

Delayed and immediate allergy caused by methylhexahydrophthalic anhydride

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Epoxy resin compounds (ERC) include a large number of chemicals, such as epoxy resins (ER), reactive diluents and hardeners. Many hardeners, e.g., aliphatic polyamines, are well-known sensitizers. Another type of ER hardeners are the phthalic anhydrides, such as methylhexahydrophthalic anhydride (MHHPA) and methyltetrahydrophthalic anhydride (MTHPA), which have been reported as causing immunologically-mediated respiratory diseases and contact urticaria, but not allergic contact dermatitis. Here, we present a horizontal boring-machine worker who developed allergic contact dermatitis, as well as allergic rhinitis and an immediate contact skin reaction from MHHPA. Patch testing with a dilution series of MHHPA in pet. elicited the following results: 2%, 1% and 0.5%, ++; 0.25% and 0.125%, + (3- to 6-day readings). An immunohistochemical and electron microscopic study also indicated that the patch test reactions were conventional-delayed allergic reactions. Interleukin 8 was observed in the epidermal cells, whereas interleukin 4 immunoreactivity was detected in the dermal cells. Immunoreactivity to interleukin 5, granulocyte/macrophage-colony stimulating factor (GM-CSF) or eosinophil cationic protein was not seen. In conclusion, the patient developed both Type I and Type IV allergy to MHHPA. The clinical data, patch test results, immunohistochemical and electron microscopic observations indicated that the MHHPA allergy detected by the patch test reaction was a conventional delayed-type hypersensitivity reaction. The patient also had an allergic patch test reaction to para-phenylenediamine and diaminodiphenylmethane, possibly representing occupational sensitization.

Key words: methylhexahydrophthalic anhydride; plastics; epoxy compounds; rhinitis; contact urticaria; occupational; electron microscopy; immunohistochemistry; interleukin 4; interleukin 5; interleukin 8; granulocyte/macrophage-colony stimulating factor; eosinophil cationic protein; respiratory effect. © Munksgaard, 1997.

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Epoxy resin compounds (ERC) include a large number of chemicals, such as epoxy resins (ER), hardeners and reactive diluents. Many of the epoxy hardeners have caused allergic dermatitis, namely aliphatic polyamines, e.g., ethylenediamine, diethylenetriamine, triethylenetetramine, 3-dimethylaminopropylamine and trimethylhexamethylenediamine; cycloaliphatic polyamines, e.g., isophoronediamine and 3,3'-dimethyl-4,4'-diaminodicyclohexylmethane; and aromatic amines, e.g., 4,4'-diaminodiphenylmethane, m-phenylenediamine and 1,3-xylylenediamine (1).

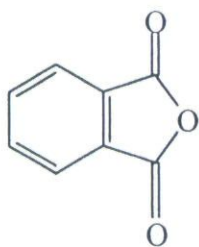
Another type of ER hardeners are the phthalic anhydrides, such as methylhexahydrophthalic anhydride (MHHPA) and methyltetrahydrophthalic anhydride (MTHPA) (Fig.1) (2, 3). MHHPA and MTHPA typically require an elevated curing temperature (50–200 °C), which facilitates escape of the

anhydride vapours. Anhydrides have been reported as causing immunologically-mediated respiratory diseases (2–4) and contact urticaria (5–7), but not allergic contact dermatitis. Here, we present a patient who developed allergic contact dermatitis, contact urticaria and allergic rhinitis from MHHPA. The allergic nature of the patch-test reactions was confirmed by immunohistochemical and electron microscopic observations.

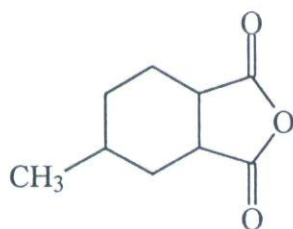
Case Report, Methods and Results

Case report

The patient was a 45-year-old horizontal boring-machine worker, who had been exposed to mineral oils, cutting fluids, black rubber products and plastic chemicals for 26 years when he started to develop dermatitis on his wrists, lower arms, and the



Phthalic anhydride



Methylhexahydrophthalic anhydride

Fig. 1. Molecular structure of phthalic anhydride (PA) and methylhexahydrophthalic anhydride (MHHPA).

backs of his hands, as well as rhinorrhea and some nasal blockage at work. Within some months, he started to have dermatitis also on his forehead, cheeks, earlobes and neck, as well as swelling of the eyelids. The dermatitis and rhinitis cleared at weekends and during sick leaves, but always relapsed at work.

Patch testing

Patch testing was performed and scored as previously described (8–10). A total of 5 patch test sessions were performed because all relevant allergens were not detected earlier. An allergic patch test reaction was elicited by para-phenylenediamine (PPD; +++) in the standard series, whereas the oil series, antimicrobial series, epoxy resin series and rubber series (9) were negative.

After the PPD allergy had been verified, it was believed that exposure to black rubber was the main cause of the symptoms. The patient was advised to avoid contact with black rubber products, and rubber materials were removed from his work site. Despite this, his skin symptoms persisted. Consistent with this finding, the gas chromatography determination of PPD from 2 black rubber products did not detect PPD in the products (detection limit below 0.001%).

