Carvone Contact Allergy in Southern Sweden: A 21-year Retrospective Study

Liv KROONA1, Marlène ISAKSSON2, Camilla AHLGREN1, Jakob DAHLIN2, Magnus BRUZE2 and Gunnar WARFVINGE1

1Department of Oral Pathology, Malmö University, 2Department of Occupational and Environmental Dermatology, Lund University, Skåne University Hospital, and 3Department of Prosthodontics, Malmö University, Malmö, Sweden

Carvone (l-carvone), a mint flavour in spearmint oil, is considered a mild skin sensitizer. Carvone-sensitization may be linked to oral/perioral signs and oral lichen planus, but studies are sparse. The prevalence of patch test reactions to carvone and relevant findings from the positive group were investigated. Records for patch-tested patients at the Malmö clinic, for the period 1996 to 2016, were studied. Carvone-positive and carvone-negative patients were compared regarding patch test data from baseline series and dental series. Dental series-tested carvone-positive patients were also compared with a matched group. A total of 147 out of 4,221 referred patients had a positive patch test to carvone. Sensitized patients had higher mean age and were primarily women; 72% had oral signs and 57% had oral lichen. Concomitant patch test reactions to gold, nickel and mercury were common. In the matched group-comparison carvone-positive patients had a higher frequency of oral lichen, but no difference was found in sensitization to gold and mercury.

Accepted Jul 27, 2018; Epub ahead of print Aug 7, 2018
Acta Derm Venereol 2018; XX: XX–XX.
Corr: Liv Kroona, Oral Pathology, Malmö University, Faculty of Odontology, SE-205 06 Malmö, Sweden. E-mail: liv.kroona@mah.se

The monoterpenic carvone (l-carvone; Fig. S1a) is the chief component of spearmint oil and is widely used as a mint-flavouring agent (1). The flavour is typically found in oral healthcare products and foodstuffs, such as chewing gum and sweets. In addition to isolation from natural oils, carvone can be synthesized from d-limonene (Fig. S1b) (2) and it is also one of several oxidation products found in auto-oxidized d-limonene (3).

Oral healthcare products are a major source of exposure, and carvone is a constituent of most toothpastes (4, 5). Carvone is considered a weak sensitizer (6) and the prevalence of contact allergy in patch-tested cohorts has been estimated to be 1.6–2.8% (7, 8). A few papers on patients’ adverse reactions to carvone or spearmint oil have reported both perioral and intraoral lesions from oral healthcare products, with intraoral lesions described as stomatitis or erosions associated with oral lichen planus (OLP) (9–13). Studies on patients with OLP or oral lichenoid lesions (OLL) have shown an association with carvone or spearmint contact allergy (14–16). However, these reports have not made a clear distinction between OLP, which is considered to be an autoimmune disease, and OLL, such as contact reactions to dental materials or other lichenoid lesions not fulfilling the criteria of OLP (17, 18).

The aim of this retrospective study was to estimate the prevalence of individuals with a positive patch test to carvone in Southern Sweden and to assess the characteristics of this group with regard to other contact allergies and clinical signs with special reference to OLP and OLL.

METHODS

Study population
Data were obtained from a clinical database comprising all patients subjected to patch testing at the Department of Occupational and Environmental Dermatology, Skåne University Hospital in Malmö, Sweden (19). All patients patch-tested with carvone during a 21-year period between 1996 and 2016 were included in the study.

Data recording
Information retrieved from the database included age at time of investigation, sex, type of patch test series in which carvone was included, grading of carvone test reaction and other contact allergies. From patients with a positive carvone patch test, information was also collected regarding the referrer’s profession, diagnosis, clinical signs, and localization of signs related to the investigation. It was not possible to deduce from the referrals or patient records whether the patients had OLP or OLL. Therefore, all variants of lichenoid lesions will henceforth be denoted as oral lichen (OL) unless otherwise specified.

SIGNIFICANCE
Carvone is a mint flavour found in most oral healthcare products. This study investigates the prevalence of contact allergy to carvone and describes common features of affected patients. In a cohort of 4,221 tested patients 3.5% had contact allergy to carvone. These patients often had oral signs and variants of oral lichenoid lesions, which otherwise only affects a few percent of the population. These data show that soluble allergens such as flavours and fragrances can cause oral contact allergy.

Key words: l-carvone CAS: 6485-40-1; spearmint; patch test; allergens: contact allergy: oral lichen planus: oral lichenoid lesions.

1https://www.medicaljournals.se/acta/content/abstract/10.2340/00015555-3009
Descriptive and comparative analysis

During the investigated period carvone was used in various patch test series. A descriptive analysis was performed on the collected data of carvone-positive patients regardless of which series they were tested with.

