

Influence of a detergent on skin response to methyldibromo glutaronitrile in sensitized individuals

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The present study was undertaken to evaluate the combined effect of the preservative methyldibromo glutaronitrile (MDBGN) and sodium lauryl sulfate (SLS) on the elicitation response of allergic contact dermatitis. 20 volunteers with contact allergy to MDBGN were patch tested with 5 concentrations (10, 50, 100, 500 and 1000 p.p.m.) of MDBGN alone and in combination with 0.25% SLS on the upper arms for 24 h. Skin reactions were evaluated by clinical scoring, and data were evaluated by logistic dose–response models. Additionally, evaluation of skin reactions was performed by measurement of transepidermal water loss (TEWL) and skin colour. Measurements were obtained at baseline and at D3 and D7. As evaluated by clinical assessment, allergic reactions to MDBGN were elicited at lower concentrations when applied in combination with SLS than when applied alone. The response was augmented by a factor of 6.4. An increased response to combined exposure to SLS and MDBGN as compared with MDBGN alone was confirmed by TEWL and colour measurements. Effects of exposure time and concentration of the detergent are discussed. In conclusion, an augmented response was found after concurrent application of MDBGN and SLS. The response was augmented by a factor of 6.4, with confidence limits of 2.8–14.6 ($P < 0.0001$). This result is important in relation to the determination of threshold values and the risk assessment of contact allergens in consumer and industrial products, where allergens are often present in combination with surfactants.

Key words: allergic contact dermatitis; bioengineering methods; colorimetry, irritant contact dermatitis; methyldibromo glutaronitrile; sodium lauryl sulfate; transepidermal water loss.
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Methyldibromo glutaronitrile (MDBGN) is used as a biocide in consumer products. An exponential increase in clinical cases of allergic contact dermatitis to MDBGN has been found in European dermatological clinics over the last 2 years (1). Measures have currently been taken to reduce the problem. The EU Commission has laid down a prohibition on the use of MDBGN in leave-on cosmetic products, such as creams and lotions (2), while it still may be used in wash-off products, such as shampoo and liquid soaps. Even though the time of contact is shorter for a wash-off than a leave-on cosmetic product, the presence of detergents in soaps may alter the skin response, so that the skin becomes more susceptible to the induction of contact allergy and the threshold value for elicitation changes. Knowledge about such combined effects is necessary for accurate safety assessments. The current study was undertaken to

evaluate the effect of concurrent application of MDBGN and a detergent on threshold values for elicitation of allergic contact dermatitis.

Materials and Methods

20 volunteers (12 women and 8 men) with contact allergy to MDBGN participated in the study after its approval by the local ethical committee. Informed consent was obtained from each participant. Inclusion criterion was a positive patch test to MDBGN within the last 3 years (1999–2002). Persons with a history of atopic dermatitis and persons with current eczema were excluded.

Test method

Patch tests were performed on the volar side of the upper arm with MDBGN aq. at the following

concentrations: 10, 50, 100, 500 and 1000 p.p.m. with and without 0.25% aq. sodium lauryl sulfate (SLS, >99% purity) (Sigma Chemical Co., St Louis, MO, USA) and 1 patch with SLS 0.25% aq. alone. Small Finn Chambers (8 mm) on Scanpor[®] tape (Epitest, Tuusula, Finland) were used with 15 µl in each patch. The dose of MDBGN per cm² was the same when applied alone or in combination. Patches were removed after 24 h (D1). A diagnostic MDBGN [0.3% petrolatum (pet.)] patch was applied on the back of each participant and removed after 48 h (D2) and read at D3. Clinical evaluation of the skin reactions was performed at D1, D3 and D7. Bioengineering methods were performed at baseline, D3 and D7. The study was undertaken in December 2002 and January 2003. Environmental temperature during measurements was kept at 20°C and the relative humidity was 38.6% (mean value).

Assessment of skin reactions

Clinical scoring was performed according to International Contact Dermatitis Research Group (ICDRG) guidelines (3).

Evaluation by bioengineering methods

Transepidermal water loss (TEWL) was measured by the Dermalab[®] system equipped with a TEWL probe by Cortex Technology Aps (Hadsund, Denmark) according to the guidelines of the ESCD on measurement of TEWL (4).

