The preservative 2-(thiocyanomethylthio) benzothiazole: a potential allergen in leather products

Anne Herman[1], An Goossens[2], Dominique Tennstedt[1], Ola Bergendorff[3], Marléne Isaksson[3], Martin Mowitz[3], and Marie Baeck[1]

[1]Department of Dermatology, Cliniques universitaires Saint-Luc, Brussels, Belgium.

[2]Department of Dermatology, University Hospitals, KU Leuven, Leuven, Belgium.

[3]Lund University, Department of Occupational and Environmental Dermatology, Skane University Hospital, , Malmö, Sweden.

Correspondence:

Dr. Anne Herman

Department of Dermatology

Cliniques universitaires Saint-Luc (UCL)

Avenue Hippocrate 10, B-1200 Brussels, Belgium

Tel: +32 764 7956 / Fax: +32 (0)764 89 58

E-mail: anne.herman@uclouvain.be

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Abstract

Background

Allergic contact dermatitis (ACD) from leather is common, and several responsible allergens, such as tanning agents, glues, mercaptobenzothiazole- derivatives, dyes, but also antimicrobials and antifungals are involved.

Material and methods

Three female patients were referred to the Departments of Dermatology in a Belgian university hospital following skin reactions caused by leather products (shoes, belt, and car seats). They were patch tested with the European Baseline Series and samples of suspected leather products, and additionally with 2-(thiocyanomethylthio)benzothiazole (TCMTB), an antifungal agent previously reported as a contact allergen in footwear. Chromatographic analyses of samples of all the leather materials tested were performed at the Department of Occupational and Environmental Dermatology in Malmö, in Sweden.

Results

The patients reacting to the leather samples were shown to be sensitized to TCMTB, the presence of which could be confirmed by chemical analyses of samples obtained from three of them.

Conclusion

Patch tests with TCMTB should be considered in patients with contact dermatitis from leather items.

1. Introduction

Allergic contact dermatitis (ACD) from leather materials is frequent, caused by several responsible allergens, such as chromium (tanning), cobalt salts (dyeing), *p-tert*-butylphenol-formaldehyde resin (glues), mercaptobenzothiazole- derivatives, and dimethyl fumarate, octylisothiazolinone, and 2-(thiocyanomethylthio) benzothiazole (TCMTB) (antimicrobials and antifungals) (1), the latter compound involved in the three cases described here. TCMTB (CAS no. 21564-17-0) is also known as Tolcide 2230 (Santa Cruz Biotechnology, Dallas,Texas).

2. Patients and methods

2.1 Patients

Case 1: A 15-year-old girl, with no history of personal or familial atopy, presented with an eczematous dermatitis located at three different body sites, namely, on the back of the feet, two days after wearing a new pair of shoes, on the abdomen underneath her leather belt, and on the back of both thighs, the latter which had started shortly following the first contact with the leather seat of her father's new car (Fig. 1).

Case 2: A 34-year-old woman, suffering from atopic dermatitis, presented with an erythematous and squamous eruption limited to the upper and back sides of both thighs (Fig. 2), which had appeared following repeated contact with the leather seats of her new car.

Case 3: A 51-year-old woman, schoolteacher, without any history of atopic dermatitis, had suffered for more than three years from dermatitis on the back of her feet, sometimes spreading towards the legs. This only appeared a few days following contact with some leather shoes, but never with shoes made from canvas, other fabric or rubber.

2.2 Patch testing

The three patients were evaluated at the Department of Dermatology at the Cliniques universitaires Saint-Luc in Brussels, Belgium, and patch tested using the European Baseline Series (Chemotechnique Diagnostics, Vellinge, Sweden and/or Allergeaze, SmartPractice, Phoenix, Arizona). Additional series were also tested, that is, a rubber, a plastic and a glues series (patients 1-3), and a dye and (meth)acrylate series (patient 2), all allergens from Chemotechnique Diagnostics and/or Allergeaze. Own materials (tested "as is", humidified with physiologic saline) and fixed on acrylic tape were tested as well: case 1 with a sample of the leather belt, the leather interior part of a shoe, and a sample of the leather car seat provided by the car company, the latter also tested in case 2; case 3 with pieces of different parts of the interior leather of shoes (sole and counter).

