

Free radicals generation from skin allergens in a reconstructed human epidermis 3D model

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Introduction: Skin sensitization processes involving radical intermediates are suspected for several allergens, especially allylic hydroperoxides derived from autoxidation of natural terpenes widely used in the composition of fragrances. However, radicals actually formed in the epidermis from these skin allergens and the mechanisms of action are far from being unravelled. We have developed a novel EPR spin-trapping methodology with the potential to investigate *in situ* the formation and behaviour of radicals derived from these compounds in a reconstructed human epidermis 3D model (RHE). RHE consist of normal multi-layered keratinocytes cultures, being keratinocytes the major cell type in the epidermis and playing a key role in skin inflammatory reactions. To our knowledge, EPR spectroscopy had never been used before for the study of radicals derived from skin allergenic substances in RHE.

Materials and Methods: Target compounds were hydroperoxides (ROOHs) derived from autoxidation of linalool and limonene. EpiskinTM epidermis model were initially incubated with the spin-trap DEPMPO able to trap radicals too short-lived or of too low concentration, forming this way more persistent radicals detectable by EPR and with a signature characteristic of the trapped transient reactive species. ROOHs were then topically applied to get closer to real life contact-scenarios with consumer goods. Treated RHE were placed in an EPR special tissue cell and resulting EPR spectra recorded *in situ* right afterwards. Spectra were analysed by means of computer simulation using labmade scripts based on Easyspin toolbox under Matlab (Mathworks) environment.

Results: Carbon centred radicals were mainly formed with slight hydroxyl radical suggesting a key role of carbon radicals in the antigen formation process. Control experiments without ROOHs produced no signal, suggesting that DEPMPO trapped radicals most probably originated from the hydroperoxide rather than through biomolecules present in RHE. No radical initiation was used to mimic real life skin allergy situation. One can hypothesize that reaction of organic peroxides in the skin in the presence of one-electron donor agents (i.e. amino and thiol groups present in amino acids, metal complexes, enzymes) may start the electron transfer processes and further skin proteins haptentation.

Conclusion: EPR spin-trapping allowed for the first time to monitor the formation of carbon radicals issued from ROOHs in a 3D epidermis model. This is an important achievement opening new perspectives and mechanistic insights for the molecular understanding of sensitization processes to precursors of radical intermediates. This methodology, able to follow radical production and stress induction, gives *ex vivo* indication of induced free radical processes occurring in the skin and will be valuable to study other dermato-oxidation events.