Skin colour was measured with a colorimeter (Minoltameter) according to the guidelines of the ESCD on colorimetry (5). The skin colour is given in a 3-dimensional co-ordinate system L*a*b*, where redness is measured on the a*-axis. Colorimetry is generally accepted for evaluation of irritant and allergic reactions (6, 7). 2 recordings were made for evaporimetry and skin colour.

Statistical analysis

Analysis of variance (ANOVA) was used to assess the effects of MDBGN and SLS on logarithmic transformed TEWL and colorimetry measurements. The ANOVA model included subjects as a block factor and enabled tests for interaction (synergistic or antagonistic) effects of methyldibromo glutaronitrile and SLS, as well as separate effects of the 2 agents relative to the intrasubject variation (8). The clinical readings were analysed according to a binary logistic dose-response model (9) that also allowed tests of interaction and separate effects of MDBGN and SLS in a similar way as the ANOVA. The analysis of the data was performed with computer programmes validated relative to the procedures GLM and LOGISTIC of the SAS[®] system, version 6.12 (SAS Institute Inc., Cary, NC, USA).

A significance level of ≤ 0.05 was used for assessment of effects.

Results

19 of 20 volunteers had a clinically positive reaction (defined as at least erythema and homogeneous infiltration) to MDBGN (0.3% pet.) tested on the back and continued in the study. 2 patients were taken out after evaluation on D3 because of severe and spreading patch test reactions.

Results of clinical patch test readings on D3 and D7 are summarized in Tables 1 and 2. Exposure to MDBGN and SLS in combination led to a response at lower concentrations in most subjects, as compared with exposure to MDBGN alone. The difference was largest at D3 and least at D7. For 0.25% SLS tested alone, no reaction was observed in 36.8% of the cases. In 52.6%, a reaction with slight redness (spotty or diffuse) was seen, and in 10.5% of the volunteers, a reaction of moderate and uniform redness was observed according to the irritant scale proposed by Frosch and Kligman (10).

Table 1. Clinical patch test reactions at D3 ($n = 19$)

Concentration MDBGN as p.p.m.	Number of persons with a response to MDBGN alone (%)	Distribution of responders				Number of persons with a response to MDBGN + 0.25% SLS	Distribution of responders			
		?+	+	++	+++		?+	+	++	+++
10	9/19 (47%)	5	1	1	2	17/19 (89%)	9	6	1	1
50	11/19 (58%)	4	4	1	2	16/19 (84%)	5	7	3	1
100	9/19 (47%)	4	1	3	1	16/19 (84%)	7	4	3	2
500	13/19 (68%)	5	4	1	3	18/19 (95%)	4	8	2	4
1000	15/19 (79%)	4	6	2	3	19/19 (100%)	4	7	4	4

MDBGN, methyldibromo glutaronitrile; SLS, sodium lauryl sulfate.

Table 2. Clinical patch test reactions at D7 ($n = 17$ and 2 drop-outs)

Concentration MDBGN as p.p.m.	Number of persons with a response to MDBGN alone (%)	Distribution of responders				Number of persons with a response to MDBGN + 0.25% SLS	Distribution of responders			
		?+	+	++	+++		?+	+	++	+++
10	7/17 (41%)	4	1	2	0	11/17 (65%)	7	4	0	0
50	7/17 (41%)	5	1	1	0	12/17 (71%)	8	2	2	0
100	6/17 (35%)	3	3	0	0	9/17 (53%)	4	2	3	0
500	10/17 (59%)	5	3	1	1	13/17 (77%)	6	3	2	2
1000	10/17 (59%)	4	4	2	0	14/17 (82%)	3	6	3	2

MDBGN, methyldibromo glutaronitrile; SLS, sodium lauryl sulfate.

The logistic analysis of the binary allergic response showed the frequency of positive response with fitted parallel dose-response curves (Fig. 1). The logistic analysis of response showed no significant interaction between MDBGN and SLS, indicating an additive and not a synergistic effect. The response was augmented by a factor of 6.4 expressed as an odds ratio (OR), with confidence limits of 2.8–14.6 and $P < 0.0001$.

