

Combined effects of irritants and allergens

Synergistic effects of nickel and sodium lauryl sulfate in nickel-sensitized individuals

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Knowledge of the combined effects of irritants and allergens is of interest with respect to accurate risk assessment. The threshold for elicitation of allergic contact dermatitis in previously sensitized individuals may theoretically be markedly influenced by the simultaneous presence of irritants and allergens. Combined exposures have, however, only been studied infrequently. In the present study, the combined effect of an irritant and an allergen was evaluated in a dose–response designed experimental study. 20 nickel-sensitized subjects were exposed to patch testing with varying concentrations of NiCl₂ (nickel chloride) and sodium lauryl sulfate (SLS) alone and in combination. Evaluation of skin reactions was performed by colorimetry, measurement of transepidermal water loss and clinical evaluation, and the data were analyzed by logistic dose–response models. A synergistic effect was found of combined exposure to NiCl₂ and SLS, as compared to each of the substances applied separately, as evaluated by colorimetry and clinical scoring. This means that the effect produced by the combined exposure was substantially greater than the effect produced by either of the substances alone. A synergistic effect of combined exposure on skin barrier impairment was not found, since the barrier function is significantly influenced by SLS-exposure only and not by NiCl₂. Concentration limits are used by industry and government agencies to protect consumers. The present results clearly illustrate that elicitation thresholds and concentration limits may be influenced considerably by combined exposure to allergens and irritants.

Key words: allergic contact dermatitis; irritant contact dermatitis; nickel; sodium lauryl sulfate; bioengineering methods; colorimetry; transepidermal water loss; visual assessment; synergistic effect. © Blackwell Munksgaard, 2002.

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While most experimental studies within the field of contact dermatitis concentrate on elicitation of either irritant or allergic reactions to defined single chemical substances, the situation in real life is more commonly a simultaneous exposure to a mixture of irritants and allergens. Many clinical cases of contact dermatitis are elicited by both an irritant and an allergic component. Knowledge of the combined effects of irritants and allergens is of special relevance to accurate risk assessment. Many consumer products include allergens as well as irritants, and the threshold for elicitation of allergic contact dermatitis in previously sensitized individuals may theoretically be markedly influenced by the concurrent presence of irritants and allergens. Combined exposures have, however, only been studied infrequently.

Exposure to detergents impairs the skin barrier function and facilitates penetration of water (1).

An early experimental patch test study indicated an increased reactivity to nickel and chromate in already sensitized individuals after addition of a detergent to the patch test solution (2). Subsequently, more detailed studies have been undertaken, which demonstrated that the presence of an ionic surfactant resulted in a marked increase in sensitivity to allergens (3–5). In a later study on the concentration threshold values for nickel exposure in nickel-sensitive individuals, surfactant addition of sodium lauryl sulfate (SLS) was, however, not found to increase the risk of a positive reaction to nickel applied unoccluded (6).

Pretreatment of the skin with the anionic surfactant SLS has been reported to increase the response to patch test with hydrophilic allergens, while lipophilic allergens have not been studied recently (7–9). Application of SLS after patch testing with various allergens (nickel, chromate, thiuram,

fragrance, colophonium and lanolin) in already sensitized individuals has been shown to increase the patch test response with an additive augmentation of the response (10).

An additive effect of allergens applied in combination has also been reported (11). In a later study, the increased skin response to simultaneously applied allergens was confirmed, but the augmentation of the response was in this study found to be synergistic, that is, greater than the sum of the 2 separate effects (12).

The aim of the present study has been to examine the effect of combined exposure to an allergen and an irritant with respect to additive or synergistic effects. For this purpose, data were analyzed by logistic dose-response models. Knowledge about combined effects is extremely valuable with respect to accurate risk assessment, since threshold values for elicitation of allergic reactions in already sensitized individuals may be markedly changed by combined exposures.

Materials and Methods

20 volunteers with nickel allergy, 19 women and 1 man (mean age 29.1 years, range 22–52), participated in the study. Inclusion criteria for the study were a positive patch test to nickel within the last 2 years, or a history of several, persistent reactions to nickel in metal alloys within the same period. Subjects with current eczema and atopics were excluded.

