

Relationship between 2,4-dinitrochlorobenzene elicitation responses and individual irritant threshold

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Contact dermatitis shows significant interindividual variation. We studied the relationship between an individual's irritant threshold and the elicitation of experimentally induced allergic sensitization. The results suggest that there is an association between reactivity to an irritant and the likelihood of positive elicitation reactions to lower hapten concentrations. This novel finding would be compatible with the proposal that contact allergy is best explained using a danger model with cutaneous irritancy determining sensitization. However, the enhanced allergic reactivity of individuals with a lower irritant threshold was not apparent on visual inspection, suggesting that the determinants of skin reactivity to haptens are multifactorial.

Key words: contact allergy; danger; elicitation; irritants; sodium lauryl sulphate. © Blackwell Munksgaard, 2002.

Accepted for publication 14 November 2001

Variation in sensitization to hapten occurs despite equal population exposure. For example, ear piercing is an important cause of nickel sensitization, yet in a study of 364 women with pierced ears only a third were patch-test positive to nickel (1). This may result from variation in the response of the individual skin immune system to hapten. 2,4-Dinitrochlorobenzene (DNCB) is a highly potent antigen frequently used in experimental contact dermatitis. Human experiments with DNCB have shown that clinical sensitization and elicitation of subjects is proportional to the dose of DNCB used, when applied to a constant area. For each sensitizing dose of DNCB, only a proportion of subjects is sensitized, a proportion which increases when higher doses of DNCB are used. Furthermore, the higher the sensitizing dose of DNCB used, the greater the elicitation reaction to a given challenge dose of DNCB. Increasing the area of exposure for a given DNCB dose initially raises the sensitization rate, but above a very small threshold area the key determinant of skin sensitization is dose per unit area (2).

As with skin sensitizers, reactions to irritants depend on the duration and concentration of application, the irritant capacity of the chemical and individual susceptibility (3, 4). When nonatopic humans are exposed to the irritant anionic surfactant sodium lauryl sulphate (SLS) at varying con-

centrations under occlusion for 4 h, significant interindividual variation can be demonstrated. For example, some patients will develop erythema and swelling 24 h after application to concentrations as low as 0.3%, whilst others will fail to react to concentrations as high as 20%, the approximate upper limit of aqueous solubility (5). However, the relationship between irritant susceptibility, sensitization and elicitation in the nonatopic individual is unclear. One study examined irritant reactivity and the ability to sensitize cancer patients with DNCB. The ability to react to an irritant and to be sensitized to DNCB was reduced in patients with cancer. The authors hypothesized a link between the patients' reduced irritant reactivity and sensitization (6). Kligman demonstrated that the production of clinical inflammation prior to hapten application enhanced the subject sensitization rate (7). Earlier authors did not show that irritant and allergic patch test reactions were related in patients with dermatitis (8, 9). Greater reactivity to SLS, measured by *trans*-epidermal water loss, has been linked to positive patch test reactions to nickel (10, 11).

Given the above considerations, it seems likely that the irritant properties of a hapten are also able to promote sensitization (12). In this study we compared the irritant threshold to SLS of DNCB-sensitized subjects with their elicitation reactions

Table 1. Mean change in dermal thickness by DNCB elicitation concentration for 14 SLS reactors and five non-reactors

DNCB elicitation dose ($\mu\text{g}/20\ \mu\text{L}$)	SLS threshold	Mean percentage increase in dermal thickness	95% confidence interval range	<i>p</i> -value (SLS reactor vs. non-reactor)
3.125	reactor	45	14, 77	0.04
	non-reactor	9	-13, 31	
6.25	reactor	60	31, 90	0.01
	non-reactor	15	-11, 42	
12.5	reactor	77	46, 108	0.4
	non-reactor	48	-38, 134	
25	reactor	113	17, 178	0.7
	non-reactor	97	73, 154	

to DNCB. We hypothesized that subjects with a low irritant threshold to SLS (more easily irritated skin) would experience a greater sensitization response to DNCB. Such patients may react not only to lower concentrations of DNCB at elicitation but also to a greater extent to a defined dose level.

Methods

All patients with active dermatitis or a past history of atopic dermatitis were excluded; atopic dermatitis has been associated with greater irritant reactivity (13, 14). Volunteers were sensitized to DNCB by applying 50 μL of 0.0625% DNCB (31.25 μg) in acetone using a 12-mm Finn chamber applied for 48 h to buttock skin. Then, 21 days after sensitization, DNCB was applied to their upper inner arm using 8 mm Finn chambers for 48 h. DNCB was applied in the following concentrations: 3.125, 6.25, 12.5 and 25 μg dissolved in 20 μL acetone. The test areas were assessed prior to application, at 60 min and 48 h after removal by visual subjective scoring. The diameter of the reaction was measured and visual scoring of the intensity was done using a scale of 0–5: 0 = no reaction, 0.5 = partial response with just perceptible erythema, 1 = just perceptible erythema, 2 = mild erythema, 3 = moderate erythema, with some oedema, 4 = intense erythema with oedema, 5 = extreme or spreading reaction (occasionally with blistering). The dermal thickness of each elicitation site was quantified using a high frequency 20 MHz ultrasound scanner (Quantity Medical Instruments Ltd). For each time point the percentage increase in dermal thickness was calculated and expressed as percentage change compared to pre-exposure. After the elicitation response had subsided, the subjects were tested broadly for their irritant threshold following a previously described method (5). In brief, 0.2 mL of descending concentrations of SLS from 20% to 0.1% were applied for 4 h on the upper inner arm and read at 48 h for clinical inflammation. The lowest concentration of SLS that induced erythema was taken as the subject's irritant threshold.

