjects with a history of skin lesions at sites of metal contact and negative patch results, were invited to undergo further nickel sulfate 5% pet. patch tests on SLS pretreated skin sites on the forearm.

Patients and Methods

A group of 46 women, aged 22 to 46 years, with a clinical history of nickel sensitivity, who showed negative responses to routinely performed nickel sulfate 5% pet. patch tests (Trolab Hermal, Germany) performed on the skin of the back, were

Table 1. Pre-treatment of nickel test areas with 0.1% SLS and 0.5% SLS; clinical evaluation

Test site	Results	No. subjects	+72 h mean score per area
0.1% SLS		46	0.17±0.25
0.5% SLS		46	0.38±0.37
5% NiSO ₄ pet.	negative	46	0.43±0.44
0.1% SLS+Ni	negative	17	0.39±0.21
0.1% SLS+Ni	doubtful	10	0.76±0.31
0.1% SLS+Ni	positive	19	2.53±0.61
0.5% SLS+Ni	negative	10	0.38±0.22
0.5% SLS+Ni	doubtful	13	0.79±0.35
0.5% SLS+Ni proximal	positive	19	1.74±0.94
0.5% SLS+Ni distal	positive	23	2.35±0.57

recruited from the patch test clinic from June to December 1994.

Patients underwent 6 patch tests on adjacent sites on the volar surface of the forearms. Large Finn Chambers, 11 mm in diameter (Epitest, Finland), secured to the skin by Scanpor tape (Norgesplaster, Norway) were used. 4 of the patch tests were performed with 40 mg nickel sulfate 5% pet. (Trolab Hermal, Germany). While 1 of these served as control (Ni area), 3 test areas underwent 24-h pre-treatment with 40 µl SLS (99%, SIGMA Chemical Co.), 1 with 0.1% and 2 with 0.5% aq. solution (distilled water) (SLS+Ni areas). To evaluate differences in the reactivity to SLS plus nickel sulfate related to the site on the forearm, 0.5% SLS pretreatment was carried out both on a proximal (2 cm distally from the elbow fold, on the left) and on a distal test site (8 cm distally from the elbow fold, on the left), whereas 0.1% SLS pre-treatment was performed on the distal area on the right forearm. Control tests were also performed with 0.1% SLS and 0.5% SLS. For nickel patch testing, a 72-h application time was chosen to avoid too many additional visits for the patients. A visual grading system and echographic measurements were used to quantify the responses 30–40 min after patch test removal. For clinical assess-

ment, a 5-point scale (0, 0.5, 1, 2 and 3) was used, according to the guidelines of the ICDRG (11).

Echographic evaluations were carried out using a 20 MHz B-scanner (Dermascan C, Cortex Technology, Denmark), which produces images representing a cross-section of the skin. Equipment, calibration methods and recording conditions have already been described in detail elsewhere (12, 13). 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. Numerical elaboration of the images was performed by a dedicated program, employing an 0–30 amplitude band. Extension of the hypo-echogenic (0–30) dermal area was used for assessing the intensity of the allergic reaction (13).

Statistics

The ANOVA test for repeated values was used to check differences between baseline values and 24-h and 72 h values, whereas for mutually comparing values referring to 72-h evaluations (0–30 areas and skin thickness measurements), the Student *t*-test for unpaired observations was used.

Results

Clinical evaluation

At the 72-h evaluation, 19 subjects showed positive reactions to nickel sulfate 5% pet. at 0.1% SLS pre-treated skin sites and at proximal 0.5% SLS pre-treated areas, whereas 23 subjects had positive skin responses at distal 0.5% SLS+Ni areas (Table 1). 10 and 13 test sites, respectively, which had been pre-treated with 0.1% and 0.5% SLS, were evaluated as doubtful. In these cases, the test area showed erythema, edema and, sometimes, small papules and pustules, which were interpreted as being irritant in nature. Clinical scores are also reported in Table 1. The highest clinical scores were attributed to positive 0.1% SLS+Ni areas and distal 0.5% SLS+Ni areas.

