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INVESTIGATIVE REPORT

Increased Retest Reactivity by Both Patch and Use Test with Methyldibromoglutaronitrile in Sensitized Individuals

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Hyperreactivity on re-exposure of previous allergic contact dermatitis skin areas has been previously demonstrated. This study aimed to investigate in methyldibromoglutaronitrile (MDBGN) allergic patients whether skin with previous allergic dermatitis from MDBGN showed an augmented response on re-exposure by both a patch test challenge and a use test with a liquid soap preserved with MDBGN. MDBGN dermatitis was elicited on the back and arms of sensitized individuals. One month later the previously eczematous areas were challenged with MDBGN. On the back, the test sites were patch-tested with a serial dilution of MDBGN and a use test was performed on the arms with an MDBGN-containing soap. A statistically significant increased response was seen on the areas with previous dermatitis on the back. Eight of the nine patients who developed dermatitis on the arms from the MDBGN-containing soap had an augmented response on areas with prior allergic contact dermatitis. Even though the allergic dermatitis appeared to be healed, an increased reactivity to allergen re-exposure was demonstrated both by patch test and use test challenge. *Key words: re-challenge; hyperreactivity; use test; rinse-off.*

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Onset of allergic contact dermatitis in a sensitized patient depends on substance-related factors like dose, duration and mode of exposure, as well as individual-related factors like region of exposed skin, degree of sensitivity and pre-existing skin conditions. The skin response to renewed allergen exposure under different conditions like prior exposure to allergen and irritant is less studied. Hindsén and co-workers (1, 2) showed in clinical trials that skin with healed nickel-induced allergic contact dermatitis was retest hyperreactive to nickel for several months after the dermatitis was clinically healed. Accordingly, animal studies have shown increased retest reactivity to the allergens 2,4-dinitrochlorobenzene (DNCB) and 2-hydroxyethylmethacrylate (HEMA) (3–5).

Methyldibromoglutaronitrile (MDBGN) is a preservative utilized in cosmetic products such as creams, soaps and shampoos. It is a moderate sensitizer in predictive allergy tests (6, 7) and an important allergen in Europe (8). In this study, we investigated the allergic response of skin with previous eczema from the cosmetic preservative MDBGN when re-exposed. Challenge was performed both by patch testing and a use test with a liquid soap containing MDBGN.

MATERIALS AND METHODS

Subjects

Seventeen patients sensitized to MDBGN were recruited from the Department of Dermatology, Odense University Hospital, Denmark. Exclusion criteria were dermatitis on the test areas, age below 18 years and pregnancy. Initially, 20 patients were recruited for the study, but 3 patients were excluded, one because of dermatitis on the test areas and 2 due to negative retests with MDBGN. The test group consisted of 15 women and 2 men with a mean age of 50 years (range 21–66). A use test control group consisting of seven women and three men were also recruited based on negative reactions to a patch test with 0.2% MDBGN ethanol/aqua (aq). The control group participants had a mean age of 43 years (range 29–55). The study was performed according to the Helsinki Declaration II and approval was obtained from the local ethical committees.

Test materials

The test material was manufactured by the pharmacy at Odense University Hospital: 1% sodium lauryl sulphate (SLS) in aq, 0.2% MDBGN (Bie & Berntsen A/S, Denmark) in 20% ethanol/aq, and a vehicle solution of 20% ethanol/aq. A dilution series for patch testing was produced in the concentrations: 0.2%, 0.1%, 0.05%, 0.025%, 0.0125%, 0.0063%, 0.0031%, 0.0016%, 0.0008%, 0.0004%, 0.0002% and 0.0001% MDBGN in 20% ethanol/aq. For a use test, liquid soaps containing 0.1% MDBGN were produced and delivered in small plastic bottles. A needle was used to make a puncture in the bottle cap to ensure that the soap could be applied to the test area in droplets.

