

Oxidation products and the skin – the effect of hydroperoxides

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Oxygen is pivotal for life on earth, and chemical reactions with oxygen are constantly ongoing. One of many important reactions is the autoxidation of organic compounds, which results in increased chemical reactivity of the products. As diagnostic patch testing with air-exposed fragrance terpenes has shown frequent allergic reactions in dermatitis patients all over the world, the effect of autoxidation with regard to skin contact has become a topic for the community of dermatologists, industry, and regulatory authorities. Fragrance compounds in cosmetics and everyday products are the most common sources of skin contact with terpenes in the general population. However, the significance of this exposure as the cause of skin allergy has been questioned, and other explanations have been suggested for the positive patch test reactions, for example a state of reactivity to multiple degradation products formed under oxidative conditions.

To understand the discussion, it is important to understand the oxidation process and the formation of immunogens from the primary oxidation products, that is, hydroperoxides. Chemically, autoxidation is a reaction that can be defined as the insertion of oxygen into a carbon–hydrogen bond (C–H), forming hydroperoxides (R-OOH). The insertion occurs via a free radical chain reaction, and the outcome depends on the stability of the radicals formed. Thus, it is possible to postulate from the structure of a compound where the most stable hydroperoxides should be formed. Hydroperoxides that are stable enough to accumulate have been detected after air exposure of several fragrance terpenes, and they have been shown to be highly sensitizing (1, 2). On the basis of this, fragrance terpenes that are able to autoxidize and form sensitizing compounds should be considered as prehaptenes in structure–allergy relationship (SAR) analyses. Such analyses are included in the review of fragrance allergens by the Scientific Committee on Consumer Safety of the European Commission when listing fragrance allergens of which the consumer needs to be made aware (3).

The hydroperoxides are considered to be the main sensitizers in air-oxidized limonene and linalool. However, it is important to remember that the hydroperoxides are the primary oxidation products from which secondary oxidation products and, eventually, polymeric materials are formed. Depending on the basic structure of

the parent compound, the secondary oxidation products can also be important sensitizers, for example the conjugated aldehydes geranial and neral formed from geraniol (4). Most haptens hitherto studied form immunogens via nucleophilic–electrophilic reactions with proteins in the skin, but for hydroperoxides the formation of immunogens involves radical reactions. It has therefore been hypothesized that hydroperoxides might cause less precise antigen formation, as they are formed from highly reactive radicals, but, as discussed below, no evidence of this has been found.

Specificity of the skin reactions seen to hydroperoxides

Already in the 1950s, it was concluded that hydroperoxides were the dominating haptens in a natural mixture, when Δ^3 -carene hydroperoxide was identified as the most probable major hapten in Scandinavian turpentine (5). Furthermore, in the 1980s, hydroperoxides of abietic acid and dehydroabietic acid were shown to be the major haptens in colophonium (rosin) (6). Since the 1990s, the skin-sensitizing activity of hydroperoxides from various fragrance monoterpenes has been investigated both experimentally and clinically, and it has been repeatedly established that the hydroperoxides are allergenic (in contrast to hydrogen peroxide, which is non-sensitizing) (2, 7). It has also been shown that the hydroperoxides act as specific haptens, and mechanistic studies have shown the formation of specific adducts (8). Specific reactions to different hydroperoxide haptens have been recorded in experimental studies on possible cross-reactivity in guinea-pigs and mice, and in clinical studies (9). For cross-reactivity to be identified, great structural similarity was needed; that is, the same pattern was found as could be expected from SAR analyses of traditional haptens. Even structurally closely related haptens, such as limonene-1-hydroperoxide and limonene-2-hydroperoxide, have been shown to give specific reactions experimentally in mice and in clinical patch test studies (10, 11). This said, the importance of danger signals from radicals and the involvement of reactive oxygen species (ROS) in skin sensitization from various haptens should be further elucidated. It has been shown that haptens that do not form immunogens via a radical mechanism can induce skin inflammation via

ROS production (12). The importance of this with regard to hydroperoxides is an interesting topic to investigate, but it does not provide an alternative explanation to specific antigen formation.

