

Patch testing with thin-layer chromatograms of chamomile tea in patients allergic to sesquiterpene lactones

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Patients with contact allergy to sesquiterpene lactones (SLs) are usually hypersensitive to Asteraceae plant products such as herbal teas. The objective of this study was to show sensitizers in chamomile tea by patch testing with thin-layer chromatograms. Tea made from German chamomile was separated by thin-layer chromatography. Strips of the thin-layer chromatograms were used for patch testing SL-positive patients. 15 (43%) of 35 patients tested positively to 1 or more spots on the thin-layer chromatogram, with many individual reaction patterns. Patch testing with thin-layer chromatograms of German chamomile tea showed the presence of several allergens.

Key words: chamomile; contact allergy; parthenolide; sesquiterpene lactones; thin-layer chromatography; tea. © Blackwell Munksgaard, 2007.

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There are anecdotal cases of individuals with contact allergy to sesquiterpene lactones (SLs), who experience aggravation/flare of dermatitis when drinking tea made of herbs containing SL (1–5). Recently, we reported on contact allergy to tea made of German chamomile (6). Clinically, many of our patients hypersensitive to SL suffer from hand dermatitis localized to the palms (7). This pompholyx type of hand dermatitis is a possible manifestation of systemic allergic contact dermatitis (8). We have therefore started a project in patients hypersensitive to SL, with 2 major aims, namely (i) investigation of the possible significance of oral intake of SL-containing plants/plant products for hand dermatitis and (ii) detection of sensitizers in tea made from German chamomile based on patch testing with thin-layer chromatograms (Fig. 1). This study is part of this project and refers to point 2.

Materials and Methods

Study design

The design of the whole study is depicted in Fig. 1. The study took place on 2 occasions with an inter-

val of 6 weeks. During the first week, the patients were patch tested with chamomile tea, petrolatum preparations, and thin-layer chromatograms. They answered questions in a questionnaire together with the doctor. Their hands were photographed.

On the second occasion, that is 6 weeks later, the patients' back and hands were examined, and the patients answered new questions. Their hands were photographed once more. Blood samples were taken, and finally, the patients had to ingest capsules with or without 'chamomile tea'. The present study only reports the results of the patch testing with chamomile tea, pet. preparations, and thin-layer chromatograms.

Patients

45 patients from southern Sweden, earlier patch tested with our standard series at the Department of Occupational and Environmental Dermatology in Malmö or at the Department of Dermatology in Halmstad, because of eczema and suspected contact allergy and found positive to SL-mix, were eligible for the study and therefore asked to join. 35 patients accepted to participate.

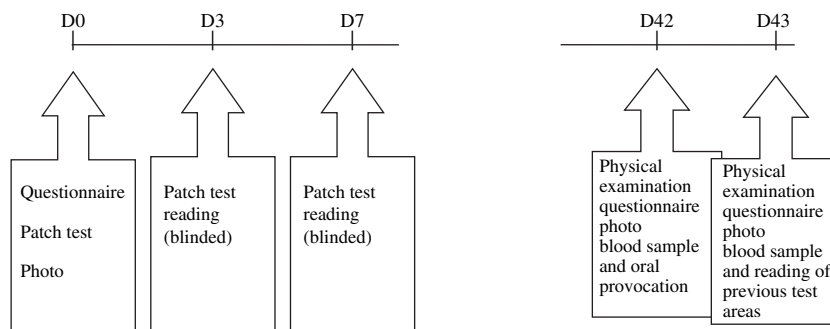


Fig. 1. Study design.

There were 26 women and 9 men, with mean age of 59 years (range 37–77 years). 22 patients (17 women and 5 men, mean age 52 years, range 36–71 years) served as controls, who were patch tested at the Malmö department and found negative to SL-mix. Informed consent was obtained from patients and controls. The study was approved by the Ethics Committee of Lund University Medical Faculty.

Patch test material

The 35 patients and 22 controls were patch tested with an aqueous (aq.) extract of tea made from German chamomile (*Matricaria recutita*) in stock solution and 4 dilutions, pet. preparations of German chamomile 2.5% w/w, Roman chamomile (*Chamaemelum nobile*) 1.0% w/w, and the SL parthenolide 0.1% w/w. They were also tested with SL-mix 0.1% w/w from the European standard series containing alantolactone 0.033% and the combination of costunolide and dehydrocostuslactone, each at 0.033%. The test preparations of German chamomile were supplied by Trolab, Hermal, Reinbeck, Germany, and the others by Chemotechnique Diagnostics, Vellinge (formerly Tygelsjö), Sweden.