The preservative TCMTB (obtained in 90% purity from Toronto Research Chemicals, North York, Ontario, Canada), prepared at 0.1% pet. by the Department of Occupational and Environmental Dermatology in Malmö, was tested at a second occasion, after its identification by chemical analyses. From January 2018 to January 2019, 12 other dermatitis patients were patch tested with the same test substance

TCMTB 0.1% pet., who may be regarded as control subjects. Patch tests were applied on the upper back and occluded for 2 days with IQ Ultra test chambers from Chemotechnique Diagnostics; they were fixed with Fixomull stretch (BSN Medical, Hamburg, Germany). Readings were performed on day (D) 2 and D4, based on the ESCD criteria (2).

2.3 Chemical investigations

Chemical investigations of the patients' leather items were carried out at the Department of Occupational and Environmental Dermatology in Malmö. Acetone extracts of the belt from case 1, the piece of car seat leather provided by the car company from case 2, and a shoe from case 3 were analysed by gas chromatography-mass spectrometry (GC-MS)(3). For these analyses ~10 g of each material was extracted in acetone in an ultrasonic bath for 5 min. The extracts were then evaporated to a volume of 0.5 mL using a rotary evaporator before being analysed by GC-MS. The National Institute of Standards and Technology (Gaithersburg, Maryland) library of mass spectra was used for identification of substances.

Quantitative analyses of TCMTB were performed with a high-performance liquid chromatography (HPLC) method suitable for identifying allergens in rubber items (4) as MBT and other chemically related benzothiazole derivatives are easily analysed in this system.

3. Results

Patient characteristics and patch tests results are shown in **Table 1**. All three patients reacted positively to TCMTB 0.1% pet., one patient extreme positive (+++) (Fig. 3), one patient strong positive (++), and another one with a doubtful reaction (?+). All three reacted positively to the individual leather samples tested (seat car, pieces of belt and/or shoes). Among the 12 control subjects tested with TCMTB 0.1% pet., none reacted positively.

According to GC-MS analyses TCMTB was identified in the car seat leather, as well as in the leather belt, the latter also containing octylisothiazolinone. Possible traces of TCMTB were observed in the extract of the shoe from case 3 as well. HPLC analysis did not reveal any other known contact allergens beyond TCMTB. The retention time of TCMTB was 12 minutes and it displayed a UV-spectrum characteristic for sulfur-bound mercaptobenzothiazoles. By comparison of the areas in the chromatograms we determined the concentration of TCMTB to be 0,017 mg/g in the belt from case 1, 0.250 mg/g in the leather sample of the car seat from case 2, and 0.005 mg/g in the shoe from case 3, respectively. In no other samples could TCMTB be detected under the used conditions with an estimated detection limit of 0.001 mg/g. Results of the HPLC analyses are summarised in **Table 2**.

4. Discussion

Three patients suffering from allergic contact dermatitis from leather goods were reported. Besides many other allergens responsible for ACD from leather (1, 5), also anti-

microbials/antifungals are potential culprits. In the past, dimethyl fumarate (DMF) has been responsible of an epidemic of ACD due to its presence in small sachets, used during storage and transport of leather goods, such as shoes (6), and sofas (7) imported from China, in particular. More recently, Vandevenne et al (8) described methylisothiazolinone, present in leather care products for sofas, as the responsible allergen in a case of severe ACD mainly located at the posterior sides of the thighs. Moreover, also other isothiazolinone derivatives, such as octylisothiazolinone, have been implicated as responsible allergens in leather goods, i.e. sofas, belts, and shoes (9-11). The GC-MS analysis of the belt from case 1 showed a likely presence of octylisothiazolinone, but the patient did not test positive to it. Testing with leather items remains sometimes positive, while no sensitizing culprits can be identified, neither when testing with the European Baseline Series, nor with additional series. Hence, chemical analyses of the leather products may be useful to identify the responsible allergens, as was TCMTB in the cases described here, since it was detected in three of the leather articles that had caused ACD.

TCMTB is a fungicidal biocide used during the leather-tanning process, since it inactivates the growth of fungi encountered in tanneries, i.e. *Aspergillus flavus*, *Aspergillus niger*, *Peacilocyces variotii*, *Trichoderma viridi*, and *Penicillium* spp. It has been used in leather since the emergence of legislation restrictions of phenol contents (including pentachlorophenol) (12). In a Belgian study carried out in the Department of Dermatology at the KU Leuven university hospital in Leuven between 1990 and 2001, TCMTB was considered as the second most frequent allergen in leather footwear (1): during this period, 781 patients suffering from foot dermatitis had been tested with TCMBT, of whom 37 had a

positive reaction to it, and 27 of these cross-reacting to mercapto-mix and/or mercaptobenzothiazole. However, because of lack of this test chemical afterwards, no data on its frequency are available during the period 2002-2015. Since 2016, 12 patients were tested with TCMBT obtained from the Malmö department, and 2 positive cases were observed as well.