Patch tests were performed with dilution series of nickel chloride (NiCl_2) and SLS alone or in combination, using large Finn Chambers (12 mm) on Scanpor tape. Large Finn Chambers were used to obtain reliable measurement conditions for the bioengineering methods. Patch tests were applied to the volar side of the upper arm, and left on for 24 h.

Group A. 10 volunteers were tested with sodium lauryl sulfate (SLS Sigma, >99% quality (13)) 0.12%, 0.25% and 0.50% aq with and without 1% nickel chloride (NiCl_2), and 1 patch with NiCl_2 1% aq alone.

Group B. Another 10 volunteers were tested with NiCl_2 0.25%, 0.50% and 1% aq with and without 0.5% SLS, and 1 patch with SLS 0.5% aq alone. Patches were removed after 24 h, and evaluation of the skin reactions was performed after 48 and 72 h.

Clinical scoring was performed according to ICDRG (International Contact Dermatitis Research Group) guidelines (14).

Evaluation by bioengineering methods

Skin color was measured with a colorimeter (Minoltameter) according to the guidelines of the

ESCD on colorimetry (15). The skin color is given in a 3-dimensional co-ordinate system, where redness is measured on the a^* -axis. Colorimetry is generally accepted for evaluation of irritant and allergic skin reactions (1, 16).

Transepidermal water loss (TEWL) was measured by an Evaporimeter (Servomed) according to the guidelines of the ESCD on TEWL (17). TEWL is the passive diffusion of water through the stratum corneum, and, when measured under stable conditions, it is generally accepted as being an indicator of skin barrier function.

Evaluations were performed at baseline, at day (D) 2 (48 h) and D3 (72 h). Patch tests were removed after 24 h (D1) by a nurse to make sure that the patches had been satisfactorily applied. All measurements were undertaken in February–May 2000. Environmental temperature during measurements was 20°C, and relative humidity varied from 27 to 40%.

Statistical methods

Analysis of variance was used to assess the effects of NiCl_2 and SLS on the colorimetry measurements and TEWL, and dose-response analysis was applied to test for synergistic effects of NiCl_2 and SLS (12). The clinical readings were analyzed according to an ordinal logistic dose-response model to assess the joint effects of the 2 agents (18, 19).

The additive dose-response model describes the response as $f(x + rz)$ where x is the dose of NiCl_2 , z is the SLS dose, and r is the potency of SLS relative to NiCl_2 . For example, if $x = rz$ the model implies that the response to a mixture of x NiCl_2 and rz of SLS will be the same as to $2x$ without SLS or $2rz$ without NiCl_2 . This model is compared to the nonadditive model $f(x + rz + k \cdot \sqrt{xr})$. If $k > 0$ the mixture will give a larger response than the additive model ($k = 0$), i.e. synergism, whereas $k < 0$ corresponds to antagonism. For the analysis of the colorimetric and TEWL data, a quadratic polynomial was used for $f(\cdot)$, while the clinical assessment was adequately described by a cumulative ordinal logistic model (19).

A significance level 0.05 was used for assessment of effects.

Results

19 of the 20 volunteers had a clinically positive or doubtful positive reaction to NiCl_2 1%, while 1 (from group B) only reacted positively to NiCl_2 in combination with SLS. Statistical calculations were not significantly influenced by exclusion of the person without a reaction to Ni alone.