Results of evaporimetry on D3 for MDBGN alone and in combination with SLS are given in Fig. 2. Combination with SLS caused increased TEWL at all concentrations. A positive dose-response trend of MDBGN was found for TEWL values at D3 ($P = 0.0049$). On D7, no statistically significant positive dose-response relationship was found for TEWL measurements ($P = 0.26$). The ANOVA showed no statistically significant interaction between MDBGN and SLS at D3 and D7 for TEWL ($P = 0.98$).

Results of colorimetry measurements for MDBGN alone and in combination with SLS on

D3 are illustrated in Fig. 3. Colorimetric measurements showed increased redness with increasing concentrations of MDBGN ($P = 0.0079$), despite the large interindividual variation in response. On D7, colorimetric measurements showed no statistically significant positive dose-response relationship ($P < 0.0001$). Combination with SLS caused increased redness at all concentrations. No statistically significant interaction between MDBGN and SLS was found by ANOVA at D3 ($P = 0.71$) or D7 ($P = 0.74$), indicating an additive response.

Discussion

Our results illustrate that the dose-response curve for elicitation of allergic contact dermatitis is shifted in parallel to the left when the allergen MDBGN is combined with a detergent, as compared with MDBGN applied alone. The response is augmented by a factor of 6.4 and thus less MDBGN is needed for the production of a response in combination with the detergent than alone. This result is relevant for everyday exposures

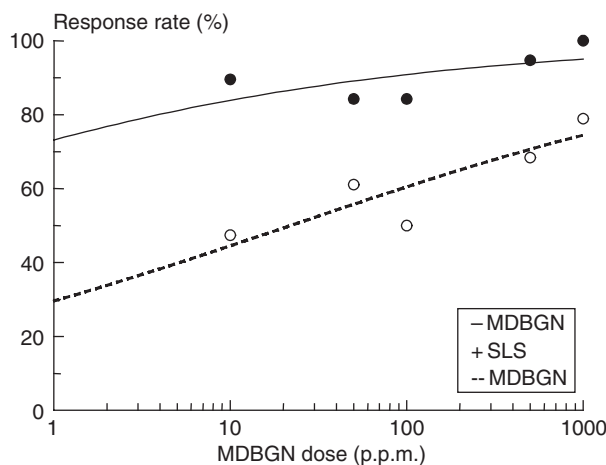


Fig. 1. Dose-response curve on D3 for clinical allergic response $\geq ?+$ to methyldibromo glutaronitrile (MDBGN) alone and in combination with 0.25% sodium lauryl sulfate (SLS). Black points illustrating dose-response curve for MDBGN + 0.25% SLS. White points illustrating dose-response curve for MDBGN separately.

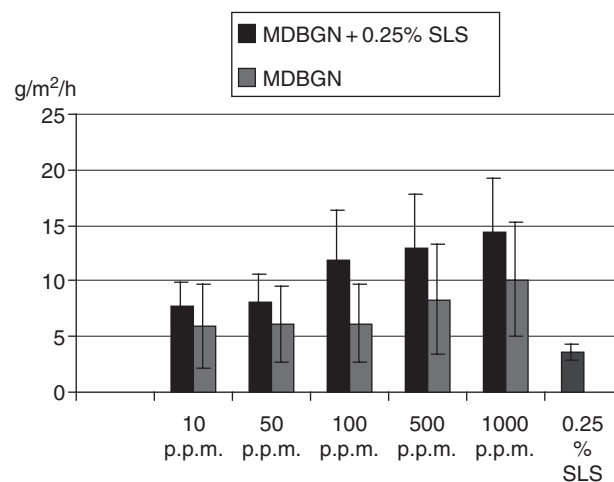


Fig. 2. Transepidermal water loss responses at D3 (means and SEM). Data are given as delta values (D3 – basal values). MDBGN, methyldibromo glutaronitrile; SLS, sodium lauryl sulfate.

