

Methyldibromo glutaronitrile contact allergy: effect of single versus repeated daily exposure

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Some types of cosmetic products such as hand soaps and creams are commonly used several times a day, especially in occupational use situations. Little has experimentally been shown of how the daily frequency of the application of an allergen in a product influences the allergic response. This study investigates the allergic responses elicited in presensitized individuals when exposed to a specific amount of allergen applied either in 1 application per day or distributed over 4 applications per day. As model allergen, the cosmetic preservative methyldibromo glutaronitrile (MDBGN) is used. 19 contact allergic individuals and 12 controls participated in a double-blind, randomized use test. To areas on the forearms were applied 2 drops either once daily of a solution containing 0.04% MDBGN or 4 times a day of a solution containing 0.01% MDBGN. 14 of 19 patients developed dermatitis following the application of approximately equal amounts of MDBGN on both arms. Controls were negative. In this experiment, applications of 0.04% MDBGN once daily or 0.01% MDBGN 4 times daily had, in a use test, approximately equal capabilities of provoking allergic contact dermatitis. This adds new knowledge to the risk assessment and regulation of cosmetic allergens. However, these results may be highly allergen dependent, and further studies are needed before more general conclusions can be made.

Key words: allergic contact dermatitis; contact allergy; methyldibromo glutaronitrile; repeated daily application; use test. © Blackwell Munksgaard, 2005.

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Exposure to cosmetic products is a common cause of induction and elicitation of allergic contact dermatitis. To minimize the risk of inducing such a condition, the risk assessment of ingredients and products is carried out prior to manufacturing and marketing of a product. To enable proper risk assessment, it is important to understand the behaviour of human skin when exposed to an allergen in a use situation. It is generally accepted that dose per unit area is a relevant measure of exposure opposed to the concentration of the allergen in a product (1), and dose–response relationships have been established for several allergens (2–4). It is also known that certain body areas are more sensitive to exposure than others (5), and vehicle and occlusion effects are recognized as factors influencing the development of allergic contact dermatitis (6, 7).

As model allergen in this study, the preservative methyldibromo glutaronitrile (MDBGN) is used, which has been utilized in cosmetic products in Europe since the mid-1980s. An increasing number

of eczema patients sensitized to MDBGN have been found in dermatological clinics in Europe (8–12), and based on this, the Scientific Committee on Cosmetic Products and Non-Food Products of the EU Commission has recommended that the use of MDBGN is prohibited in leave-on products and restricted to rinse-off products until an improved risk assessment establishes safe use concentrations of MDBGN (13).

The current study examines whether 1 high-dose exposure a day of MDBGN is more or less potent than several low-dose exposures with regard to elicitation of allergic contact dermatitis. This knowledge may be useful in the risk assessment and regulation of cosmetic ingredients in products that may be used several times a day.

Materials and Methods

Subjects

19 patients sensitized to MDBGN from the Department of Dermatology, Odense University

Hospital, Denmark, participated in this investigation. The inclusion criterion was a previous diagnosis of MDBGN contact allergy. A total of 22 patients were recruited for the study, but 3 patients were excluded because a positive patch test reaction to MDBGN 0.3% in petrolatum (pet.) could not be reproduced. The test group consisted of 17 women and 2 men, with a mean age of 51 years (range 20–83). The patients had either no dermatitis or a mild degree of dermatitis outside the test area in the trial period. A control group of 12 individuals, with negative patch test reactions to 0.3% MDBGN pet., were recruited from the hospital staff. The control group included 10 women and 2 men, with a mean age of 43 years (range 28–56). Age below 18, pregnancy and dermatitis on the forearms were exclusion criteria. The study was performed according to the Helsinki Declaration II, and ethical approval was obtained from the local ethical committees.

Materials used in patch testing and use test

Test material was manufactured by the pharmacy, Odense University Hospital. Patch test solutions were made with MDBGN (Bie & Berntsen A/S, Rødovre, Denmark) at the concentrations: 0.2%, 0.1%, 0.05%, 0.025%, 0.0125%, 0.0063%, 0.0031%, 0.0016%, 0.0008%, 0.0004%, 0.0002%, 0.0001%, 0.00005%, 0.000025%, 0.000013% and 0% in ethanol/aqua (eth./aq.) (20:80). For the use test, solutions of 0.04%, 0.01% and 0% MDBGN in eth./aq. (20:80) were made and delivered in glass droplet bottles. The use-test solutions were allocated to the left and right arm at random in 2 sets of 2 solutions, by the pharmacy, Odense University Hospital. 1 set consisted of a 0.04% and a 0% MDBGN solution and the other of 2 0.01% MDBGN solutions. The randomization code was not available to the investigator until the trial was completed. The concentrations of MDBGN in 2 0.04% and 4 0.01% solutions were analysed by high-performance liquid chromatography and were measured to an average of 0.036% and 0.0089%, respectively.