Elicitation of experimental allergic dermatitis on back

An area of dermatitis was produced on the lower back of the test patients. A method described by Hindsén et al. (1) was used with a few small alterations to produce an area of homogeneous dermatitis. All volunteer patients were patch-tested with a dilution series of MDBGN in ethanol/aq in the range 0.2% to 0.0001% to establish the degree of sensitivity of the patient. Fifteen µl of each patch test solution was micropipetted onto filter paper discs of small Finn Chambers (Epitest Oy, Helsinki,

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Finland) on Scanpore Tape (Alpharma A/S, Vennesla, Norway) and mounted on the backs of the subjects. The patches were removed on day 2 and read on day 3 according to the International Contact Dermatitis Research Group (ICDRG) guidelines (9). A 5×8 cm^2 filter paper was saturated in 800 µl of MDBGN solution of a concentration equal to the lowest concentration of MDBGN to produce a ++ reaction. Another identical filter paper was saturated in 800 µl of the vehicle solution (20% ethanol/aq) to be used as a control. The filter papers were placed symmetrically in a randomized manner on either side of the spine on the lower back of the patients. To ensure a degree of occlusion, hydrocolloid dressings (Duoderm, Convatec, Denmark) of 11×14 cm^2 were used to fix the filter papers onto the skin. The positions of the filter papers were carefully recorded by measuring the distance to the spine, hip bone, collar bone and, if possible, to birth marks and other characteristics on the skin. The patches were removed by the patient on day 2, and on day 3 the dermatitis was evaluated at our clinic.

Challenge patch testing on back

One month later, when the dermatitis had healed, the test sites were challenged. (Two patients (nos 13 and 15) needed 1.5 and 2 months, respectively, for the areas of dermatitis to heal clinically. One because the allergic reaction was stronger than expected and the other because of a delayed reaction.) The test sites were challenged by patch testing with six consecutive dilutions of MDBGN within a range determined according to the sensitivity of the patient found 1 month earlier. Patch test concentration ranges are shown in Table I. The study design is illustrated in Fig. 1. Fifteen ml of each of the solutions were micropipetted onto filter paper discs of small Finn Chambers (Epitest Ltd Oy, Finland) on Scanpore Tape (Norgesplaster A/S, Norway) and mounted on the test sites on the subjects' backs. Patches were removed on day 2 and read on day 3 by a person who did not know the localization of the previous dermatitis. The following scoring system was used: negative=0; doubtful=0.5; erythema and infiltration=1; erythema, infiltration and a few papules=1.5; erythema, infiltration, papules=2; erythema, infiltration, papules and a few vesicles=2.5; and erythema, infiltration, papules, vesicles=3 (10).

Table I. *The range of methyldibromoglutaronitrile patch test concentrations used in the dilution series for each patient and the sum of scores of the two sites with previous eczema and normal skin, respectively*

Patient no.	Patch test concentration range (%)	(ppm)	Sum of scores Previous eczema	No previous eczema
1	0.0002–0.0063	(2–63)	10.5	7.5
2	0.0008–0.0250	(8–250)	15.5	12
3	0.0004–0.0125	(4–125)	10	5
4	0.0004–0.0125	(4–125)	5	5
5	0.0002–0.0063	(2–63)	11	10
6	0.0008–0.0250	(8–250)	13	13.5
7	0.0004–0.0125	(4–125)	8.5	8
8	0.0002–0.0063	(2–63)	16	10
9	0.0004–0.0125	(4–125)	16.5	12
1	0.0004–0.0125	(4–125)	5	5.5
11	0.0002–0.0063	(2–63)	9.5	10.5
12	0.0031–0.1	(31–1000)	5.5	7.5
13	0.0002–0.0063	(2–63)	7	4.5
14	0.0008–0.0250	(8–250)	6	2.5
15	0.0031–0.1	(31–1000)	3	3
16	0.0001–0.0016	(1–16)	6	5
17	0.0031–0.1	(31–1000)	18	17.5
Total			166	139

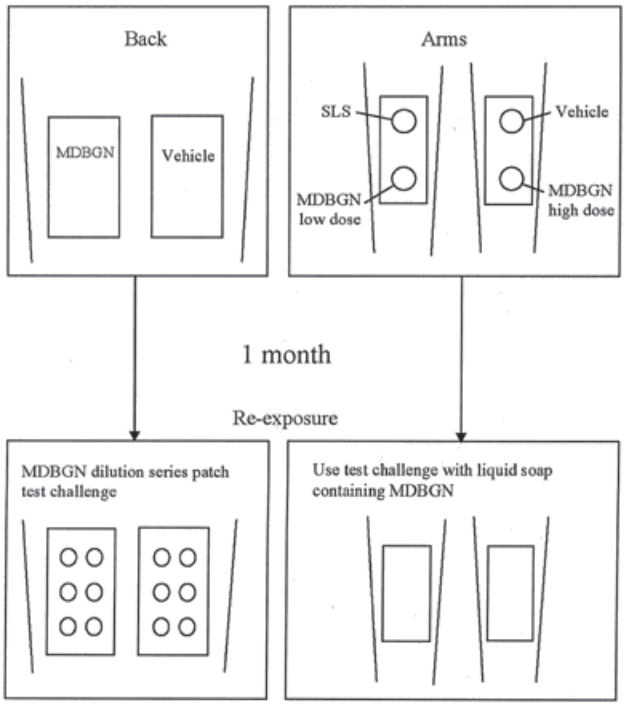

Fig. 1. Diagram of the study design with methyldibromogluteonitrile (MDBGN).