In 2007, Domingo et al (13) reported a case of ACD on the hands and wrists of an employee manipulating wood packaging that had previously been treated with a varnish containing a 30% concentration of TCMTB as the active wood preservative, i.e. Mirecide- TC/45- Glicolo (Lamirsao, Terrassa, Spain). Patch test performed with serial dilutions 1/100, 1/200, 1/400 of TCMTB in pet. were positive; the authors recommended a patch-test concentration of 1% in pet. (13). Because MBT is a metabolite produced by degradation of TCMTB, cross-reactions with MBT may be observed (1). Our analyses could not detect MBT in the leather samples though. The dermatitis in the three patients sensitized to TCMTB eventually resolved after cessation of contact with the causal leather objects.

In conclusion, we present three patients suffering from allergic contact dermatitis from leather goods, who had positive patch test reactions to the antifungal agent TCMTB. Chemical investigations revealed the presence of TCMTB in the leather samples obtained from them. Patch testing with TCMBT should thus be considered in patients suspected of leather dermatitis, particularly, as the chemically related MBT or mercapto-mix are not always cross-reacting. Therefore, its inclusion in the shoe series is suggested.

References

1. Nardelli A, Taveirne M, Drieghe J, et al. The relation between the localization of foot dermatitis and the causative allergens in shoes: a 13-year retrospective study. Contact Dermatitis. 2005;53:201-6.
2. Johansen JD, Aalto-Korte K, Agner T, et al. European Society of Contact Dermatitis guideline for diagnostic patch testing - recommendations on best practice. Contact Dermatitis. 2015;73:195-221.
3. Herman A, Aerts O, Baeck M, et al. Allergic contact dermatitis caused by isobornyl acrylate in Freestyle(R) Libre, a newly introduced glucose sensor. Contact Dermatitis. 2017;77:367-73.
4. Bergendorff O, Persson C, Hansson C. High-performance liquid chromatography analysis of rubber allergens in protective gloves used in health care. Contact Dermatitis. 2006;55:210-5.
5. Herro E, Jacob SE. p-tert-Butylphenol formaldehyde resin and its impact on children. Dermatitis. 2012;23:86-8.
6. Gimenez-Arnau A, Silvestre JF, Mercader P, et al. Shoe contact dermatitis from dimethyl fumarate: clinical manifestations, patch test results, chemical analysis, and source of exposure. Contact Dermatitis. 2009;61:249-60.
7. Rantanen T. The cause of the Chinese sofa/chair dermatitis epidemic is likely to be contact allergy to dimethylfumarate, a novel potent contact sensitizer. Br J Dermatol. 2008;159:218-21.
8. Vandevenne A, Vanden Broecke K, Goossens A. Sofa dermatitis caused by methylisothiazolinone in a leather-care product. Contact Dermatitis. 2014;71:111-3.
9. Raison-Peyron N, Amsler E, Pecquet C, et al. Severe allergic contact dermatitis caused by octylisothiazolinone in a leather sofa: two new cases. Contact Dermatitis. 2017;77:176-8.
10. Aerts O, Meert H, Romaen E, et al. Octylisothiazolinone, an additional cause of allergic contact dermatitis caused by leather: case series and potential implications for the study of cross-reactivity with methylisothiazolinone. Contact Dermatitis. 2016;75:276-84.
11. Alipour Tehrany Y, Quenan S, Bugey A, Piletta P. Allergic contact dermatitis caused by octylisothiazolinone in a leather sofa. Contact Dermatitis. 2018;79:188-9.
12. Muthusubramanian L MRB, Sundara Rao V.S. 2-(thiocyanomethylthio)-benzothiazole fungicide on leather: A review. Journal- Society of Leather Technologists and Chemists.1998;82 :22-23.
13. Domingo H, Carrascosa JM, Boada A, Leal L, Ferrandiz C. Allergic contact dermatitis to 2(thiocyano-methylthio) benzothiazole present in a packaging wood. Contact Dermatitis. 2007;57:121-2.