In order to compare the group data of the carvone-positive patients with a more general patch test population, data from the 2 test series with the largest collections of carvone-positive patients, the baseline series (extended) and the dental series (dental patients), were investigated further (Fig. S2). Carvone was included in the Malmö extended baseline series during 1997 to 1998 for research purposes and prevalence assessment. In this paper, “baseline series” will subsequently refer to the extended baseline series. Patch test data from the baseline series were analysed by comparing carvone-positive patients with carvone-negative patients. For the dental series, which included carvone during the whole investigated period, a similar comparison was made between carvone-positive and carvone-negative patients.

In addition, carvone-positive patients within the dental series were compared with a matched group of carvone-negative patients (Fig. S2). The presence of OL, atopy and patch test data were compared. Patients were considered atopic if they had present or previous atopic dermatitis, allergic rhinitis and/or allergic asthma. The matching criteria were sex, age ± 6 years and date of testing ± 3 months except for 2 cases where the criteria were extended to age ± 10 years and date of testing ± 4 months. Patients were chosen by using a random number generator on sets of patients meeting the required criteria.

The study was performed in accordance with the Declaration of Helsinki.

Patch testing

The patch test method for carvone and other investigated allergens has been consistent throughout the investigated period. Patch testing was carried out using 8-mm diameter Finn chambers (Epitest Ltd Oy, Tuusula, Finland or SmartPractice, Phoenix, AZ, USA) attached to Scanpore tape® (Norgeplaster A/S, Oslo, Norway). Finn chambers with 20 mg of petrolatum test preparation were applied for 48 h and discarded by the patients themselves. For liquid preparations 15 μl were used, applied to the chambers with a micropipette. Reactions were read on days 3 or 4 and on day 7. Occasional late reactions beyond 7 days were also recorded. Test reactions were graded by an experienced dermatologist according to the International Contact Dermatitis Research Group guidelines (20).

Test preparations of carvone (l-carvone, Acros Organics, Geel, Belgium, CAS: 6485-40-1) were prepared at the Department of Occupational and Environmental Dermatology, Malmö University Hospital, Sweden at a concentration of 5% in petrolatum.

Statistical analysis

The frequencies of the patients’ different diagnoses, clinical signs and co-reactivity to different allergens were analysed with the χ2 test, or with Fisher’s exact test when expected values were small. A 2-sided p-value < 0.05 was considered statistically significant.

RESULTS

Of 4,221 referred and patch-tested patients, 147 (3.5%) had a positive test reaction to carvone. The positive reactions were most commonly detected by the dental series, followed by the baseline series and cheilitis series (Table I). Complete records were available for 145 patients, whereas 2 only had information on patch test reactions. The mean age at testing was 66.2 ± 11 years. The male-to-female ratio in the carvone-positive group was 1:5.1 compared with 1:2.6 in the carvone-negative group.

Referring information

Based on the 145 patients with clinical data, the carvone-positive patients were primarily referred by dentists (n = 99; 68%), a majority of whom were oral surgeons (n = 52; 36%). Physicians referred 36 (25%) of the patients and 10 (7%) patients were enlisted as research subjects in projects on oral lichen. Patients referred by dentists were mainly tested with the dental series (87 of 99), whereas patients referred by physicians were tested with a variety of patch test series.

Diagnoses and clinical signs recorded in the referrals were predominantly localized intraorally (n = 106; 73%), whereas the perioral area was affected to a lesser degree (n = 20; 14%). Eczema except in the perioral area was present in 34 (23%) patients. Of these, 19 (13%) displayed dermal signs only, i.e. no oral or perioral involvement, often in the form of hand eczema. The main referring inquiry was “contact allergy to dental materials?”. Common words used to describe the clinical presentation were “redness”, “swelling” or “eczematous”. The presence of OL was recorded in 82 (57%) of the patients. OL often coincided with intraoral signs, where 81 of 106 patients had OL, whereas only 5 of the 20 patients with signs from the perioral area had OL. Lichen planus affecting the skin or genital area was recorded in 11 (8%) patients. Patients with OL or intraoral signs were predominantly tested with the dental series, whereas most patients with dermal signs were tested with the baseline series. Most patients with perioral signs were tested with either the dental series or the cheilitis series (Table II).

Patch test reactions

The number of positive test reactions to carvone at day 3 and at day 7, respectively, were essentially the same (110 vs. 111) and 31 patients (22%) tested positive only at day 7. Six patients had late reactions, from 10 days
of Peru (Table III(b)). Sixteen patients were tested with their private toothpaste and 11 of these displayed a positive reaction (Table III(c)). The tested toothpastes were, for the most part, diluted in water, often at 50% w/v, but several dilutions were often tested, ranging from 5% to 50%, and they varied over the investigated years. Four patients were also tested with undiluted toothpaste. Irritant reactions were noted in 4 patients, of whom 3 were tested with 50% toothpaste and 1 was tested with undiluted toothpaste. No specific brand was singled out as giving more frequent reactions.