Patch test studies have shown the overall frequencies of contact allergy to different oxidized fragrance compounds to range between 0.1% and 8% of the tested patients (2, 7, 13–16). These frequencies are largely comparable to the frequencies of reactions observed for fragrance mix I and fragrance mix II in the established baseline series for the diagnosis of contact allergy (17). Regarding the specificity of the reactions, extensive clinical multicentre studies using patch test preparations with oxidized limonene containing limonene hydroperoxides and oxidized linalool containing linalool hydroperoxides in standardized concentrations have shown that only 25% of consecutive dermatitis patients with positive reactions to either patch test preparation also had simultaneous reactions to the other preparation. In other words, the majority of patients (~75%) reacted to only one of the hydroperoxides, thus further supporting the specificity of the reactions (15, 16, 18).

A new study from Bennike et al. presented in this issue adds support to the specificity in antigen formation from terpene hydroperoxides. The study investigated whether patients sensitized to *p*-phenylenediamine (PPD) are at significantly increased risk of having concomitant positive patch test reactions to either limonene hydroperoxides or linalool hydroperoxides. PPD is another common chemical that can cause allergic contact dermatitis because of oxidation and radical reactions. Theoretically, PPD-sensitized individuals could therefore be at increased risk of having patch test reactions to terpene hydroperoxides, owing to concomitant unspecific reactions. However, no concomitant sensitization to PPD and the terpene hydroperoxides beyond that expected by chance could be identified (19).

Clinical relevance and exposure

The overall clinical relevance of positive patch test reactions to either of the standardized patch test materials for oxidized limonene or oxidized linalool (containing limonene hydroperoxides or linalool hydroperoxides in standardized concentrations) has ranged from 36% to 49% in recent extensive multicentre studies; however, there were large variations between the different test centres (15, 16, 18). There are various ways in which dermatologists can study the clinical relevance of contact allergy detected at patch testing. The patient's history and concomitant reactions to other markers of fragrance allergy are important to assess. All clinical studies have shown

that ~40% of the patients reacting to oxidized fragrance terpenes also react to fragrance markers in the baseline series (2, 7). Also, the study by Bennike et al. showed a clear association between reactions to the terpene hydroperoxides and reactions to the fragrance markers in the baseline series (19). It should be noted that significantly more patients reacting only to oxidized limonene with no other fragrance allergies reported problems with fragranced products than those without a patch test reaction to any fragrance patch test material in the multicentre study performed by Bråred Christensson et al. (18).

In recent years, there has been an increasing focus on the precise exposure assessment and products containing the culprit chemicals, in order to prove clinical relevance, not least from a producer perspective. It can be difficult to identify a certain product when the culprit chemical is ubiquitous and present in many products at low concentrations, a scenario that is especially true for fragrances. The concentrations of fragrance compounds are generally low in scented products, except for fine fragrances, in which up to 15–30% of the total volume can consist of fragrance materials. In liquid detergents and toilet soaps, 0.1–2% of the total volume may consist of fragrance material (3), but there is great diversity among the scented products. Also, ecologically based or home-made products derived from natural materials, for which controls are limited, have become popular. A major question is whether oxidation products, and especially the sensitizing hydroperoxides formed from fragrance compounds, are present in concentrations high enough to cause sensitization from scented products. Exposure to hydroperoxides is challenging to prove analytically, and this has forced the development of new analytical methods. A persistent problem is posed by the matrices in the complex cosmetics and everyday products, making detection and quantification of hydroperoxides even more challenging. Hitherto, it has been observed that hydroperoxides are easily formed from fragrance compounds of natural origin in essential oils; for example, limonene-2-hydroperoxide was detected in natural sweet orange oil, and linalyl acetate hydroperoxides were already identified in petit-grain oil at the time of delivery from the producer (20).