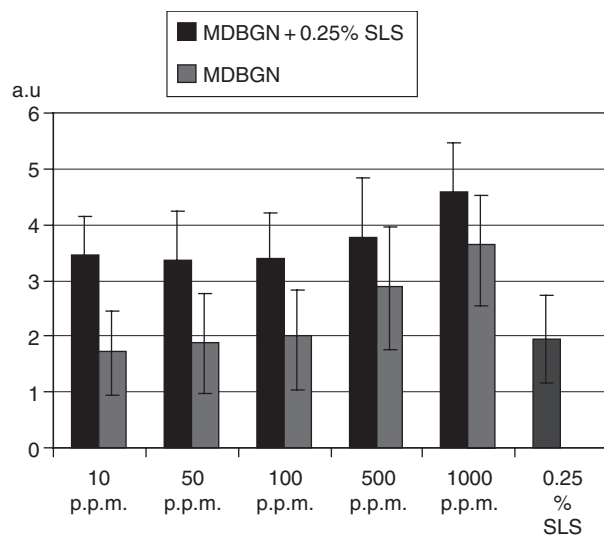


Fig. 3. Colorimetric response at D3 (means and SEM). Data are given as delta values (D3 – basal values). MDBGN, methyldibromo glutaronitrile; SLS, sodium lauryl sulfate.

to soap, shampoo and cleansing agents, where biocides and detergents commonly occur together. Evaluation by colorimetry supports the clinical findings, as increased redness was found after combined exposure to MDBGN and SLS as compared with MDBGN alone.

Other studies have investigated the combination of metals, e.g. chromium or nickel, and surfactants. Chromium may also be present in household products and thresholds for allergic reactivity to chromium have been studied in the presence of SLS (11). It was shown that the threshold of response was altered from 10 p.p.m. chromium to 1 p.p.m. chromium in the presence of SLS (11). This is within the same order of magnitude as in the present study. SLS acts as a skin barrier disrupter, as shown by TEWL measurements, and may cause increased penetration of the allergen. The lack of dose–response in TEWL measurements shows that the allergen MDBGN did not influence barrier function. This is different from other allergens, e.g. nickel, and may be due to the more irritant effect of MDBGN (12).

The penetration profile of an allergen may differ according to the chemical properties of the allergen and combination with different doses of SLS. With respect to the allergen, previous studies on effect of combination have concentrated on metals (13–16). MDBGN is an organic chemical. A synergistic response was found to nickel chloride in combination with SLS in a recent study by our group (12). In the current study, the combined response was additive rather than synergistic, as the effect of SLS on MDBGN response,

expressed as an OR, was constant. However, in principle, it might be asserted that a synergistic effect exists, because the same added dose of SLS has a multiplicative increase on the dose of MDBGN. The augmentary effect of SLS seems to become less at higher concentrations of MDBGN, possibly because the irritant effect of MDBGN at higher concentrations is more pronounced, and therefore addition of a relatively weak concentration of SLS is not so augmentary. While nickel chloride is very water soluble, this is not the case for MDBGN. It has been shown that nickel penetrates via the hair follicles (13). The route of penetration for MDBGN, however, may be different from that of nickel and thus affected in a different way by SLS.

The combined response may also depend on the concentrations of SLS and the allergen. In the current study, only 1, relatively weak concentration of SLS was used together with low concentrations of allergen and with an exposure time of only 24 h compared to the 48 h normally used for patch testing. The weak concentration of SLS used will mainly affect the barrier function and hardly cause any significant inflammation (17). However, even a low concentration of SLS may have a pro-inflammatory effect and thereby may augment the elicitation reaction, e.g. by cytokine release. Together with the short time of exposure this may, at least theoretically, act together differently with the allergen than higher concentrations of SLS. The short time of exposure may also have influenced the possibilities for interaction, giving less time for penetration. Thus, another design using higher and different concentrations of SLS or a longer exposure time may further clarify the type of combination effect. The present doses of SLS and MDBGN were, however, chosen because they reflect daily life exposures.

The present study illustrates that the threshold value for elicitation of allergic reactions in MDBGN-sensitized individuals is influenced by the presence of a detergent at low concentration. The results are relevant to real-life exposure to many types of products such as wash-off cosmetics. They show that the presence of detergents in a product needs to be considered in assessing the risk for elicitation of allergic contact dermatitis and in deciding cut-off values for protection of the allergic individual.

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