Elicitation of experimental allergic and irritant dermatitis on arms

On the inside of the lower part of each arm on the test and control subjects, an area of 5×10 cm^2 was marked and divided into two squares of 5×5 cm^2, giving a total of four squares on the two arms (Fig. 1). Fifty µl of four different solutions were applied to the squares using large Finn Chambers (12 mm) on Scanpore. The four solutions applied were: MDBGN ethanol/aq in a high and a low concentration, 1% SLS aq and 20% ethanol/aq (control). The two concentrations of MDBGN were chosen with regard to the sensitivity of the patients with a factor 4 of difference between the two concentrations to get a stronger and a weaker reaction. The range of concentrations used for the low concentration was 0.0004–0.05% MDBGN and for the high concentration 0.0016–0.2% MDBGN. The control and the high concentration of MDBGN were always placed on the same arm, due to the possibility of increased reactivity in the proximity of a strong reaction (11). Also, the control solution was placed in the upper test area of the arm, as the skin has increasing sensitivity to irritant exposure from the wrist to the elbow (12). Randomization to the left and right arm was performed to avoid influence from left–right arm variations in reactivity. The patches were removed on day 2 and the reactions were scored on day 3.

Challenge use test on arms

After 1 month healing of the dermatitis areas, the test and control subjects participated in a provocative use test. They were provided with a liquid soap containing 0.1% MDBGN and asked to wash the marked 5×10 cm^2 test areas on the arms twice a day for up to 3 weeks or until dermatitis appeared. According to instructions, the test area was moistened with water and three drops of soap were applied to the area. The test area was washed by moving a small water-soaked nylon sponge back and forwards over the area 10 times and the soap was then

rinsed off with running water and the arm was dried. No other soaps or moisturizers were allowed on the test area during the study. If dermatitis appeared first in one half of the test area the patients were asked to continue the washing of the other half of the test area. The soap bottle was weighed at every visit to determine the amount of soap used. In each patient, reactions in the test areas were compared visually according to the strength of the reactions by noting the extent of erythema and oedema and the number of papules and vesicles.

RESULTS

Challenge patch testing on back

All patients developed dermatitis from the initial exposure to MDBGN on the lower back. The dermatitis was at least characterized by erythema and infiltration (Fig. 2). No response was seen on the control test area exposed to vehicle. The scores for the six patch test reactions were summed for both test areas for each patient, resulting in a summed score for the previously eczematous area and one for the control area (Table I). Eleven patients had a higher summed score for the pretreated skin and four had the highest summed score for the normal skin. Two subjects had equal summed scores for both areas. The difference in summed scores for the pretreated area and the area of normal skin is shown in Fig. 3, and the highest score was given to the formerly eczematous area. A statistically significant difference was found between the summed scores of the pretreated and the normal skin (p=0.02, Wilcoxon matched-pairs signed-ranks test (two-tailed)).

Challenge exposure on arms

All test subjects and no controls developed dermatitis from the two MDBGN patches on the arms, and both test

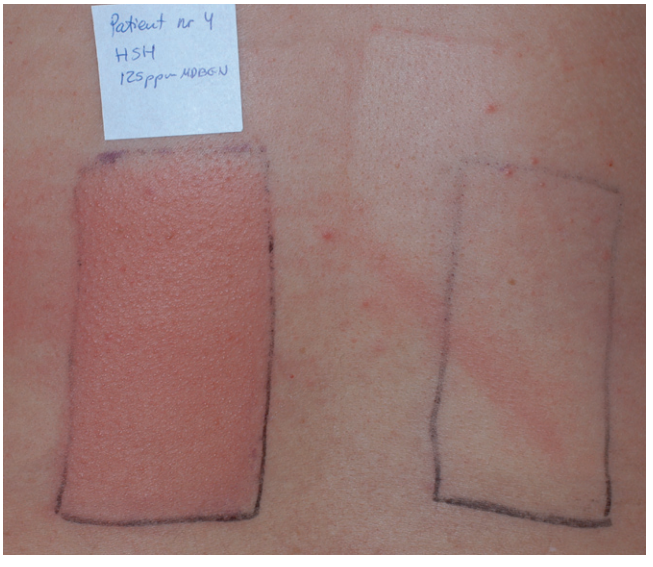

Fig. 2. Example of an area of provoked methyldibromoglutaronitrile dermatitis (left) on the lower back next to a control area previously exposed to vehicle only (right).