Patch testing

15 µl of each patch test solution was micropipetted onto filter paper discs of small Finn Chambers[®] (Epitest Oy, Helsinki, Finland) on Scanpor tape (Alpharma A/S, Vennesla, Norway) and mounted on the subject's back. The patches were removed after 2 days, and readings were taken on day 3 and day 7 according to the International Contact Dermatitis Research Group guidelines (14). The

test patients were patch tested with a series of concentrations of MDBGN in eth./aq., and the patch test threshold value was determined. This is the lowest patch test concentration to give a positive reaction in a patient, and it is a measure of the sensitivity of the patient to the allergen.

Use test

The subjects participated in a provocative use test. They were provided with randomized sets of 2 × 2 droplet bottles containing eth./aq. solutions to be applied to a 3 × 4 cm² area on the inside of their forearms for up to 3 weeks. The subjects were instructed to apply 2 solutions to each arm, one of the solutions once daily and the other thrice daily. They were instructed to allow a minimum of 3 h between each application. The 2 solutions for one arm contained 0.04% MDBGN (to be applied once a day) and no MDBGN, respectively. The solutions for the other arm both contained 0.01% MDBGN. This resulted in an application of an approximately equal amount of MDBGN on both arms, applied either in 1 application of 0.04% or distributed over 4 applications of 0.01%. This design was necessary to ensure that the test was performed in a blinded, randomized fashion, so that neither the test subject nor the investigator knew which arm had been exposed to which set of solutions. No moisturizers were allowed on the test area during the study. The droplet bottles were weighed every week to determine the amount of solution used. The number of applications prior to a positive use test was recorded. A positive response was erythema covering at least 25% of the test area and infiltration represented by papule(s) regardless of number.

Results

Of the 19 subjects who participated in the study, 14 developed dermatitis on both arms while 5 were completely negative on both arms at the termination of the study after 3 weeks. In 7 of the 14 reacting patients, the total amount of allergen applied to provoke dermatitis was lower for the 0.01% MDBGN solution than for the 0.04% solution, while in 6 patients, the opposite was found. For 1 patient, equal amounts of MDBGN had been applied to both arms when dermatitis appeared. In Figs. 1 and 2, the total amount of MDBGN applied and the number of days until a positive use test are illustrated for the two solutions. The 14 subjects developed dermatitis after application of an average total of 7.9 µg/cm² MDBGN from the 0.01% solution

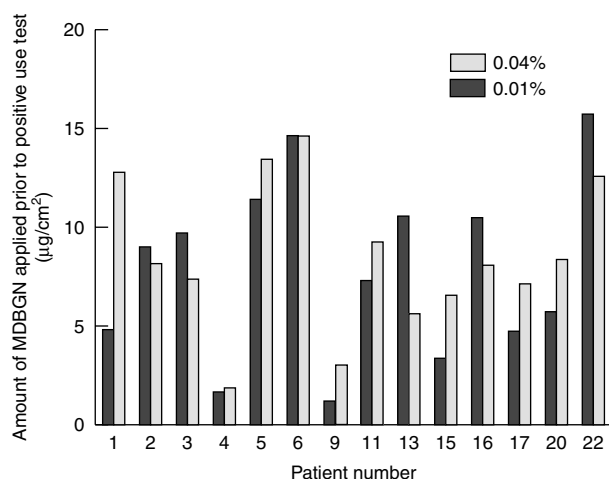


Fig. 1. Amount of methyldibromo glutaronitrile (MDBGN) applied until an allergic response was seen from the 4 times 0.01% MDBGN and the single 0.04% MDBGN applications a day.

and $8.3 \mu\text{g}/\text{cm}^2$ MDBGN from the 0.04% solution (Table 1). This was distributed in averagely 15.0 applications (4 daily) for the 0.01% MDBGN solution and in 3.9 applications (1 daily) for the 0.04% MDBGN solution. Most patients developed dermatitis on both arms within 4 days. None of the controls showed any skin changes in the use test.

The patch test threshold values of the participants ranged from 0.0016% to 0.05% MDBGN. There was a significant correlation between the patch test threshold value and the total amount of MDBGN applied to produce a positive provocative use test. A dose–response curve is shown in Fig. 3. The more sensitive the

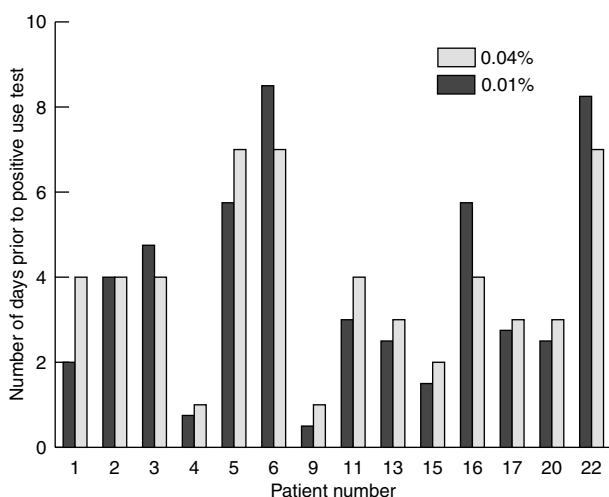


Fig. 2. Number of days until an allergic response was produced from the 4 times a day applications of 0.01% methyldibromo glutaronitrile (MDBGN) and the single application a day of 0.04% MDBGN.

subject was according to patch test data, the earlier an allergic response developed in the use test. A Spearman rank correlation (non-parametric) was statistically significant for both the 0.01% ($P=0.006$) and 0.04% ($P \leq 0.0001$) solutions. In concordance with the results mentioned above, the trend lines are close to coinciding for the two solutions.

