Autoxidized limonene as a contact hapten, a multi-center prospective study of contact sensitization to hydroperoxides of limonene in Spain

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Introduction

Limonene is a common fragrance terpene present in a wide variety of products. Limonene is known to be a prehapten, and its primary oxidation product formed after air exposure, the hydroperoxides, has been recognized as a contact allergen of growing interest. Significant rates of contact allergy to hydroperoxides of limonene (Lim-OOHs) have been reported in recent studies. This fragrance, however, are not routinely patch tested either in Spain or in other Eropean countries. Moreover, the best patch test preparation and the optimum concentration for testing remain to be established, considering the high number of irritant/doubtful reactions recorded in previous studies.

Objectives

To investigate the prevalence of contact allergy to limonene hydroperoxides in Spain, and to define the optimal concentration for detection of contact allergy to this fragrance terpene in consecutive patients.

Materials and methods

During the period from May 2015 to February 2016, 3639 consecutive patients undergoing patch testing because of suspected allergic contact dermatitis at 22 Departments of Dermatology belonging to the GEIDAC (Spanish Group of Research for Contact Dermatitis and Cutaneous Allergy), were screened with three different concentrations of Lim-OOHs (0.1, 0.2 and 0.3% pet.), simultaneously, in addition to regular patch testing. Standard allergens used for patch testing were provided from Chemotechnique Diagnostics (Vellinge, Sweden). Patch tests were applied on the patients' upper back using IQ Ultimate chambers (Chemotechnique) and occluded for 48 hours. Visual readings were made twice, on days 2 and 4-5, and were scored by using the European Society of Contact Dermatitis (ESCD) patch test guidelines

Results

The number of patients with positive, doubtful and irritant patch test reactions to Lim-OOHs at each tested concentration is shown in Table 1. For the higher concentration tested (Lim-OOHs 0.3% pet.), 187 (5.1%) patients showed positive patch test reactions. Sixty-three of these 187 (33.7%) patients would not have been diagnosed using only the immediate lower patch test concentration. A wide range in the frequency of positive reactions to Lim-OOHs 0.3% pet. (range: 0- 24.8%) between the 22 centres was found. The overall frequency of concomitant reactions to other fragrance markers in the baseline series and/or colophonium in patients with positive reactions to Lim-OOHs 0.3% pet. was 31.0% (58/187) (Table 2).

The mean age of the patients with positive reactions to Lim-OOHs 0.3% pet. (n=187) was 46 years (range 2-89). One hundred and thirty-one (70.1%) patients were female and 34 (18.2%) showed occupational contact allergy. The most common sites affected were the hands (40.6%) and the face (27.3%). Regarding relevance, a present exposure to one or several products containing limonene, used on the dermatitis area, was registered in 86 of the 187 (46.0%) patients with positive reactions to Lim-OOHs 0.3% pet., while a past exposure was registered in 5 (2.7%) patients. The most common products containing limonene of clinical relevance were cosmetics and fine fragrances (n=57), soaps (n=21), hair products (n=18), moisturizers (n=17), and detergents (n=12).

TABLE 1. Number and percentage of patients with positive, doubtful and irritant patch test reactions to hydroperoxides of limonene (Lim-OOHs) at each respective concentration. The distribution of the strength of positive reactions is also shown.

Fragance terpene	Patch test concentration	No. of positive patch test reactions (%)			No. of doubtful patch test reactions (% *)	No. of irritant patch test reactions (% *)
		Total (% *)	+	++/+++		
Lim-OOHs	0.1% pet.	51 (1.4)	34/51 (66.7)	17/51 (33.3)	25 (0.7)	11 (0.3)
	0.2% pet.	124 (3.4)	90/124 (72.6)	34/124 (27.4)	21 (0.6)	30 (0.8)
	0.3% pet.	187 (5.1)	112/187 (59.9)	75/187 (40.1)	14 (0.4)	55 (1.5)

* Regarding the total of patients patch tested (n=3639)

TABLE 2. Number of patients showing positive patch test reactions to hydroperoxides of limonene (Lim-OOHs) at each respective concentration who also reacted to other fragrance markers (fragrance mix I [FM I], fragrance mix II [FM II], hydroxyisohexyl 3-cyclohexene carboxaldehyde [HICC] and *Myroxylon pereirae*) and/or colophonium in the baseline patch test series tested concomitantly.

Fragrance terpene	Patch test concentration	No. patients with positive reactions	Concomitant positive patch test reactions					[3]1 fragrance marker and/or colophonium No. (%)
			FM I No. (%)	FM II No. (%)	HICC No. (%)	*Myroxylon pereirae* No. (%)	Colophonium No. (%)	
Lim-OOHs	0.1% pet.	51	6 (11.8)	4 (7.8)	2 (3.9)	2 (3.9)	3 (5.9)	13 (25.5)
	0.2% pet.	124	14 (11.3)	12 (9.7)	6 (4.8)	14 (11.3)	4 (3.2)	30 (24.2)
	0.3% pet.	187	23 (12.3)	23 (12.3)	10 (5.3)	18 (9.6)	9 (4.8)	58 (31.0)

Discussion

Limonene is one of the most common fragrance ingredients. The present study shows a significant rate of contact allergy to limonene in the Spanish population: 5.1% of patients tested had a positive patch test reaction to Lim-OOHs 0.3% pet. Similar rates of contact allergy have been found in a study from the U.K. (5.0%) and in a recent multicentre European study (5.2%). These rates place limonene hydroperoxides among the most common contact allergens throughout Europe. A large difference in the prevalence of contact allergy to limonene hydroperoxides between the participating test centres was observed, and is most likely attributable to regional variations in exposure (i.e. different preferences regarding fragrance notes) and/or differences of tested populations.

Testing to the terpene hydroperoxides, which have been demonstrated to be the main hapten in the oxidation mixture, is a good tool to diagnose contact allergy to limonene. In the present study, the higher patch test concentration used for Lim-OOHs (0.3% pet.) allowed diagnosing the largest number of cases of contact allergy. In addition, with higher patch test concentrations, only a slightly increase of irritant reactions were observed. Moreover, a large number of doubtful reactions were interpreted as positive reactions at higher concentrations, thus indicating that a certain number of the doubtful reactions are very weak positive patch test responses. Based on these results, and according to previous recommendations, it seems that the patch test preparation of Lim-OOHs 0.3% pet. is a useful tool for screening of contact sensitization.

Contact allergy to limonene was assessed as being likely to be relevant in almost 50% of the patients. Fine fragrances and cosmetics were the most frequently products containing limonene and/or linalool judged to be relevant for the patients' dermatitis. Other products, such as soaps, shampoos, deodorants and moisturizers were also frequently listed as sources of these terpenes.

Conclusions

Lim-OOHs can actually be considered as a common cause of contact allergy, and therefore, their inclusion in an extended baseline patch test series seems appropriate. The patch test preparation of Lim-OOHs 0.3% pet. would be useful method for screening of contact sensitization. Since individuals could be potentially in contact with these compounds from many sources in a day, the identification of allergic patients by using specific patch tests would be necessary.

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