On further testing, diaminodiphenylmethane (MDA) elicited a + allergic reaction in the plastics and glues series (10). Previous exposure to MDA

chemicals during the long years of work is possible. When patch tested to several of his own substances, methylhexahydrophthalic anhydride (MHHPA) elicited an allergic patch test reaction (1% pet., ++). When MHHPA was retested with a dilution series (pet.), the following results were obtained: 2%, ++; 1%, ++, 0.5%, ++, 0.25%, + and 0.125%, + (3–6-day readings). Control patch tests were performed in 20 control persons. 2% caused slight irritation in 1/2 of the patients; 1% caused slight irritation in 2 control persons, whereas 0.5% did not cause any irritation in the 4–6-day readings.

During patch testing with the plastics and glues series, the patient developed a flare-up reaction on day 21. Retesting showed that this was due to para-tertiary-butylcatechol (PTBC), which induced a +++ reaction (++ on D 2; +++ on D 3 and D 6).

Prick testing and radioallergosorbent tests

Prick testing with 20 common environmental allergens, including natural rubber latex, polyester chemicals and the epoxy resin chemicals, was negative. Prick testing with human-serum albumin conjugated acid anhydrides (MHHPA from Aldrich Chemical Co., Milwaukee, Wis, USA, and MTHPA from K & K Laboratories ICN Biomedicals, Inc. Cleveland, Ohio, USA; 7), gave the following reactions (mean diameter in parentheses): methylhexahydrophthalic anhydride (MHHPA, 10 mm), hexahydrophthalic anhydride (6 mm), MTHPA (5 mm), maleic anhydride (5 mm), phthalic anhydride (negative), trimellitic anhydride (negative), histamine hydrochloride 10mg/ml (7 mm). The negative controls (vehicles) were negative. Total IgE was 29 kU/l and radioallergosorbent tests (RAST) to all of the above anhydrides (7) were negative (i.e., below 0.3 kU/l).

Chamber provocation

A chamber provocation test (7, 11) with MHHPA, to test for occupational rhinitis, induced erythematous skin symptoms within 15 min on the upper arms and the sites of the patient's previous dermatitis. These were still present 24 h later. There were no changes in the peak expiratory flow (PEF) or FEV1 values. Neither were there changes in the nasal status nor in the nasal volume measured by acoustic rhinometry after the provocation.

Work-site visit

A visit was made to the patient's work place. The patient's work consisted mainly of tooling metals,

and exposure to cutting fluids and cutting oils also took place. Having been diagnosed as having allergy to MHHPA, the patient was transferred to other work, but even working in the same hall elicited relapses of his skin and respiratory symptoms, indicating airborne allergy. Away from the work hall he was symptom-free.

Immunohistochemistry and electron microscopy

Biopsies for immunohistochemistry and electron microscopy were taken from the 6-day allergic patch test reactions caused by PTBC (+++), MHHPA (1%, ++), and from a negative patch test site (urea-formaldehyde resin 10%).

Cryostat sections and immunohistochemistry (an avidin-biotin-complex immunoperoxidase method) were used to study the rôle of cytokines in the MHHPA patch test reaction. Interleukin (IL) 8 and granulocyte/macrophage-colony stimulating factor (GM-CSF) immunoreactivities were studied, because IL-8 is an important chemotactic factor in allergic contact dermatitis, and GM-CSF potentiates the function of Langerhans cells. Antibodies to IL-4, IL-5 and eosinophil cationic protein (ECP) were included in this study as indicators of Type I allergy. A polyclonal rabbit anti-IL-8 antiserum (kindly provided by Dr. Marco Baggiolini, Bern, Switzerland) was used as described earlier (12). Monoclonal mouse antibodies to GM-CSF, IL-4 and IL-5 (ZM-213, 1842-01 and 2374-01 from Genzyme Diagnostics, MA, USA) as well as the ECP antibody (10-9196-01 from Pharmacia, Uppsala, Sweden) were used on sections fixed in 4% paraformaldehyde. An additional blocking of endogenous peroxidase was performed with 0.45 % H₂O₂ in methanol for 40 min. Isotype controls for the monoclonal antibodies were negative.

The immunohistochemical findings have been summarized in Table 1. IL-8 immunoreactivity was detected in all epidermal cell layers in the biopsy taken from the negative patch test reaction. IL-4

immunoreactive cells were seen in the dermis of the MHHPA reaction. However, the staining pattern of the sections from the negative patch test reaction was similar. No immunoreactivity to IL-5, ECP or GM-CSF was detected in any skin sections.

Electron microscopy after 2.5% glutaraldehyde fixation with post-osmification from the 6-day allergic patch test reactions were consistent with delayed allergic reactions (13). In the +++ reaction elicited by PTBC, there was much spongiosis, oedema of the upper keratinocytes, and disruption of their cell membranes. Langerhans cells appeared to be activated in both the PTBC and the MHHPA reaction, with active Golgi areas, increased rough and smooth endoplasmic reticulum, Birbeck granules, dense cored and clear vesicles, lipid droplets and microvilli, and a large cytoplasm/nucleus ratio. The microvilli showed close apposition to both keratinocytes and intraepidermal lymphocytes. There was a slight increase of intraepidermal lymphocytes in the negative patch test reaction with urea-formaldehyde resin, compared to untreated skin.