### Comparative analysis of contact allergies within the baseline and dental test series: carvone-positive vs. carvone-negative patients

Carvone-positive patients had significantly higher frequencies of several contact allergies compared with the carvone-negative patients when investigating both series (Table SI1). For carvone-positive patients tested with the dental series, positive test reactions to gold and mercury were highly allocated to patients with OL (Table SI1).

Positive test reactions to other allergens in the dental series, however, did not differ when comparing the 99 carvone-positive patients to the matched group of carvone-negative patients, but the frequency of OL was significantly higher in the group of carvone-positive patients: 67/99 vs. 28/99 (p<0.0001; Table IV).

### DISCUSSION

The majority of patients with a positive patch test reaction to carvone were referred for oral or perioral signs, indicating that the main exposure to carvone was from oral healthcare products (5). In addition, OL was prevalent in the studied group, with over half of the patients being affected; an association also seen in previous studies on patients with OL (14–16). Patients with OL...
were, for the most part, referred by dentists, and patients with dermal signs, such as eczema, were primarily referred by physicians. However, the data may be biased as dentists and physicians are primed to search for clinical signs in different areas. The clinical signs seem also to have raised a suspicion of contact allergy against dental restorative materials rather than rinse-off products, such as dental healthcare products.

A previous study by Paulsen et al. (7) investigated carvone sensitization in patients patch-tested with the baseline series, and found 15 out of 541 (2.8%) consecutively tested patients. The proportion was higher than in the present study, in which 14 patients were positive to carvone out of 1,355 patients (1.0%) tested with the baseline series. When considering all investigated carvone-allergic patients in our study, regardless of test series, the proportion was 3.5% (147 of 4,221), but compared with Paulsen’s study in which no clinical relevance was seen in a majority of the patients the presently investigated group seems to have been skewed towards patients with oral or perioral signs. In addition, the mean age was higher in the present study and there was a greater predominance of women among carvone-positive patients, indicating that the sampling may have affected the outcome. The strength of the patch test reactions was similar between the 2 studies, with approximately 50% having a “++” reaction or higher. Paulsen found an association between carvone and Compositae allergy (sesquiterpene lactones), and the reaction to carvone was stronger in patients with both allergies. Of all series investigated in the present study, only the baseline series had sesquiterpene lactone mix included and 11 of 1,355 tested patients had a positive patch test reaction to this. Of the carvone-positive patients, only one patient reacted to sesquiterpene lactone mix.

A positive patch test to carvone on day 7 only was observed in 31 of the 147 patients. This demonstrates the importance of performing more than one patch test reading, since over 20% of the carvone-positive patients would otherwise have been left undiagnosed. Furthermore, 2 of the 6 patients with a late patch test reaction to carvone, day 10 or later, had positive test reactions on day 3 when re-tested. This indicates a possible active sensitization at the initial patch test. However, we have recently observed that carvone-positive patients may show positive reactions as late as 21 days upon re-testing, raising the possibility that a late appearing patch test reaction to carvone may not need to be a sign of active sensitization (to be published).

Overall, the 147 carvone-positive patients showed a high rate of additional contact allergies, especially against metals and fragrance substances (Table III). The high prevalence of spearmint oil sensitization is not surprising, since carvone is the main constituent of spearmint oil (1). Interestingly, a majority of the carvone-positive patients tested with their own toothpaste had a positive test reaction. The toothpastes were of various brands and we do not know what toothpaste constituent the patients reacted to, but most toothpastes contain carvone, though at variable concentrations, as our previous work has shown (4). Sensitization to other flavours found in oral healthcare products, such as peppermint, cinnamal, cinnamyl alcohol and limonene, were not common in the investigated group, but a considerable number of patients had reactions to fragrance mix I and balsam of Peru, which contain flavour substances such as cinnamal and cinnamyl alcohol. Spearmint oil also contains 10–15% limonene (d-limonene) (1), a common fragrant constituent in cosmetic products (21) and also present in toothpastes (4). During a large part of the investigated time period the test preparation of limonene was not intentionally oxidized and it is essentially the oxidation products in limonene that are sensitizers, e.g. carvone (3). Of the carvone-positive patients only 4 of 90 tested with limonene had a positive reaction (Table IIIb) and 2 of these were tested with oxidized limonene. There is also a possibility that patients may have been sensitized to carvone from oxidized limonene, either orally or via the skin, but in a study by Matura et al. only a few patients sensitized to oxidized limonene reacted to carvone in patch tests (22).