Clinical patch test readings at D2(48 h) are given

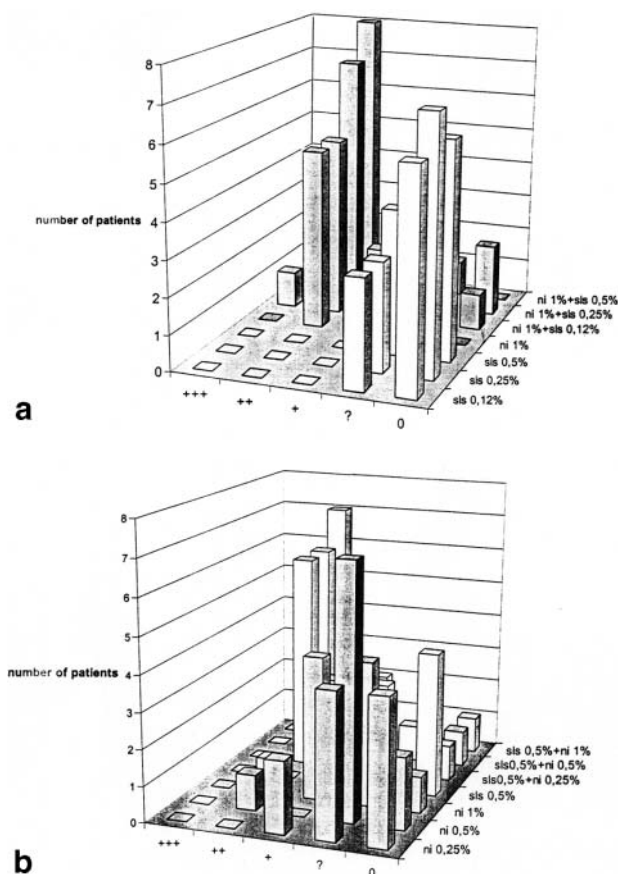


Fig. 1. A. Clinical patch test reactions at D1. 10 Subjects, group A. White columns indicate SLS only, gray columns indicate nickel or nickel and SLS in combination. B. Clinical patch test reactions at D2. 10 Subjects, group B. White columns indicate SLS or SLS and nickel in combination, while gray columns indicate nickel alone.

in Fig. 1(A, B). Exposure to SLS and NiCl_2 in combination leads to an increased response as compared to exposure to each of these substances separately. In the dose-response analysis the non-additive model gave a significantly ($p < 0.0001$) better description of the clinical assessments than the additive model both on D2 and D3, thus confirming a synergistic effect. With respect to clinical readings, results obtained at D3 did not significantly differ from those at D2.

Results of colorimetry measurements generally showed increased redness with increasing concentrations of nickel and SLS, respectively, as would be expected. Reactions to nickel and SLS in combination were enlarged compared to what would be expected from addition of the response to nickel and SLS applied separately in corresponding concentrations. Figure 2(A, B) illustrates the colorimetric response to combined exposure to nickel and SLS at D2 and D3, respectively. Responses are compared to corresponding expected values estimated from the additive dose-response model

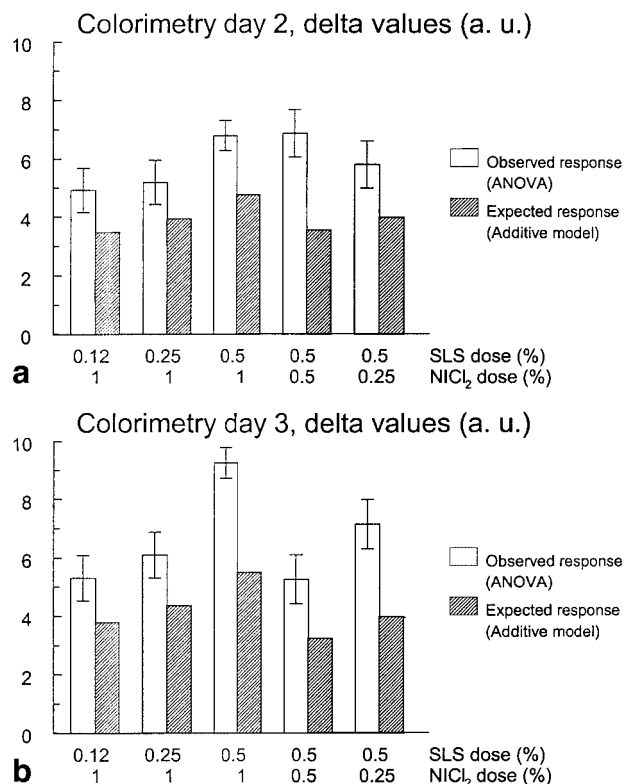


Fig. 2. A. Comparison of colorimetric responses on D2 (means and SEM from analysis of variance) obtained with mixtures of NiCl_2 and SLS are illustrated in white columns. Data are given as delta values (D2 – basal values). Corresponding expected estimates based on the additive dose-response model without the synergistic (nonadditive) term are illustrated in gray columns. B. Comparison of colorimetric responses on D3 (means and SEM from analysis of variance) obtained with mixtures of NiCl_2 and SLS are illustrated in white columns. Data are given as delta values (D3 – basal values). Corresponding expected estimates based on the additive dose-response model without the synergistic (nonadditive) term are illustrated in gray columns.