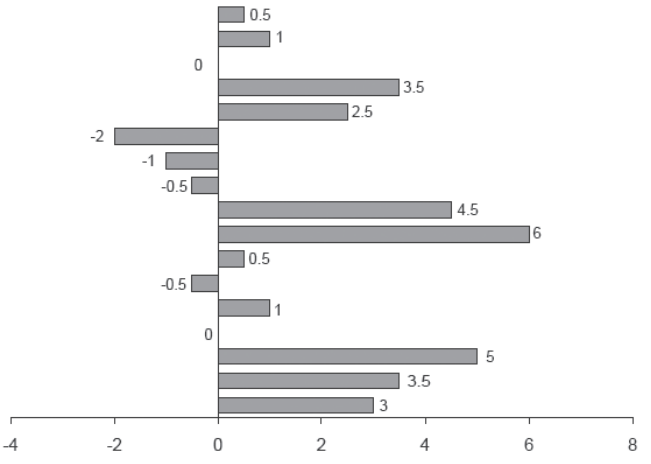

Fig. 3. Difference between the summed patch test scores for the retreated and the normal skin areas for each of the 17 patients. A positive value signifies a higher score for the area with previous methyldibromoglutaronitrile-eczema.

and control subjects developed irritant contact dermatitis from the SLS patch.

In the use test, 9/17 patients had a positive response to the use test with dermatitis developing on the test areas on both arms (Table II). Of these nine subjects, eight (nos 2, 5, 6, 8, 9, 11, 16 and 17) developed earlier and/or stronger dermatitis at the location of the formerly positive MDBGN patch on both arms compared with the vehicle-treated control area and the untreated skin of the test site (Fig. 4). In one patient (no. 10), a moderate dermatitis developed on the entire test site without an augmented development at the areas with previous MDBGN dermatitis. No increased responses were seen at the patch test areas with previous irritant dermatitis. The dermatitis on the MDBGN pretreated areas appeared stronger on one of the arms in seven of the subjects with an augmented response. In six of these, this concurred with the arm previously patch-tested with the high concentration of MDBGN. In the control group all use tests were negative. The total amount of MDBGN applied until generalized eczema appeared on the test area of the nine use test positive patients was an average of 22.6±17.9 (mean±SD range 7.6 to 61.5) μg/cm^2.

We compared the retest responses on the back and on the arms for each patient and no intra-patient correlations were observed. Six of eight patients with an increased retest response to the soap on the arms showed retest hyperreactivity on the back, but two did not. Five of nine

Table II. *Results of use test challenge with a methyldibromoglutaronitrile (MDBGN)-containing soap on test areas on the arms previously patch-tested with MDBGN*

Parameter	Subjects	Controls
Positive use test (Augmented response 8)	9	0
Doubtful use test	2	0
Negative use test	6	10

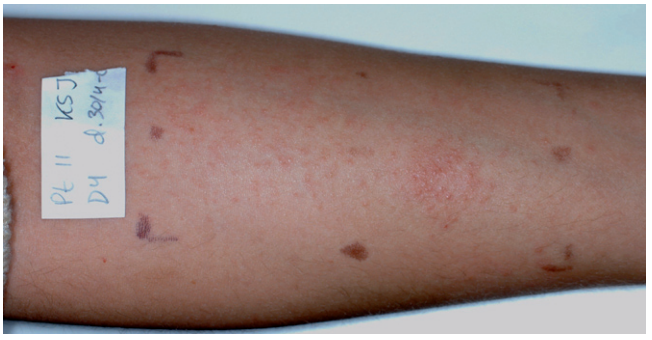

Fig. 4. Example (day 4) of an increased use test response on an arm with a previous positive patch test reaction to methyldibromoglutaronitrile (right side). Dermatitis first appeared on day 2 on the site of the previous patch test reaction and spread to the entire test area on day 4. The dermatitis is clearly more strongly localized to the area of the prior patch test reaction.

patients with no retest hyperreactivity on the arms showed an increased retest response on the back, while two were hyporeactive, and in two there was no difference between the summed scores.