It has been shown previously that patch-tested patients with OL often have contact allergies both related to their dental restorations and to carvone (15, 23). The present study also demonstrates a relationship between contact allergy to carvone and metals used in dental restorations. Of the 147 carvone-positive patients studied 55 (35.4%) had a concomitant patch test reaction to gold, and these patients also often had a strong test reaction to carvone. The frequency was comparable to observations on patients with dental gold restorations (24, 25). However, we do not have data regarding the presence of dental materials in the studied group, but the mean number of restorations was probably high, as dental restorations increase with age and the mean age in the group was over 65 years. The 147 investigated patients also had a slightly higher frequency of patch test reactions to mercury (13.6%) compared with studies on dental series tested or patients with OL (15, 26).

Patch test reactions to gold and mercury were more common in carvone-positive patients, when comparing them with all carvone-negative patients tested within the baseline or dental series and the frequency of test reactions was even higher in carvone-positive patients with OL (Table SI). In contrast to what was observed in the unmatched comparative analysis there was no difference in the proportions of contact reactions between the 2 matched groups of carvone-positive and carvone-negative patients tested with the dental series (Table IV). The observed high ratio of women and high mean age in the carvone-positive groups were corrected in the matched comparison. Still, OL was strikingly over-represented.
in carvone-positive patients and thus this relationship is neither connected with concomitant contact allergy to gold nor to mercury. Furthermore, regardless of reactivity to carvone, patients with OL in both groups had a high proportion of reactions to mercury.

The oral mucosa differs from the skin, in that it seems to be more tolerant to sensitization and elicitation (27, 28). Still, one must assume that the sensitization route of carvone is primarily through the oral mucosa. Factors that disturb the mucosal environment, such as OL make the oral mucosa more sensitive to external elements and could possibly increase the risk of sensitization (29). Previous studies on oral contact allergy have primarily focused on dental materials, such as acrylates and metals, and only sporadic studies have approached allergy to soluble agents in oral healthcare products, food and beverages. This is especially evident when it comes to lichenoid reactions, although it recently has been shown that contact allergy to fragrances is common among patients with OL (16). The present study demonstrates that contact allergy to fragrances is common among patients with OL (16). The present study demonstrates that contact allergy to carvone is closely related to oral signs and OL, and that oral exposure to carvone is a probable sensitization route.

The authors have no conflicts of interest to declare.

REFERENCES

Fig. S1. Representation of chemical structures of: (a) l-carvone and (b) d-limonene.

Fig. S2. Graphical depiction of the various study cohorts in the baseline series and the dental series that were subjected to comparative analysis. C+: carvone-positive patch test; C–: carvone-negative patch test.

Table SI. Positive patch test reactions in the baseline series 1997 to 1998 and the dental series 1996 to 2016 with the number of carvone-positive (with oral lichen (OL)) demonstrated

<table>
<thead>
<tr>
<th></th>
<th>Baseline series</th>
<th>Dental series</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All tested n</td>
<td>Carvone-positive (OL) n (%)</td>
</tr>
<tr>
<td>Number of tested</td>
<td>1,355</td>
<td>14 (1)</td>
</tr>
<tr>
<td>Patch test reactions to:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gold sodium thiosulphate</td>
<td>298</td>
<td>8** (1)</td>
</tr>
<tr>
<td>Palladium chloride</td>
<td>39</td>
<td>2</td>
</tr>
<tr>
<td>Nickel sulphate</td>
<td>283</td>
<td>2</td>
</tr>
<tr>
<td>Potassium dichromate</td>
<td>78</td>
<td>2</td>
</tr>
<tr>
<td>Cobalt chloride</td>
<td>110</td>
<td>4*</td>
</tr>
<tr>
<td>Balsam of Peru</td>
<td>72</td>
<td>2</td>
</tr>
<tr>
<td>Fragrance mix I</td>
<td>90</td>
<td>2</td>
</tr>
<tr>
<td>Thiuram mix</td>
<td>24</td>
<td>2*</td>
</tr>
<tr>
<td>Ethylenediamine</td>
<td>41</td>
<td>2</td>
</tr>
</tbody>
</table>

Significant values are shown for the comparison between carvone-positive and carvone-negative groups. *p < 0.05; **p < 0.01; ***p < 0.001; (*)p = 0.0510. Allergens are displayed when ≥ 2 patients had a concomitant positive patch test for carvone.

HEMA: hydroxyethyl methacrylate; EGDMA: ethylene glycol dimethacrylate.

www.medicaljournals.se/acta