(without the nonadditive (synergistic) term). A synergistic effect of nickel and SLS in combination was found with all 5 combinations of concentrations of nickel and SLS, and the synergistic, nonadditive dose-response model was statistically significantly superior to the additive model ($p < 0.001$). No significant differences were found between measurements D2 and D3 with respect to additive/synergistic effects.

TEWL measurements showed a positive dose-response relationship for increasing SLS concentrations and TEWL values, as expected from previous studies (1). Exposure to nickel alone only slightly influenced the barrier function, as was also reported earlier (20). Figure 3(A, B) illustrates the TEWL response to combined exposure to nickel and SLS at D2 and D3, respectively. Responses are compared to corresponding expected values estimated from the additive dose-response model. The observed response is almost similar to the response

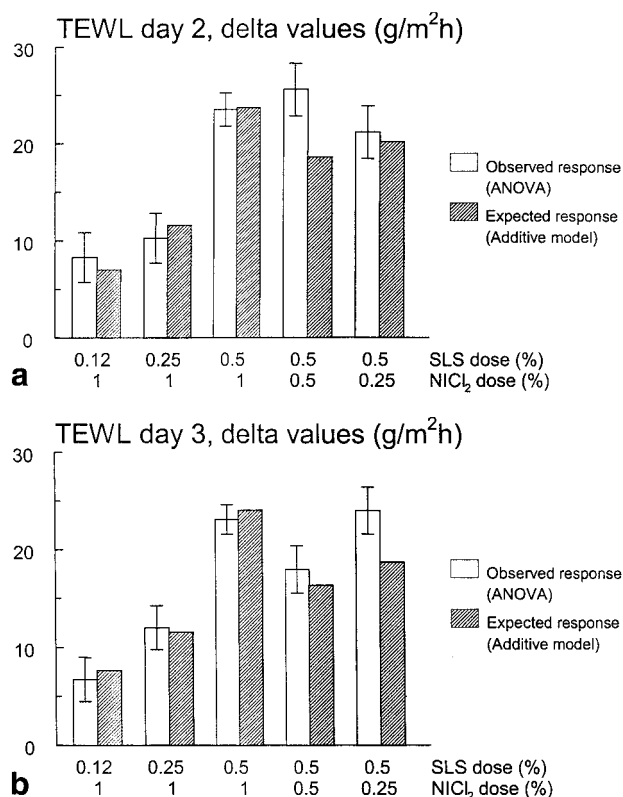


Fig. 3. A. Comparison of evapometric responses on D2 (means and SEM from analysis of variance) obtained with mixtures of NiCl₂ and SLS are illustrated in white columns. Data are given as delta values (D2 – basal values). Corresponding expected estimates based on the additive dose–response model without the synergistic (nonadditive) term are illustrated in gray columns. B. Comparison of colorimetric responses on D3 (means and SEM from analysis of variance) obtained with mixtures of NiCl₂ and SLS are illustrated in white columns. Data are given as delta values (D3 – basal values). Corresponding expected estimates based on the additive dose–response model without the synergistic (nonadditive) term are illustrated in gray columns.

calculated from the additive dose–response model. A synergistic effect of nickel and SLS in combination was not found for skin barrier damage as assessed by TEWL measurement. With respect to TEWL measurements the synergistic, nonadditive dose–response model was not statistically significantly superior to the additive model. No significant differences were found between measurements D2 and D3 with respect to additive/synergistic effects.

In summary, the results showed a synergistic effect of combined exposure to NiCl₂ and SLS when evaluated by clinical reading and colorimetry. When evaluated by colorimetry, the combined application of SLS and NiCl₂ caused a synergistic response equivalent to about 4× the dose of each substance when applied separately. When evaluated by TEWL, the response was found to be additive.