Discussion

The dose–response relationship for the elicitation of allergic contact dermatitis is influenced by a number of factors, such as inter-individual variations, including skin barrier integrity, vehicle and occlusion effects, duration of exposure and number of applications. In this study, we wanted to examine the effect of the frequency of applications. We questioned whether equivalent total doses in a day applied as either 1 exposure a day or 4 exposures a day had equal elicitation potential. Results showed that the 2 modes of exposure had approximately equal capabilities of producing allergic reactions in presensitized individuals. Thus, according to this experiment, the accumulated total dose from multiple exposures over short time seems to be of considerable importance. Few studies have been published about cumulative exposure. One study investigated exposure duration of the allergen *p*-phenylenediamine (PPD). In a use test with 5 min of daily exposure to 1% PPD, the percentage of subjects who responded in 6 days was comparable to the responses to a single 30-min exposure to the equivalent concentration of PPD (15).

In the current study, we also demonstrated a correlation between the patch test threshold value and the amount of MDBGN needed to elicit a reaction in the use test. Thus, the more sensitive the patient is, the less allergen is needed for elicitation. This relationship was equivalent for the 2 test concentrations (Fig. 3), again showing that exposures to the 2 solutions were equally eliciting using the present test design.

However, more experiments are needed before any general conclusions can be made. The results from this study may be allergen dependent, and it cannot be determined from this experiment how the time span between applications and the duration of the exposure period affects the cumulative elicitation potential. Also, in this experiment, most patients had positive use tests on both arms within only 4 days. Lower test concentrations resulting in later use-test reactions would have been preferable, as this would have made a difference in the reaction time to the 2 solutions, if present, more easily distinguishable. The

Table 1. Average amount of methyldibromo glutaronitrile (MDBGN) applied and average number of applications of the 2 solutions prior to an allergic response in the use test

	Mean $\pm$ SD (range)	
	0.01% solution (4 applications a day)	0.04% solution (1 application a day)
Average amount of MDBGN applied ($\mu\text{g}/\text{cm}^2$)	7.9 $\pm$ 4.5 (1.2–15.7)	8.3 $\pm$ 4.1 (0.8–14.6)
Average number of applications	15.0 $\pm$ 10.2 (2–34)	3.9 $\pm$ 2.0 (1–7)

concentrations of MDBGN used in this experiment are, however, of a level that may be found in marketed products (16).

Speculations concerning the influence of the time span between applications and the exposure period duration on the cumulative elicitation potential are interesting with regard to the risk assessment of cosmetic products, as cosmetics are often used frequently over long periods of time. It would seem feasible that elicitation simulates the reaction pattern of cumulative irritation. Repeated skin exposure to irritants can be tolerated for a while until the regeneration ability of the skin has been transcended and eczema develops. The latency period may be short or long, and if the tolerance limit is not exceeded, no eczema will develop. In this experiment, 4 applications in a day seemed sufficiently frequent to build the response on the previous one.

Risk assessment of cosmetic products is usually performed using experimental data from exaggerated exposure studies that are not directly

comparable to actual use situations. Extrapolation from such experimental studies involves a number of uncertainty factors that must be considered, such as unintended use, inter-individual and regional skin differences, vehicle and product matrix effects and product usage patterns such as single or multiple daily exposures (17). According to this study, particular care must be taken in the risk assessment of products containing MDBGN that may be applied to the skin several times in 1 day, as, for instance, occupational products like soaps and hand-care products. A daily accumulated exposure may elicit eczema in allergic individuals even when low concentrations of the allergen are used.

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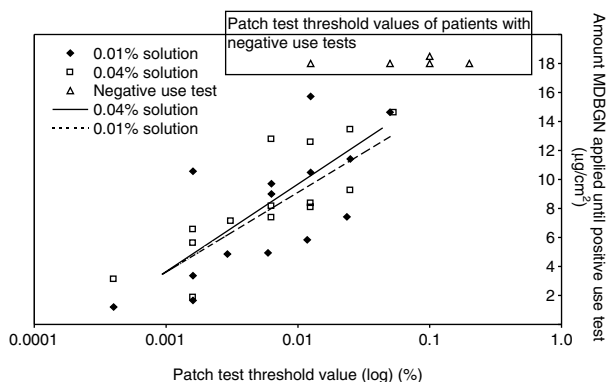


Fig. 3. Patch test threshold value in relation to the amount of methyldibromo glutaronitrile (MDBGN) applied to elicit dermatitis in the use test from the 0.01% and 0.04% solutions. Trend lines have been drawn. The triangles represent the patch test threshold values of the patients that did not react in the use test. They have mostly the largest threshold values which fit nicely with the dose-response relationship.

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