Another important consideration concerns different formulations, which may influence the sensitizing potency of a compound. An enhanced sensitizing potency of mixtures of fragrance chemicals as compared with that of the corresponding single-fragrance chemicals must be addressed, as must an enhancement of elicitation of the allergenic effect when the chemical is combined with irritants, as shown for sodium lauryl sulfate (21). Care must be taken that new topical formulations will not

increase the formation and uptake of sensitizing oxidation products. Ethosomes, which are used to enhance the uptake of active components in topical formulations, have also been shown to enhance the uptake of fragrance chemicals (22).

Although analyses of hydroperoxides in complex products remain a challenge, some evidence has been provided that the hydroperoxides are present in the formulations. For oxidized linalool, a repeated open application test (ROAT) has shown allergic reactions to a concentration not far from the concentrations detected in fine fragrances recalled from the consumers in an investigation by the fragrance industry (23, 24). A recent report described a case of a small girl with contact allergy to oxidized linalool who had persistent eyelid dermatitis, which healed only when exposure to a heavily scented shampoo ceased; the shampoo was analytically confirmed to contain linalool hydroperoxides (0.2 µg/g) (25). If further studies confirm that such low concentrations can induce dermatitis with repeated exposures, then keeping fragrances and scented products at safe levels regarding hydroperoxides is indeed a great problem.

It can be expected that repeated exposure of the general population to low concentrations of terpene hydroperoxides in fragranced products that come into close contact with the skin, many times a day, and on many days throughout life, may well cause both skin sensitization and elicitation of dermatitis. The importance of this type of exposure in causing sensitization in the population has been repeatedly observed also for nickel and preservatives. Further research using new analytical methods and exposure tests such as the ROAT is needed to investigate the exposure, with the aim of reducing the risk of sensitization.

State of the art – further outlook

There is an urgent need to identify sources of exposure to the population from skin-sensitizing oxidation products (hydroperoxides) from terpenes. New analytical methods for the identification and quantification of hydroperoxides from fragrance compounds in consumer products are therefore being developed within the ongoing joint programme International Dialogue for the Evaluation of Allergens between industry, academia, and dermatologists (26). It is important to consider not only limonene

and linalool hydroperoxides, but also other sensitizing oxidation products and hydroperoxides from various fragrance compounds in scented products (7).

It is established that many compounds, for example geraniol and cinnamyl alcohol, can act both as prehap- tens and prohaptens (be activated both outside and in the skin), but hydroperoxides have so far not been discussed in the studies on bioactivation in skin. Instead, other important haptens, mainly aldehydes and epoxides, have been reported. It is well known that lipids (most often the unsaturated fatty acid moieties oleic, linoleic, and linolenic) are easily oxidized in tissue, forming hydroperoxides, so specific investigations on hydroperoxide formation from terpenes in skin could be enlightening. The fact that non-oxidized compounds cause very few reactions when patch tested may be a result of the low concentrations of haptens produced, which might be too low to be detected on patch testing with the pure compounds. Increased knowledge of the metabolic mechanisms in skin, better possibilities of using skin models and new, specific, sensitive analytical methods have increased the possibilities of studying this topic.

Another interesting question is whether autooxidation can take place on the skin. Scented stay-on products are commonly applied on the skin at least once a day by a great proportion of the normal population in the Western world. Investigations of what happens with easily oxidized compounds on the skin after application should be performed with specific, sensitive techniques. Moreover, a fragrance compound must be volatile to act as a scent. Therefore, airborne exposure from scented products or in natural settings is also of interest. For example, the phenomenon of atmospheric oxidation of limonene has been of interest to atmospheric chemists, because of the emission of limonene from pine forests.

In conclusion, skin sensitization from terpene hydroperoxides is a topic that needs further research by experimental and clinical researchers, as well as increased efforts to elucidate relevant exposures by cooperation between clinicians and industry.

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