Table 1: Demographic data, tests series and allergens tested, and patch-test results for the 3 patients examined

Patients	Age (Years)	Sex	2-(thiocyanomethylthio) benzothiazole 0.1% D2/D4	Pieces of leather products D2/D4	Baseline series D2/D4	Additional series D2/D4
1	15	F	++/+++	Belt ++/++ Shoes ++/+	-	P&G: - Rubber: -
2	33	F	+/++	Car seat -/+	-	P&G: - Rubber: - MA: - Dye: -
3	51	F	-/ ?+	4 different shoes +/++	PTBP-FR +/+	P&G: - Rubber: -

F, Female; MA series, (meth)acrylate series; P&G series, plastic & glues series; PTBFR: *p-tert*-butylphenol-formaldehyde resin; −, negative; ?+, doubtful; + to +++, positive patch test reactions.

Table 2: Results of chromatographic analysis of the leather products involved

Patients	Leather products analysed by CGMS	Presence of 2-(thiocyanomethylthio) benzothiazole
1	Belt	0,017 mg/g
2	Car seat	0.250 mg/g
3	Shoes	0.005 mg/g

Fig 1. Allergic contact dermatitis in case 1 caused by shoes on the back of the feet (A), and caused by a leather car seat on the thighs (B.

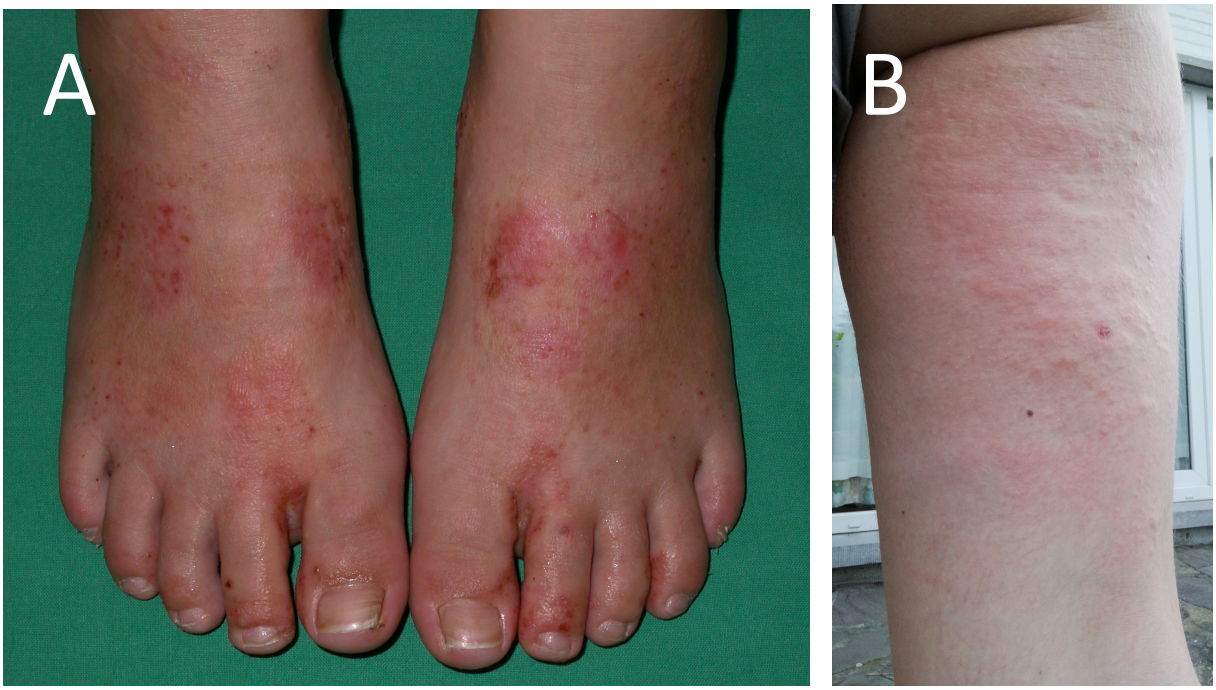

Fig 2. Allergic contact dermatitis caused by leather car seats on the posterior sides of the thighs in case 2.

Fig 3. Positive patch-test reaction to thiocyanomethythio benzothiazole 0,1% (+++) in case1 (Day 4-reading)