Table 2. Pre-treatment of nickel test areas with 0.1% SLS and 0.5% SLS; skin thickness evaluation (mm)

Test site	Results	–24 h	0	+72 h
0.1% SLS		1.09±0.08	1.17±0.15	1.20±0.17
0.5% SLS		1.01±0.13	1.05±0.24	1.14±0.16
5% NiSO ₄ pet.	negative	—	1.04±0.11	1.15±0.16
0.1% SLS+Ni	negative	1.07±0.12	1.20±0.18	1.30±0.19
0.1% SLS+Ni	doubtful	1.07±0.09	1.19±0.12	1.34±0.09
0.1% SLS+Ni	positive	1.08±0.13	1.17±0.15	1.64±0.29
0.5% SLS+Ni	negative	0.96±0.07	1.05±0.18	1.16±0.15
0.5% SLS+Ni	doubtful	1.02±0.11	1.14±0.15	1.28±0.22
0.5% SLS+Ni proximal	positive	0.96±0.12	1.03±0.11	1.34±0.23
0.5% SLS+Ni distal	positive	1.08±0.13	1.16±0.15	1.65±0.29

Echographic evaluation

Table 2 shows the results of skin thickness evaluation. In respect to baseline values (-24 -h values), skin thickening at 72 -h was significant at all skin areas. However, 72 -h values of positive SLS+Ni test sites were also significantly higher than 72 -h values of negative patches and control areas. The skin thickness increase was highest for 0.1% SLS+Ni areas and for distal 0.5% SLS+Ni areas. Values of positive distal 0.5% SLS+Ni areas were significantly higher than those of positive proximal 0.5% SLS+Ni areas.

Figs. 1, 2 illustrate the results of the 0 – 30 band elaboration of 0.1% and 0.5% SLS pre-treatment, respectively. In respect to values of baseline skin, a significant increase was observable at 72 -h at all test areas. Values of positive SLS+Ni areas at 72 -h were significantly higher than 72 -h values of negative SLS+Ni areas and of nickel and SLS areas, confirming clinical evidence of increased response to NiSO_4 after SLS pretreatment. For posi-

tive 0.5% SLS+Ni sites, extension of the hypo-echogenic dermal area of distal test sites was significantly greater than that of proximal ones.

Discussion

Allergen identification can be compromised by false-negative results of patch tests. Negative responses on epicutaneous investigations performed with nickel salts have been reported in a fair % of subjects with a history of metal allergy. (1–5, 14). They can depend both on high sensitization threshold, and on lack of bioavailability of nickel ions from the patch test material.

In practice, sensitivity to nickel shows different degrees of intensity, as demonstrated by dose-response profile studies performed on the population of nickel-allergic individuals. However, whereas minimal eliciting concentrations of nickel salts have been thoroughly studied on both normal and compromised skin (9, 10, 15, 16), little information is available on the frequency of negative 5% nickel