Discussion

The hypothesis that a synergistic effect exists for the skin response to NiCl₂ and SLS in combination, as compared to separate exposures to the 2 substances, was tested. Advanced statistical methods were used to test whether the dose–response analysis fitted best with an additive or with a synergistic (nonadditive) model. The results show that a synergistic effect of combined exposure to nickel and SLS exists on the inflammatory response, as evaluated by clinical scoring and measurement of skin redness. This means that the effect produced by the combined exposure was substantially greater than that expected based on the effect produced by either of the substances alone. Concentration limits are used by industry and government agencies to protect consumers. Knowledge of concentration limits and threshold values for elicitation of contact dermatitis comes from experimental studies of skin exposure to one substance at a time, while development of contact dermatitis in real life is often due to simultaneous exposure to multiple substances. The present results clearly illustrate that elicitation thresholds and concentration limits may be considerably influenced by combined exposure to allergens and irritants.

With respect to effect on the skin barrier function impairment as evaluated by TEWL measurements, the combined exposure to nickel and SLS had an additive effect only and, as expected no synergistic effects were found here. SLS and NiCl₂ influence the skin barrier function in different ways. SLS may elicit a significant increase in TEWL without any significant inflammatory reaction (1), while contact allergens in most cases, including NiCl₂, only influence the barrier function secondarily to the inflammatory response and have no direct influence on the barrier function (20). This explains why a synergistic response should not be anticipated with respect to impairment of skin barrier function.

SLS was chosen for the present study since it is a commonly used model surfactant irritant, and NiCl₂ was chosen since Ni is as a frequent allergen, being the most common contact allergen among women. NiCl₂ was preferred to nickel sulfate since it is a more real-life exposure and known to penetrate the skin more easily (3).

Due to a low concentration of NiCl₂ some subjects only reacted with a doubtful positive reaction to NiCl₂ alone. However, the combined exposure to SLS and NiCl₂ in some subjects led to quite strong reactions, and an increase in NiCl₂ concentration to more than 1% when using large Finn Chambers may cause severe skin reactions and is not recommended. Clinical reading of the patch

test reactions was performed according to the IRCDG recommendations (14), developed for reading of allergic reactions. When the scoring system is applied to combined allergic/irritant reactions, as in the present study, difficulties in interpretation of the reactions may arise, especially considering the irritant reactions. Using a traditional score for irritant reactions would, on the other hand, impair the interpretation of the allergic reactions. The augmented response following combined exposure to irritant and allergen, either as a simultaneous exposure or when the irritant is applied as a pre or post-treatment, has been described earlier in the literature. Only in 1 study (6) was simultaneous exposure to SLS and NiCl_2 found to reduce the skin response, and this result may be due to open application and use of quite low concentrations of both substances. Other studies unanimously found augmented responses after combined exposure or that addition of a detergent was applicable to elicit allergic reactions that would otherwise remain latent (1, 5, 7–11).

Application of SLS, either as a pretreatment or simultaneously with nickel, may influence the patch test reaction in several ways. SLS is known to impair the skin barrier function and facilitate penetration of hydrophilic substances, which is one way to promote the skin response to nickel patch testing. SLS has also been shown to cause a progressive depletion in the number of epidermal Langerhans cells, which may theoretically weaken the likelihood of eliciting a contact allergic reaction (21). Since the depletion is an incomplete and slow process, taking place over several days, this seems unlikely in practice to influence the patch test reactivity significantly. That mild physical and chemical insults to the skin may act as provocative agents for elicitation of contact hypersensitive reactions was proposed by Kligman (7), and evidence that non-specific proinflammatory effects determine the concentration-dependent elicitation of allergic reactions was later obtained in experimental animal studies (22, 23). Ultimately, the concept that a key function of the irritant is to provide 'danger signals' has been applied to allergic contact dermatitis (24). A Swedish study highlights the fact that timing of irritant and allergen application may be important, and that pretreatment with SLS several weeks before Ni patch testing may cause hardening and actually diminish the response (25). The combined effect of an allergen and an irritant on the skin is influenced by factors related to the immune system as well as to the skin barrier function. In the present study, the simultaneous exposure to NiCl_2 and SLS was illustrated to cause not an additive but a synergistic effect on the ultimate inflammatory response, though not on skin barrier

impairment (Figs 2 and 3). In reality, SLS increases the allergenic effect of Ni beyond the SLS-induced irritation, and this is probably due to both increased penetration of nickel and non-specific proinflammatory effects induced by SLS, which lower the threshold value for elicitation of allergic reactions. This observation has important practical consequences for the determination of threshold values and the risk assessment of contact allergens in consumer and industrial products, where allergens may often be present in combination with surfactants.

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