To secure a blind reading of the tests and to have a control of the specificity of any positive flare-up reactions in the oral provocation, the 27 patients earlier patch tested and found positive to nickel (16 SL positive and 11 controls) were tested with nickel sulfate hexahydrate 5.0% pet. w/w. The remaining 30 patients were tested with sodium lauryl sulfate (SLS) 2.0% aq. (Acros Organics, Geel, Belgium).

The 35 SL-positive patients were also epicutaneously tested with thin-layer chromatograms of German chamomile tea (9). The 22 controls were also tested with 'thin-layer chromatograms' but only with blank strips of the plastic roll with silica gel, that is without chamomile tea because it was not possible to manufacture freshly prepared thin-layer chromatograms for all the 57 patients.

Test preparation of chamomile tea for patch testing

To make an aq. extract of tea made from German chamomile, we used mainly flowers labelled to be of Swedish origin. They were acquired from a health food shop, and according to the health shop dealer, 1.5 g of tea ('dried flowers') is needed for making 1 cup (=200 ml) of tea. 3 cups of tea were made at the time. The 3 cups of tea were prepared as follows: 600 ml of boiling water was poured on 4.5 g of tea; the extract was allowed to draw for 10 min; the flowers were strained off and the tea was filtrated through filter paper number no. 3 (Munktell Filter AB, Grycksbo, Sweden); the tea was then evaporated in a rotary evaporator to a volume of 2 ml, constituting the stock solution. The stock solution was then diluted to 32% aq. v/v, 10% aq., 3.0% aq., and 1.0% aq.

Test preparation of chamomile tea for thin-layer chromatography

3 cups of tea were prepared and evaporated under vacuum as described above. 10 ml ethanol (99.5% v/v; Kemetyl AB, Haninge, Sweden) was added to the residue and the mixture treated in an ultrasonic bath for 30 seconds and the ethanol extract collected. The residue was once more treated with 10 ml ethanol, and this second ethanol extract was combined with the first one. Not all the residue could be dissolved in ethanol. The extract containing 20 ml ethanol was evaporated under vacuum to a final volume of 2 ml, resulting in a solution in which substances that had been extracted from the tea residue and dissolved completely in the ethanol had the same concentration as in the patch test preparation of the tea.

Preparation of thin-layer chromatograms

The method was recently developed (9). The chromatograms were prepared 1 day before use and kept refrigerated within an aluminium foil.

A plastic roll 500 × 20 cm with silica gel 60F254 (Merck KGaA, Darmstadt, Germany)

was cut into 18- × 18-cm strips. 3 µl of the ethanol solution of chamomile tea was deposited using a pipette for every 2.5 cm of the baseline. This was repeated 25 times, which makes 75 µl on each spot. The chromatogram was developed in a glass tank with chloroform (Scharlau Chemie S.A., Barcelona, Spain)/acetonitrile (Labscan Ltd, Dublin, Ireland), 86 : 14 v/v as the mobile phase. The chromatograms were developed for about 45 min and finally examined in ultraviolet (UV) radiation. All spots detected in 254 and/or 360 nm wavelength were marked with a pencil. 9 different spots were identified including the application spot. The spots were numbered from 1 to 9, where no. 1 indicates the site of application. The chromatogram was cut into 2.5- × 18-cm strips, with a band of spots on each.

Patch testing and testing with thin-layer chromatograms

The pet. test preparations and test solutions of German chamomile tea, 15 µl of each dilution, was applied on the back with Finn chambers with a diameter of 8 mm (Epitest OY, Tuusula, Finland) on Scanpor tape (Norgesplaster A/S, Vennesla, Norway) and removed after 2 D.

The spots on the chromatograms were marked out before applying the thin-layer chromatography (TLC) strips to the patients' back. The chromatograms were attached with Scanpor tape and removed after 2 D.

The tests were read and scored according to the International Contact Dermatitis Research Group (10) on D3 and D7. The reading was blinded.

