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Dermal provocation study with 4,4'-diphenylmethane diisocyanate led to active sensitization

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Abstract

Background: To investigate the dermal uptake of 4,4'-diphenylmethane diisocyanate (4,4'-MDI) a study was performed where two female volunteers were exposed to 10 and 25 mg respectively of 4,4'-MDI by applying 2.0% 4,4'-MDI in petrolatum over areas where the surface concentration corresponded to 800 $\mu\text{g}/\text{cm}^2$. 10 days later they developed eczematous dermatitis at the area of application.

Objectives: To investigate if the dermal application caused active sensitization to 4,4'-MDI.

Methods: Chemical analysis of the 4,4'-MDI preparation used in the application and the amount of 4,4'-MDI not absorbed in the skin was performed using liquid chromatography-mass spectrometry. The volunteers were tested with serial dilutions of 4,4'-MDI and the potentially cross-reacting substances 4,4'-diaminodiphenylmethane (4,4'-MDA), *p*-phenylenediamine (PPD) and dicyclohexylmethane-4,4'-diisocyanate (DMDI).

Results: Patch test results implied that the volunteers were actively sensitized to 4,4'-MDI following the dermal uptake study since they reacted positively to 4,4'-MDA, a marker for MDI allergy. No positive reactions were seen to PPD or DMDI. Chemical investigation confirmed that the correct concentration had been used for the dermal uptake study and showed that about 70% of the applied 4,4'-MDI was not absorbed.

Conclusions: A dermal uptake study with 4,4'-MDI in 2.0% petrolatum with an occlusion time of 8h induced active sensitization to 4,4'-MDI and subsequently to 4,4'-MDA.

Key words: 4,4'-MDI; MDI; 4,4'-diaminodiphenylmethane; MDA; *p*-phenylenediamine; PPD; dicyclohexylmethane-4,4'-diisocyanate; DMDI; patch test; polyurethane.

Introduction

Isocyanates are established as a cause of occupational asthma and there are specific threshold limit values for airborne exposure to them. However, there are suggestions that dermal exposure may be of importance for the induction of respiratory diseases (1). Little is known about the dermal uptake of isocyanates and to study this topic further, a study was initiated in order to investigate the cutaneous uptake of a commonly used industrial isocyanate, 4,4'-diphenylmethane diisocyanate (4,4'-MDI) (2). In a pre-study, two volunteers were recruited to determine the exposure necessary to enable detection of markers of exposure in blood and urine. After this pre-study both volunteers developed an eczematous dermatitis corresponding to the exact areas of application. This indicated 4,4'-MDI sensitization and the study was interrupted. The aim of the present investigation was to determine if the application had caused active sensitization to 4,4'-MDI in the two volunteers.

Materials and Methods

Volunteers

Both volunteers gave written informed consent to participate in the study, which was approved by the Research Ethics Committee of Umeå University (Dnr 09-214M), Sweden. They were healthy females at the age of 19 and 35 years, with no previous history of contact

allergy to isocyanates or any respiratory symptoms that could be related to isocyanates. The original exposures and the investigation of the suspected sensitization were carried out at the Department of Occupational and Environmental Dermatology, Malmö.

Chemicals and materials

The chemicals that were used in this study were the following: 4,4'-MDI and 4,4'-diaminodiphenylmethane (4,4'-MDA) (TCI Europe, Zwijndrecht, Belgium), *p*-phenylenediamine (PPD), toluene and dicyclohexylmethane-4,4'-diisocyanate (DMDI) (Sigma-Aldrich Chemie GmbH, Schnellendorf, Germany), petrolatum USP/NF Kval E (Apoteket production & laboratories, Gothenburg, Sweden), filter paper Quality 1F (Munktell filter AB, Falun, Sweden), Scanpo® tape (Norgesplaster A/S, Vennesla, Norway), aluminium foil (Duni AB, Malmö, Sweden), acetone (Fisher Scientific, Leicestershire, UK), dibutylamine (Acros Organics, Thermo Fisher Scientific, Geel, Belgium), methylene chloride (Mallinckrodt Baker B.V., Deventer, The Netherlands), acetonitrile multisolvent grade (Scharlau Chemie SA, Barcelona, Spain), deionised water (18.2 MΩ), MILLI-Q® system ZFMQ23004 (Millipore SA, Molsheim, France), formic acid, analytical reagent grade (Riedel-deHaën, Seelze, Germany), 4,4'-MDI-reaction product with dibutylamine (4,4'-MDI-DBA) and 4,4'-MDI reaction product with deuterated dibutylamine-d₉ (4,4'-MDI-DBA-d₉) (IFKAN AB, Hässleholm, Sweden). The chemical structures of the substances used for patch testing (4,4'-MDI, 4,4'-MDA, DMDI and PPD) are shown in Figure 1.