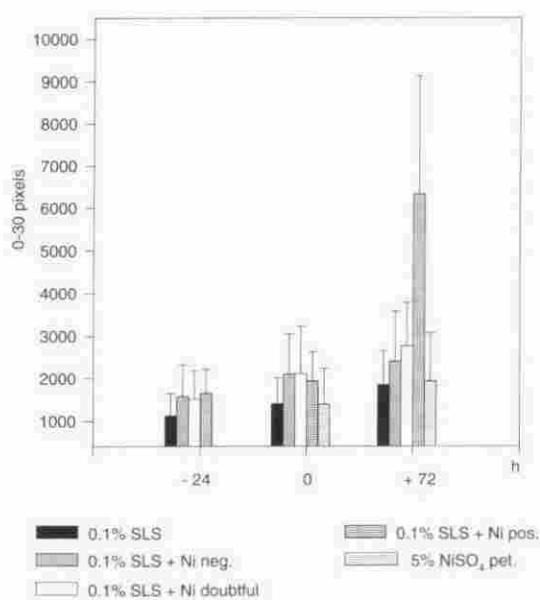


Fig. 1. Results of echographic evaluation of NiSO_4 5% patch tests performed with 24 -h pre-treatment with 0.1% SLS on 46 patients: comparison with control areas (0.1% SLS) and with non-pre-treated NiSO_4 areas. Extension of hypo-echogenic dermal areas is expressed in number of 0 – 30 pixels. 0.1% SLS = values referring to control tests performed with 0.1% SLS. 0.1% SLS+Ni neg. = values referring to areas showing negative reactions to NiSO_4 5% pet. after 24 -h pre-treatment with 0.1% SLS. 0.1% SLS+Ni doubtful = values referring to areas showing doubtful reactions to NiSO_4 5% pet. after 24 -h pre-treatment with 0.1% SLS. 0.1% SLS+Ni pos. = values referring to areas showing positive reactions to NiSO_4 5% pet. after 24 -h pre-treatment with 0.1% SLS. 5% NiSO_4 pet. = values referring to negative patch test reactions to NiSO_4 5% pet. performed without 0.1% SLS pre-treatment.

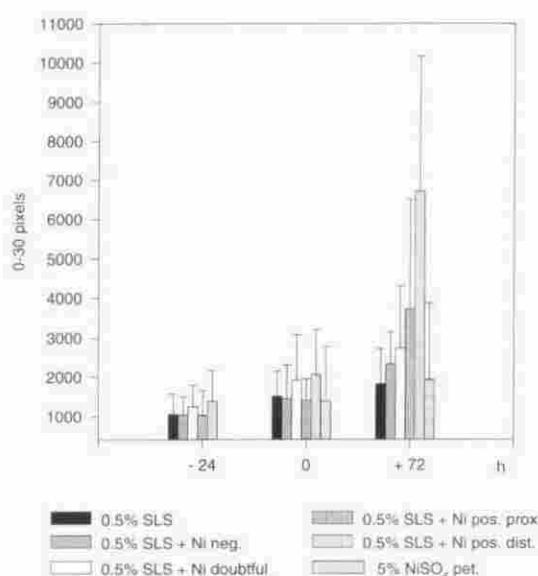


Fig. 2. Results of echographic evaluation of NiSO_4 5% patch tests performed with 24 -h pre-treatment with 0.5% SLS on 46 patients: comparison with control areas (0.5% SLS) and with non-pre-treated NiSO_4 areas. Extension of hypo-echogenic dermal areas is expressed in number of 0 – 30 pixels. 0.5% SLS = values referring to control tests performed with 0.5% SLS. 0.5% SLS+Ni neg. = values referring to areas showing negative reactions to NiSO_4 5% pet. after 24 -h pre-treatment with 0.5% SLS. 0.5% SLS+Ni doubtful = values referring to areas showing doubtful reactions to NiSO_4 5% pet. after 24 -h pre-treatment with 0.5% SLS. 0.5% SLS+Ni pos. prox. = values referring to proximal areas showing positive reactions to NiSO_4 5% pet. after 24 -h pre-treatment with 0.5% SLS. 0.5% SLS+Ni pos. dist. = values referring to distal areas showing positive reactions to NiSO_4 5% pet. after 24 -h pre-treatment with 0.5% SLS. 5% NiSO_4 pet. = values referring to negative patch test reactions to NiSO_4 5% pet. performed without 0.5% SLS pre-treatment.

sulfate patches in individuals reacting clinically with an eczematous dermatitis to nickel contact (1–5, 14).

Insufficient delivery of the allergen to the effector cells in the skin can depend both on skin factors and on the manner in which the test substance is presented to the skin (5). Less than 7% of nickel enters the skin from standard patch test samples with nickel sulfate in pet. (17). To increase the bioavailability of the test substance, modifications of the standard testing procedure have been suggested, including change of vehicle (18, 19), increase in concentration of allergen (20), employment of nickel chloride instead of nickel sulfate (2, 21), and pre-treatment of the patch test area by occlusion (22). Intracutaneous testing also represents a method for increasing the response rate during investigations of nickel sensitivity (23).

