

Clinical and Experimental Studies on Oxidized Fragrance Terpenes as Contact Allergens

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademien vid Göteborgs
Universitet kommer att offentligen försvaras
Torsdagen den 19 mars 2009 kl 13.00
Hörsal Arvid Carlsson, Academicum, Medicinaregatan 3, Göteborg

av
Johanna Bråred Christensson
Leg. läkare

Opponent: Thomas Rustemeyer, MD, PhD
Department of Dermatology–Allergology, VU University Medical Centre,
Amsterdam, The Netherlands

This thesis is based on the following papers:

- I. Bråred Christensson J, Forsström P, Wennberg A-M, Karlberg A-T, Matura M. Air oxidation increases skin irritation from fragrance terpenes. *Contact Dermatitis*: 2009; 60: 32–40.
- II. Bråred Christensson J, Matura M, Gruvberger B, Bruze M, Karlberg A-T. Oxidized linalool- a significant contact sensitizer. *Manuscript*.
- III. Bråred Christensson J, Matura M, Bäcktorp C, Börje A, Nilsson JLG, Karlberg A-T. Hydroperoxides form specific antigens in contact allergy. *Contact Dermatitis*: 2006; 55(4): 230-7.
- IV. Bråred Christensson J, Johansson S, Hagvall L, Jonsson C, Börje A, Karlberg A-T. Limonene hydroperoxide analogues differ in allergenic activity. *Contact Dermatitis*: 2008; 59(6): 344-52.



UNIVERSITY OF GOTHENBURG

Clinical and Experimental Studies on Oxidized Fragrance Terpenes as Contact Allergens

Johanna Bråred Christensson,

Department of Dermatology and Venereology, Institute for Clinical Sciences at Sahlgrenska Academy, University of Gothenburg, Göteborg, Sweden

Abstract :

The work presented in this thesis aims to improve the possibilities for diagnosing contact allergy to oxidized limonene and linalool and to, in patients, study contact allergy from structurally closely related hydroperoxides with regard to their specificity, potency and cross-reactivity. The fragrance terpenes limonene (from citrus oil) and linalool (from lavender oil) are widely used in household and industrial products. For both limonene and linalool, exposure to air at normal handling results in autoxidation and thus formation of allergenic oxidation products among which the primary oxidation products, the hydroperoxides, are the most potent allergens. Concomitant positive patch test reactions to oxidation mixtures where the main allergens are hydroperoxides are recorded in specific patch test studies. However today, oxidized terpenes are not included in routine patch testing for contact allergy.

An irritation study was performed for non-oxidized and oxidized limonene and linalool, showing that air oxidation increased irritation of both limonene and linalool and that oxidized limonene was more irritating than oxidized linalool at similar concentrations. Based on these results, a dose-response study was conducted in 3418 consecutive dermatitis patients investigating four patch test concentrations of oxidized linalool 2.0-11.0% in petrolatum (pet.). 5-7% of the patients showed positive patch test reactions to oxidized linalool. Oxidized linalool at 6.0% pet. concentration is suggested for future screening.

Concomitant positive patch test reactions and cross-reactivity of hydroperoxides were investigated in clinical studies and experimental procedures. No evidence for general cross-reactivity or formation of non-specific antigens upon administration of the investigated hydroperoxides was found. Cross-reactivity was only seen between hydroperoxides with great similarity in structure. Furthermore, three limonene hydroperoxide analogues were investigated as to allergenic potential in a modified murine local lymph node assay (LLNA) calculating on lymph nodes from individual mice. A statistically significant difference between sensitizing capacities of the investigated limonene hydroperoxides was established. Two of the limonene hydroperoxide analogues were assessed in individuals allergic to oxidized limonene. The analogue with the highest sensitizing capacity in the modified LLNA (limonene-1-hydroperoxide) gave more reactions in the tested individuals.

In conclusion, this thesis shows that it is important to study the contact allergenic potential of chemicals to which we are exposed. Seemingly harmless products may cause or worsen ACD. Such risks can only be detected by testing of relevant allergenic compounds in appropriate concentrations. The frequency of positive patch test reactions to autoxidized linalool observed among the dermatitis patients, places this material among the most common contact allergens tested today. In addition, a new main allergen in the oxidation mixture of limonene, limonene-1-hydroperoxide, is proposed. The thesis confirms the effect of air oxidation on the allergenicity of common fragrance terpenes. Furthermore, the studies show the great specificity of the contact allergens *in vivo*. Finally, the impact on the allergenic potency by the primary oxidations products, the hydroperoxides is re-established in humans.

Key words: autoxidation; contact allergy; limonene; linalool; colophonium; limonene-1-hydroperoxide; limonene-2-hydroperoxide; 15-hydroperoxyabietic acid; 15-HPA; terpenes; local lymph node assay; LLNA; Freund's complete adjuvant test; FCAT; patch testing; laser Doppler imaging; skin irritation; visual reading.

ISBN: 978-91-628-7716-3