Results

The results of the epicutaneous testing with thin-layer chromatograms are shown in Table 1. Of the 35 previously SL-positive patients, 31 (88%) tested positively to SL-mix. 1 of them (no. 18) did not test positively to any of the test preparations, just a doubtful reaction to chamomile tea 100% aq.

30 (86%) of the 35 patients tested positively to the German chamomile tea. All these patients except 1 (no. 5) were hypersensitive to SL-mix, but all 30 were positive to parthenolide. 33 (94%) of the 35 patients tested positively to parthenolide.

22 (63%) of the 35 previously SL-positive patients tested positively to the pet. test preparation of German chamomile, whereas 15 of the 35 (43%) were positive to the preparation of Roman chamomile.

All the 27 formerly nickel-positive patients tested positively to nickel sulfate. 30 patients were tested with SLS; in 4 of them, the result was negative. 2 controls tested positively to the chamomile tea (2 of the 22 controls versus 30 of the 35 patients hypersensitive to SL-mix; $P < 0.001$, Fisher's exact test, two sided).

15 (43%) of the 35 patients tested positively to at least 1 spot/compound on the chromatogram (Table 1 and Fig. 2). Besides the application spot, there were 8 identified spots (Fig. 3). Patient no. 6 (Table 1) had so strong confluent reactions so that individual spots could not be identified. The patient is therefore excluded in Fig. 3. Most positive reactions were observed to spot no. 8. In addition to that spot, many patients tested positively to the application spot no. 1, 6, and 3, sometimes also including the area between spot no. 2 and 3 (Fig. 3). The patients showed different reaction patterns to the spots (Table 1).

1 patient (no. 35) who tested positively to parthenolide and to the thin-layer chromatogram (spot no. 6) tested negatively to the German chamomile tea. All the other patients who tested positively to the thin-layer chromatogram also tested positively to the tea.

No obvious relationship was found between test reactivity to the chromatogram on the one hand and plant-related (German chamomile tea, Roman chamomile, parthenolide, and SL-mix) allergy or degree of hypersensitivity to an individual sensitizer (German chamomile tea, Roman chamomile, parthenolide, and SL-mix) on the other hand.

Discussion

Patch testing with thin-layer chromatograms has proven useful in the separation, detection, and identification of individual sensitizers in compound material (11). The present study shows that TLC is also a useful method for separating and showing allergenic constituents in chamomile tea.

We have shown that there are at least 9 substances in the chamomile tea. However, it is possible that a separate spot on the chromatogram, maybe particularly the application spot, consists of more than 1 substance. Furthermore, substances not absorbing UV radiation (254 and 360 nm, respectively) can be present anywhere on the chromatogram. Yet, it is unlikely that such at present untraced substances are contact sensitizers as the positive reactions to the chromatograms mainly corresponded to identified spots. Strong reactions were never observed for possible substances appearing between the identified spots, only weaker ones that may be interpreted as spreading

Table 1. The strongest reactions to extracts of German chamomile tea, test preparations of German and Roman chamomile, parthenolide, and SL-mix and distribution of positive reactions to different spots on the thin-layer chromatogram in 35 SL-positive patients and 22 controls^a

Patient number	German chamomile tea					German chamomile, 2.5% pet.		Roman chamomile, 1.0% pet.		Parthenolide, 0.1% pet.		SL-mix, 0.1% pet.		Thin-layer chromatogram (TLC), German chamomile											
	100% aq.	32% aq.	10% aq.	3.0% aq.	1.0% aq.	2.5% aq.	1.0% pet.	0.1% pet.	0.1% pet.	0.1% pet.	0.1% pet.	1	1-2	2	2-3	3	4	4-5	5	5-6	6	6-7	7	8	9
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42 C	++	++	++	++	++	++	++	++	++	++	++	++	++	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
43 C	++	++	++	++	++	++	++	++	++	++	++	++	++	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
44-57 C														nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt

aq., aqueous; C, control; nt, not tested; SL, sesquiterpene lactone.

^aEmpty cells indicate a negative test reaction. The strength of the TLC reaction is not indicated.



Fig. 2. Positive test reactions to the spots on the thin-layer chromatogram in patient no. 32.