Dermal Uptake Study

The 4,4'-MDI preparation 2.0 % w/w in petrolatum, for the dermal uptake study was made at the Department of Occupational and Environmental Dermatology, Malmö. A total cutaneous

exposure dose of 10 mg 4,4'-MDI on one volunteer and 25 mg 4,4'-MDI on the other with the surface concentration of 4,4'-MDI at 800 $\mu\text{g}/\text{cm}^2$ was achieved. The different doses were obtained by using filter paper with areas of 12.5 cm^2 and 31.0 cm^2 , evenly covered with the 4,4'-MDI in petrolatum. The amounts of the 2% 4,4'-MDI petrolatum preparation applied were 500 mg and 1250 mg, respectively. The filter paper was placed on the outer aspect of one upper arm of each volunteer, and in order to mimic the patch test technique used in Malmö (the Finn Chamber® technique) the filter paper was covered with aluminium foil and then secured with Scanpor® tape. The exposure time was 8 hours.

Sampling and chemical analysis

After 8 hours the aluminium foil backing and filter paper were removed and the excess of petrolatum on the skin was wiped off using 2-3 compresses saturated in acetone. The removed filter paper with its aluminium foil backing and the contaminated compresses were placed in an impinger flask containing 60 ml 0.10 M DBA in methylene chloride and toluene, 50/50 v/v. The samples from the 4,4'-MDI preparations (2×500 mg and 2×100 mg) were treated with the same reagent solutions in impinger flasks. The impinger flasks were sent for chemical analysis to the Department of Occupational and Environmental Medicine in Örebro.

To each impinger sample an aliquot of 50 μl 2.0 $\mu\text{g}/\text{ml}$ 4,4'-MDI-D₉DBA was added as internal standard. The samples were ultrasonicated at 20°C for 5 minutes and then each sample was divided into 10 ml subsamples. The subsamples were evaporated until approximately 1.5 ml remained, then the subsamples were pooled and evaporated to dryness using a vacuum centrifuge (AES2010, Savant Instruments Inc, Holbrook, NY, USA). A set of 10.0 ml toluene (zero samples) and calibration solutions all with internal standard were evaporated with the impinger samples. The dry samples were reconstituted with 0.50 ml acetonitrile, ultrasonicated for 1 minute and filtered using a Titan® 17 mm 0.22 μm nylon

syringe filter (SunSRi, Duluth, USA). An aliquot of the impinger samples were diluted with a zero sample to calibrated range (0.010-0.56 µg/ml). The samples were analysed using liquid chromatography-mass spectrometry on an Agilent 1100 Series HPLC/MS instrument, consisting of: a vacuum degasser G1322A, a binary pump G1312A, an autosampler G1329 A with a thermostat G1330A (Hewlett Packard, Waldbronn, Germany), a mass-selective detector G1946B with an electrospray interface G1948A (Hewlett Packard, Palo Alto, USA). Data generated by the HPLC/MS were collected and evaluated using the software LC/MSD Chemstation A.10.02 (Agilent technologies, Waldbronn, Germany). 10 µl of each sample were injected using segmented injection with deionised water (25 µl water-10 µl sample-25. µl water). Chromatographic separation was performed on a Hypersil Gold™ column (100 × 2.1 mm, 3.0 µm, Thermo Electron Corporation, Waltham, USA) using gradient elution, see Table 1. The flow rate was 0.35 ml/min. Introduction into the mass spectrometer was done using electrospray in positive mode, with a capillary voltage of + 5000 V, the nebuliser pressure set to 35 psig and the drying gas flow to 12 L/min at 350°C. Detection was performed in SIM mode of m/z 509.5 and 527.5, corresponding to protonated 4,4'-MDI-DBA and 4,4'-MDI-DBA-d₉, respectively. The fragmentor voltage was set to 120 V and gain at 1. Dwell time was 98 ms.