Discussion

There are very few reports of delayed or immediate-type allergic skin reactions to phthalic anhydrides (PA) (5–7, 14), or other anhydrides (15). In earlier studies, allergic dermatoses caused by PA were believed to be due to impurities in PA (16). PA and MHHPA are also irritants of the skin and mucous membranes. Heavy exposure of the skin to dusts, fumes or vapours of PA may cause marked skin irritation, and even slowly healing caustic ulcers and blisters (16, 17). The symptoms are aggravated by moisture, which causes rapid hydrolysis of the anhydrides to respective acids (2, 16). Therefore most workers get symptoms in their eyes and upper airways but also on the skin (2).

We have earlier reported contact urticaria due to MHHPA (5, 6). Immediate allergy was verified by the open test with undiluted MHHPA (5) and the scratch chamber test using 1% MHHPA in ethanol (6). Immediate allergy to MTHPA was verified by prick tests in a patient who also had immediate and delayed-type allergy to DGEBA-based epoxy resin (11). Recently we had 2 patients who were sensitized by airborne MHHPA and MTHPA in their workplace (7). They had not had any skin contact with liquid epoxy resin or hardeners. Their immediate allergy was verified by positive skin prick tests and specific IgE determinations supported by RAST inhibition studies. Our patient also developed immediate skin reac-

Table 1. Immunoreactivity to cytokines in a positive methylhexahydrophthalic anhydride (MHHPA) patch test reaction and 2 control reactions

Cytokine	Patch test reaction		
	positive control	MHHPA	negative control
IL-8	+	+++	++
IL-4	+	+	NT
IL-5	–	–	NT
GM-CSF	–	–	NT
ECP	–	–	NT

Staining intensity graded from – to +++; IL: interleukin; GM-CSF: granulocyte/monocyte colony stimulating factor; ECP: eosinophil cationic peptide; NT: not tested.

tions after chamber provocation, consistent with a non-whealing contact urticaria.

We wanted to get further insight into the immune reaction caused by MHHPA by performing morphological studies. Using immunohistochemistry, IL-8 immunoreactivity was detected in all epidermal cell layers in the biopsy taken from the negative patch test reaction. However, a more intense immunoreactivity was detected in the epidermis of the ++ MHHPA reaction. This is partly in accordance with earlier studies on allergic patch test reactions caused by urushiol (18). Griffiths and his coworkers did not detect any IL-8 immunoreactivity in normal skin with their anti-IL-8 antiserum (18). However, the epidermal immunoreactivity pattern of the positive urushiol reaction was similar to our MHHPA reaction pattern. Differences in method sensitivity or anti-IL-8 antisera might explain why Griffiths and coworkers did not detect IL-8 immunoreactivity in normal skin (18). Thus, we suggest that the MHHPA reaction does not differ from other ++ allergic patch test reactions in IL-8 immunoreactivity. Unexpectedly, the epidermal IL-8 immunoreactivity of the +++ allergic patch test reaction caused by 4-tertiary butyl catechol was less intense than the IL-8 immunoreactivity of the MHHPA reaction and even less intense than the IL-8 immunoreactivity of normal skin. It is possible that in extreme allergic patch test reactions all IL-8 is consumed from the epidermal cells to recruit a maximal leukocytic infiltration in the skin. Another possibility is that the keratinocyte cell membranes are disrupted in +++ allergic reactions, as was evident from the electron microscopic study, and IL-8 leaks out of the cells in vivo or during processing of the skin biopsy. Immunohistochemical staining was repeated, and the differences in the staining intensities were confirmed.

The patient had had symptoms of rhinitis at work but there were no changes in nasal status after the chamber provocation. However, the chamber provocation test could not be continued as long as is usual (30 min) due to the skin symptoms. Thus the causal connection between the rhinitis symptoms and work exposure could not be verified. However, based on the work-related immediate rhinitis symptoms and positive skin tests, it is likely that the patient's symptoms were caused by allergy to MHHPA.

It was concluded that the patient had developed both Type I and Type IV allergy to MHHPA. The clinical data, patch test results, immunohistochemical and electron microscopic observations indicate that the MHHPA allergy detected by the patch test reaction was a conventional delayed type hypersensitivity reaction. The prick test reactions to

other phthalic anhydrides than MHHPA apparently represented cross-sensitivity. The allergic patch test reaction caused by MDA could have represented a past relevant occupational allergy, as MDA had been used in the same factory as an epoxy resin hardener, and the patient had a work history of 26 years. The patient also had an allergic patch test reaction to PPD, possibly representing occupational sensitization from rubber chemicals, or cross-sensitivity to MDA. During patch testing, active sensitization (19) to p-tert-butylcatechol occurred.

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