DISCUSSION

Skin areas that one month prior to re-exposure had an allergic contact dermatitis caused by MDBGN, showed enhanced re-exposure reactions to both patch and use tests with a rinse-off product preserved with MDBGN. According to our knowledge, this is the first time retest hyperreactivity has been shown with an allergen-containing rinse-off product. Pre-irritated skin did not exhibit an augmented response to allergen exposure, thus the increased sensitivity to MDBGN seems to be specific to allergic contact dermatitis. These findings document the necessity of careful avoidance of exposure to MDBGN-containing household and cosmetic products in patients previously sensitized.

Our results are in agreement with the findings of Hindsén et al. (1, 2, 10), who performed a series of important studies investigating the significance of previous dermatitis for the allergic response to nickel exposure. They observed an increased reactivity similar to our results when previous nickel-eczema sites were challenged with nickel. Moreover, these studies examined the response to nickel exposure of skin with prior eczema elicited by an allergen other than nickel. They found that skin with prior cobalt-eczema had a response to nickel comparable to non-pretreated skin (10). Also, in oral challenge studies, it has been observed that flare-up reactions were localized to sites of previous dermatitis caused by the challenging allergen only (13, 14). In guinea pig studies, similar results have been observed, as increased retest reactivity to HEMA was only observed at former HEMA skin test sites and not at previous DNCB test sites, while likewise for DNCB, retest hyperreactivity was only found at former DNCB test locations and not at previous HEMA test sites (3). Also, in these studies, sites of previous HEMA-dermatitis were retested with the irritant croton oil without resulting in hyperreactivity. These findings, together with the lack of persisting hyperreactivity of pre-irritated skin on allergen exposure, indicate that the enhanced response of earlier eczematous skin is an allergen-specific immunological phenomenon. It cannot be explained by increased penetration and bioavailability due to a compromised skin barrier, but instead it seems likely that an immunological local memory function is responsible. Experiments have shown that hapten-specific T lymphocytes may persist for several weeks at former inflammatory sites, and probably cause local hyperreactivity at challenge with hapten (4, 15). Moreover, it has recently been shown that expression of the homing chemokine CCL27 and the receptor chemokine CCR10 remains increased 3 weeks after inflammation, while neither could be detected in normal or previously irritated skin after 3 weeks. As CCL27 is involved in the recruitment of T lymphocytes during an allergic reaction it is speculated that CCL27 might also play a part in retaining CCR10+ T cells at the previously exposed area of skin (15).

Skin irritated with 1% SLS one month before re-exposure did not exhibit an enhanced response to the use test. Hyporeactivity to exposure from nickel on pre-irritated skin has been demonstrated after one month while after 6–30 h an augmented response was seen (10, 16). When challenged with irritant, both hyper- and hyporeactivity have been described on pre-irritated skin (17–21). The reactivity is possibly dependent on the time period allowed between treatments, as generally, hyperreactivity has been described when retest was performed after up to 2–3 weeks after first treatment and hyporeactivity has been described for retest after 2 weeks or more. The increased reactivity succeeding the development of irritant dermatitis may partly be due to an enhanced penetration because of a compromised skin barrier, but probably also arises from induction of immunological mechanisms like expression of cytokines or increased density of Langerhans' cells from the previous irritant reaction (22). The following hyporeactivity has been hypothesized to occur because of other mechanisms, such as thickening of the skin from hyperkeratosis and/or changes in the composition of strateum corneum lipids (19, 23).

No correlation was found between retest reactivity on the back and on the arms of the individual patients, so it is not possible to extrapolate retest responses from one experimental design to another, particularly when it concerns different body areas.

A relationship was observed between the degree of response and the intensity of the pre-existing eczema. The stronger the previous patch test reaction to MDBGN, the stronger the response at challenge. A correlation between the intensity of the previous dermatitis and a flare-up reaction on oral challenge has been described (2).

The results from this study could constitute the basis for the development of a standardized model for evaluation of rinse-off products, for instance to be used to compare the effects of different product formulations. Exposure from rinse-off products may be difficult to predict, as the product is washed off the skin and they contain irritant ingredients that may produce unpredictable combination effects with allergens (24). Also, long-term exposure is often necessary to observe the effects of the products because of a limited exposure. Alternatively, the augmented response of pretreated skin in a use test, as observed here, may be exploited in this regard.

Experimental studies have shown that previously eczematous skin, although appearing to be healed, may be susceptible to certain exposures for several months. This is important information for patients suffering from dermatitis and enables them to take the best possible precautions. It is important to keep increasing our understanding of the mechanisms of contact allergy to enable the best management and treatment of this common disease.

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