Patch test preparations and patch testing

4,4'-MDI, 4,4'-MDA, PPD and DMDI patch test preparations in petrolatum were made at the laboratory at the Malmö department. 20 mg of each preparation was applied on Finn Chambers® (8mm, Allerderm, Phoenix, Arizona, USA) on Scanpor® (Norgesplaster A/S, Vennesla, Norway) (3). The patch test concentrations of the dilution series of the substances were made equimolar to the 4,4'-MDI preparations. The dilution series were based on the factor $\sqrt{10}$. Patch test concentrations are given in table 4. However, due to a mistake regarding molecular weights the concentrations of DMDI were made 19% lower than the intended

equimolar ones and for PPD the corresponding difference was 6% higher. The tests were applied to the upper part of the back for 48 h and then discarded. The tests were read on day (D) 3 or D4 as well as on D7 and D10 by a dermatologist. The reactions were read according to the International Contact Dermatitis Research Group (ICDRG) criteria (3).

Results

Of the total amount of 4,4'-MDI applied during the dermal uptake study, i.e. 10.0 mg and 25.0 mg, 7.0 (70%) and 17.5 mg (70%) was recovered, respectively (Table 2). 7 to 10 days after this study an itchy eczema appeared corresponding to the area of application on both volunteers. The mean concentration of 4,4'-MDI found in the analysed petrolatum preparations was 1.9% w/w (Table 3). Results from patch testing of 4,4'-MDI, 4,4'-MDA, PPD and DMDI are given in Table 4.

Discussion

Major patch test preparation manufacturers have been supplying 4,4'-MDI at a stated concentration of either 1.0 or 2.0% in petrolatum. At the clinic in Malmö, we have been using such a commercial patch test preparation for patch testing and so have many other departments to test dermatitis patients when investigating suspected contact allergy to 4,4'-MDI. We included a commercially available 4,4'-MDI 2.0% w/v test preparation in our baseline series in 2002-2006 and patch tested approximately 2800 patients without observing any suggestion of active sensitization to this substance. Therefore a 2.0% 4,4'-MDI preparation in petrolatum, prepared in the Malmö laboratory was used when performing the uptake study. The amount applied at regular patch testing is 20 mg (4) containing 2.0% MDI in petrolatum in the Finn Chamber® (8 mm), corresponding to 800 µg/cm²; this surface dose

was also used in this study. The eczematous dermatitis that developed on the exact area of application 7-10 days after the application indicated active sensitization to 4,4'-MDI. Positive reactions seen on 7-10 days after application are usually considered as a sign of active sensitization. However, it has previously been shown that such late positive reactions to isocyanates may appear in already sensitized individuals (5). Therefore, it was theoretically possible that the volunteers, without them being aware, already had contact allergy to 4,4'-MDI when entering the initial dermal uptake study. To investigate this, 4,4'-MDI was re-tested in serial dilutions with potentially cross-reacting substances such as 4,4'-MDA, PPD and DMDI.

Active sensitization is traditionally defined as a negative patch test reaction followed by a flare-up reaction after D10 to D20, and with a positive reaction on D2 to D4 at retest (6). A positive reaction on the second patch test occasion could also represent an increase in the level of sensitivity, but if patch testing on this test occasion is done with the substance diluted 10-100× as compared with the original test concentration, and the test turns out positive, active sensitization is the most likely (6). Since the volunteers had showed such strong flare-up reactions at the site of the provocation they were not tested with the highest concentration of 4,4'-MDI, i.e. 2.0%, and thus no retest was performed with the same concentration as the one used at the suspected active sensitization. Only one of the volunteers reacted to the diluted preparations of 4,4'-MDI and that was on D7. In our experience at the Malmö department, patch testing with 4,4'-MDI can be difficult since there can be significant individual variation in test response from one test occasion to another and we have seen a case where the same patient exhibited both early, late, and no reaction at all to 4,4'-MDI when tested on different occasions (5). Based solely upon the testing with diluted preparations of 4,4'-MDI, the results do not fulfil the criteria described above for establishing that active

sensitization had occurred in both volunteers, since one did not react to 4,4'-MDI. Still, we consider it most certain that both volunteers had been sensitized through the dermal uptake since they both reacted to 4,4'-MDA, which is a marker of 4,4'-MDI allergy (5).

The concentration of the 4,4'-MDI preparation used for the dermal uptake study was confirmed to be 1.9% w/v, which is very close to the intended concentration of 2.0%. We also confirmed that the dose/area unit was close to the intended $800 \mu\text{g}/\text{cm}^2$, the calculated dose used earlier for 4,4'-MDI patch testing. It is considered that the vital factor for sensitization is the dose/area unit rather than the exposed area (7) and this is the background for choosing the concentration and the dose/area unit for the dermal uptake study. At this concentration 2 volunteers were sensitized and the only possible explanation is that 2.0% MDI concentration is high enough to cause active sensitization.