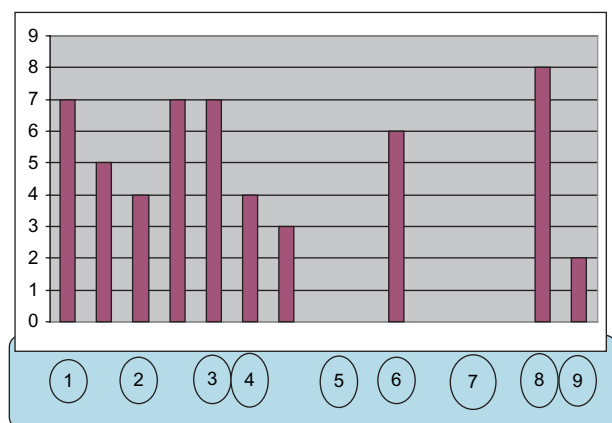


Fig. 3. Number of patients (y-axis) with positive reactions to different spots is deleted (spot no. on the x-axis), shown as spots on the thin-layer chromatogram. Patient no. 6 is excluded.

phenomena from strong reactions at the spot areas were observed.

86% of the SL-positive patients reacted positively to the aq. extract of German chamomile tea, whereas only 43% had positive reactions to thin-layer chromatograms of the tea. This could be explained by the various dissolution conditions. After the water had been evaporated from the German chamomile tea, all the residue could not be dissolved in ethanol. Water would have been a more efficient solvent but could not be used because of incompatibility between water and silica. Therefore, there may still be sensitizers present in the residue not dissolved in ethanol or, more probably, insufficiently dissolved in ethanol, resulting in a too low concentration of the individual spot/compound on the chromatogram to allow a positive test reaction to the chromatogram in those with a low degree of hypersensitivity. In

contrast, we showed in our previous study (6) that water and ethanol solutions of aq. extract of German chamomile tea gave similar results with equivalent number of positive reactions to the respective type of solution. The residue not dissolved in ethanol will be subjected to patch testing as an aq. solution on a chromatogram using a different stationary phase than silica.

Another explanation to fewer positive reactions to the TLC strips than to the chamomile tea extract may be a possible significance of additive reactions and/or synergistic effects when having several sensitizers in chamomile tea extract compared with the separate TLC spots. The separated spots may represent individual substances with lower amount of allergen than in the entire tea extract.

A higher affinity of the extract to the silica gel than to the Finn chamber paper is another possible explanation. There is also a possibility of a better contact to the skin when using Finn chambers compared with thin-layer chromatograms.

The reason why the German chamomile tea rendered more positive test reactions than the test preparation of German chamomile is not known but may be explained by differences in batches, procedures in manufacturing the test preparation and vehicle, and the concentration of sensitizers.

Anthecotulide and matricarin are SLs described in the literature as possible sensitizers in patients hypersensitive to German chamomile (12, 13). These substances were not available when performing this study.

Expectedly, and similarly to our previous study (6), not all patients previously observed to be hypersensitive to SL-mix tested positive to the same. Similarly to our previous study, more patients tested positively to parthenolide than to SL-mix.

The reason for this high frequency of positive reactions to parthenolide is at present not known. The SL parthenolide has been a suggested replacement/alternative to SL-mix (14). Concomitant reactions between parthenolide and other Asteraceae plants (e.g. German chamomile) have been described (15). So far, there is no experimental evidence supporting the principle of cross-reactions between different SLs.

2 controls tested positively to the chamomile tea. In 1 of them, it was the only positive reaction apart from a positive nickel reaction. The second patient tested positively to chamomile tea, parthenolide, and nickel, besides dilutions of the tea. This patient had a recent history of facial dermatitis owing to the use of chamomile cream. Interestingly, these 2 patients earlier tested negatively to SL-mix and also at the present testing. This

confirms the well-known fact that a negative patch test to SL-mix does not exclude an Asteraceae allergy (16).

In summary, this study shows that patch testing with thin-layer chromatograms may be a useful way to separate and show positive patch test reactions to separate constituents of a compound product. Furthermore, the thin-layer chromatograms can be used for patch testing, yielding positive reactions that will facilitate the later work with identification of individual sensitizers in German chamomile tea and probably other herbal products. When used for German chamomile tea, many sensitizers were shown. Our further investigations will focus on identification of these sensitizers.

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