An association between the elicitation reaction to DNCB and irritant threshold was sought for the group using a two-sample *t*-test with unequal variance.

Results

Nineteen nonatopic Caucasian volunteers in good health, aged 18–35 years (11 female, 8 male) were recruited. All subjects were sensitized to DNCB as judged by visual assessment of the reactions to challenge at 21 days. There was no significant association between SLS threshold and the threshold concentration of DNCB to which individual subjects reacted or with the intensity of reaction, assessed either by its diameter or by visual scoring (data not shown). However, on comparison of the percentage change in dermal thickness for each concentration of DNCB against the subject's irritant threshold, a significant difference was found between volunteers who reacted to SLS (threshold 0.5–20%, $n = 14$) and those who did not react to SLS ($> 20\%$, $n = 5$) at each of the two lowest concentrations of DNCB: $p < 0.05$. These data are summarized in Table 1.

Discussion

In this study, DNCB-sensitized subjects with a lower irritant threshold developed greater elicitation reactions at lower elicitation concentrations of DNCB when compared with subjects who had a higher irritant threshold. This is consistent with the view that irritant reactivity may influence to some degree sensitization and/or elicitation to a hapten. Recent work has shown a link between a tumour necrosis factor- α (TNF α) cytokine polymorphism and reactivity to SLS in humans. Volunteers who reacted to SLS showed a significant increase in expression of an allele that has been linked with an excessive inflammatory response associated with other clinical settings (4, 15). TNF α plays an important role in contact dermatitis (16, 17). It is known to influence sensitization (18) and

may also influence the elicitation phase (19–22). Animal experiments suggest that the ability of SLS to augment sensitization to DNCB is most evident at low concentrations of DNCB (23). A human study has demonstrated the importance of the irritant signal during the elicitation phase (24). After sensitization, subjects were tested with varying concentrations of irritant and haptens. At all dilutions a greater response was found to allergen and irritant compared to either alone. Furthermore, the combination of irritant and allergen in concentrations that alone were subclinical produced a significant response.

Most haptens have the capacity to provide two signals: antigenic and irritant. The more potent sensitizers tend to have a greater irritant capacity (12). Concentrations recommended for patch testing of haptens often can reflect the strength of these two signals (25). For example, one of the most experimentally potent haptens, chloromethylisothiazolinone, is tested at a concentration of 0.01%, while the relatively non-irritant and weak sensitizer neomycin is tested at 20%. Chemicals that are irritants (e.g. metals) are also diluted to minimize the irritant signal, which otherwise might result in a false positive test. DNCB provides a potent antigenic signal but also a nonantigenic, irritant signal. Although modest compared to the allergic response in those sensitized, it can be measured experimentally (2). We hypothesize that those subjects who reacted to SLS when exposed to DNCB produce a greater irritant/danger signal compared to non-reactors. This influence sensitization and elicitation: demonstrated in this study by their greater elicitation reactions.

This was a small study with wide confidence intervals for the elicitation mean values. Furthermore, the trend to greater elicitation reactions in the SLS reactors was only seen when DNCB was tested at 3.125 and 6.25 μ g. Two explanations can be offered. Firstly, at higher concentrations of DNCB, the magnitude of the antigenic signal may override the weaker irritant signal. Secondly, at higher concentrations, the irritant signal will be maximal regardless of irritant susceptibility. Indeed, both of these may represent the practical reality of the situation. A further, but in our view unlikely, explanation for our findings would be if sensitization to DNCB altered some subjects' irritant threshold. It is worth noting that if a closer concentration range for challenge of the DNCB-sensitized subjects had been chosen, results might have been a little clearer.

Past work examining the relationship between irritancy and allergy has tested subjects to single concentrations of chemicals (8). In this study we have tested to multiple dilutions of hapten and irri-

tant, which may explain why we have begun to identify an association. The finding that patients who react to SLS may have the potential to mount greater reactions to an allergen once sensitized may contribute to the clinical variation seen in allergic contact dermatitis. It would be consistent with the proposal that contact allergy is best explained using a danger model (26). In this model, the degree of hapten-derived irritancy plays a pivotal role as a second or 'danger' signal required for sensitization (27). However, the limited extent to which the association between irritant threshold and the elicitation of allergy could be demonstrated suggests that, ultimately, many factors may determine an individual's allergic skin reactivity.

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