It is well-known that disruption of the barrier by application of SLS enhances penetration of compounds applied to the skin and induces higher *in vitro* permeability to ions (6, 24). Application of SLS to the test area is employed in the maximization test, which utilizes prolonged magnified exposure of SLS-damaged and, therefore, more permeable skin, to the test substance (7). Post-application of SLS and anthralin was shown to amplify skin reactions to patch tests performed with various allergens in contact-sensitized subjects, reducing the minimal eliciting concentration (25), whereas pre-exposure to 0.3% SLS significantly enhanced the allergic responses produced by a nickel sulfate solution containing 112 p.p.m. Ni in water (9). Skin damage resulting from arm immersion in SLS greatly augmented reactivity to nickel on the forearm, both in the number of reactions and in the minimal eliciting concentration (8). Moreover, pre-treatment of nickel test areas in nickel-sensitized atopics and non-atopics increased both the number of patients responding with an eczematous reaction to the application of 0.05% nickel sulfate solution and the intensity of the reactions, as evaluated by echographic parameters of inflammation (26).

In our study, impairment of the barrier after 0.1% and 0.5% SLS pre-treatment was demonstrated by increase in TEWL, as measured by the Evaporimeter EPI (Servomed, Sweden), 30–40 min after removal of the SLS patch tests (Δ TEWL for 0.1% SLS = 2.88 ± 3.10 ; Δ TEWL for 0.5% SLS = 4.14 ± 3.36). Modification of stratum corneum permeability, subsequent to irritation, led to the identification and recovery of nickel-sensitive subjects, who, without appropriate information and nickel avoidance, would probably have continued to have episodes of contact dermatitis. A 72-h application time was chosen in order to devise a test-

ing procedure without imposing too many additional visits on the patients. For the same reason, a 2nd evaluation at 4–5 days was not performed. After appropriate information, no patient refused the 2 additional visits required for further investigation.

In this study, subjective evaluation of patch test results, expressed by clinical scores, was supported by echographic evaluation, allowing objective quantification of skin responses. Both skin thickness and extension of hypoechogenic dermal area are considered parameters of inflammation, suitable for evaluation of the intensity of allergic reactions (13). Echographic values of positive SLS+Ni areas were significantly higher than nickel control areas, confirming clinical evidence of increased response to NiSO₄ after SLS pre-treatment. Skin thickness and 0–30 values of positive SLS+Ni test sites were also significantly higher than those of SLS control test areas, indicating a greater inflammatory component of the allergic reaction than of the irritant one.

13 0.5% SLS+Ni and 10 0.1% SLS+Ni patch tests were defined as doubtful. In these patients, erythema, edema, small papules and, sometimes, pustules were observed. However, on the basis of clinical and echographic evaluations, it was not always possible to establish whether these reactions were to be considered as mixed ones, owing to summation of irritant and allergic reactions in a patient with a low degree of sensitivity, or as the expression of an irritant reaction in a hyper-reactive subject. Against this 2nd hypothesis is the fact that, in these subjects, baseline and post-SLS TEWL values were no different from those of patients reacting negatively to SLS+Ni at 72-h (data not shown). Clearly, the possible high number of doubtful reactions should be kept in mind, when choosing a testing procedure for identifying false-negative responders. The evaluation of nickel patch tests after SLS pre-treatment requires considerable experience in patch test reading, especially when estimation of positivity is based only on inspection and palpation.

Pre-treatment with 0.5% SLS identified 23 nickel-sensitive subjects, whereas 0.1% SLS pre-application facilitated a positive reaction to nickel in 19 patients; however, owing to the greater irritant component, 0.5% SLS+Ni reactions were sometimes more difficult to interpret than 0.1% SLS+Ni reactions.

Further studies on the SLS pre-treatment solution should be carried out, to identify the optimal SLS concentration, enabling reduction of doubtful irritant responses, at the same time as maintaining all allergic reactions to nickel sulfate.

Concerning regional variations in the reactivity

to nickel after pre-treatment with SLS, skin reactions in the distal position were more intense than those at proximal sites, as evaluated clinically and echographically by skin thickness and echogenicity measurements. These data confirm previous observations regarding skin reactivity to nickel sulfate increasing from the antecubital fossa to the wrist (27).

In conclusion, patch testing modalities, in subjects with a history of eczematous dermatitis upon nickel contact, should include procedures aimed at increasing permeation of nickel through the skin. Besides the methods already described (2, 18–23), 24-h pre-treatment of a distal forearm test area with 0.5% SLS can be recommended to experienced patch test readers for identifying nickel-sensitive subjects reacting negatively to 5% NiSO₄ patch tests performed on the back.

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