We are not aware of any studies where individuals have been sensitized by commercially available test preparations and it is possible that commercial test preparations of 4,4'-MDI used for routine patch testing may not contain the stated concentration 2.0%. This is supported by earlier investigations done by our group, which have shown that the concentration of 4,4'-MDI in commercial patch test preparations used all over the world is much lower than the stated 2.0% (8). Based on the results above, measures have already been taken to reduce the recommended patch test concentration of 4,4'-MDI to 0.50% w/w in Europe according to the European Society of Contact Dermatitis (ESCD) recommendations in September 2010 (9). It is also interesting that active sensitization occurred although only around 30% of the 4,4'-MDI in the uptake study could have been absorbed by the skin (Table 2) which indicates that 4,4'-MDI is a strong sensitizer.

PPD was tested because of a suspicion that one of the volunteers was allergic to it and the fact that PPD could have been the primary sensitizer, which could cause cross-reactions to 4,4'-MDI and 4,4'-MDA (Table 4). However, no reactions to PPD were seen. DMDI has been reported to cross-react to 4,4'-MDI (10) but this could not be shown in the present study (Table 4). The deviation from the intended equimolar concentrations of PPD and DMDI is considered too small to affect the patch test results.

Conclusion

Dermal application with preparations of 2.0% 4,4'-MDI, corresponding to a surface concentration of 800 $\mu\text{g}/\text{cm}^2$, caused active sensitization in two volunteers. Thus, there is a risk that patch testing with the same concentration can cause active sensitization. Based on these results the ESCD recommends that the patch test concentration of 4,4'-MDI should be lowered to 0.50% w/w.

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Table 1: Mobile phase gradient in the chromatographic separation.

Time minutes	Acetonitrile with 0.05% formic acid (% v/v)	Deionized water with 0.05% formic acid (% v/v)
0	30	70
7	63	37
10	70	30
12	95	5
18	95	5

Table 2: Analysis of the unabsorbed amount of 4,4'-diphenylmethane diisocyanate in the dermal uptake study.

Applied amount (mg)	Unabsorbed amount (mg)	Maximal absorbed amount (mg)
10.0	7.0	3.0
25.0	17.5	7.5

Table 3: Results from the determination of the 4,4'-diphenylmethane diisocyanate (4,4'-MDI) concentration in the petrolatum preparation (2.0% w/w) used for provocation.

Sample		Found 4,4'-MDI amount (mg)	Concentration of 4,4'-MDI (% w/w)
Number	Size (mg)		
1	500	9.4	1.9
2	500	9.7	1.9
3	100	2.0	2.0
4	100	1.8	1.8
Mean value of concentration			1.9

Table 4: Patch test results to 4,4'-diphenylmethane diisocyanate (4,4'-MDI), 4,4'-diaminodiphenylmethane (MDA), *p*-phenylenediamine (PPD) and dicyclohexylmethane-4,4'-diisocyanate (DMDI) at various concentrations in petrolatum in the 2 volunteers with reading days 3 or 4, 7 and 10.

Substances (% w/w)	Volunteer 1			Volunteer 2		
	D3/4	D7	D10	D3/4	D7	D10
4,4'-MDI						
0.20	-	-	-	-	++	-
0.063	-	-	-	-	++	-
0.020	-	-	-	-	-	-
4,4'-MDA						
0.16	++	++	++	+++	++	+
0.05	++	++	+	++	++	+
0.016	-	-	+	-	+	+
0.005	-	-	-	-	-	-
0.002	-	-	-	-	-	-
PPD						
0.091	-	-	-	-	-	-
DMDI						
0.17	-	-	-	-	-	-

D = reading day

Figure 1: Chemical structures of patch test substances.

<p>4,4'-Diphenylmethane diisocyanate</p> <p>CAS 101-68-8</p> <p>MW 250</p>
<p>4,4'-Diaminodiphenylmethane</p> <p>CAS 101-77-9</p> <p>MW 198</p>
<p>Dicyclohexylmethane-4,4'-diisocyanate</p> <p>CAS 5124-30-1</p> <p>MW 262</p>
<p>p-Phenylenediamine</p> <p>CAS 106-50-3</p> <p>MW 108</p>