

Patch test reactivity to feverfew-containing creams in feverfew-allergic patients

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Background: The Compositae plant feverfew (*Tanacetum parthenium*) has long been recognized as an important sensitizer in European Compositae-allergic patients, mainly because of its content of the sesquiterpene lactone parthenolide. Recently, a parthenolide-depleted feverfew extract with claimed anti-inflammatory properties has been developed for use in cosmetics.

Objective: The aim of the study was to test, on the basis of patch test reactions, whether persons with contact allergy to feverfew could tolerate creams containing this feverfew extract.

Patients/Materials/Methods: Seven patients with feverfew contact allergy were patch tested with two creams containing the feverfew extract. Subsequently, the creams were analysed by liquid chromatography with tandem mass spectrometry to detect parthenolide.

Results: Four of the patients tested positive to one of the creams; reactivity was associated with simultaneous positive reactions to parthenolide. This cream was analysed about 2 years later, and no parthenolide was detected, probably because of degradation of the compound.

Conclusions: Topical products containing parthenolide-depleted feverfew extracts may elicit positive patch test reactions in feverfew-sensitive patients. The reactivity may be enhanced by simultaneous testing with parthenolide, but the reactivity is lost over time, probably because of degradation of parthenolide.

Key words: allergic contact dermatitis; Compositae; cosmetics; feverfew; parthenolide; patch test; *Tanacetum parthenium*. © John Wiley & Sons A/S, 2010.

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Traditional herbalism has long recognized feverfew [*Tanacetum parthenium* (L.) Schulz-Bip.] as an important medicinal plant. It belongs to the Compositae (Asteraceae) family of plants and, because of its content of the sesquiterpene lactone parthenolide (Fig. 1), is a strong sensitizer, often implicated in Compositae allergy in Europe (1, 2).

Its clinical effect in migraine prophylaxis has formerly been attributed to parthenolide, but this has been questioned recently because of inconsistent results in clinical studies (3). However, a more recent non-clinical study suggests that parthenolide is indeed responsible for the antimigraine effect (4).

In the last few years, a parthenolide-free feverfew extract has been documented to show, among other

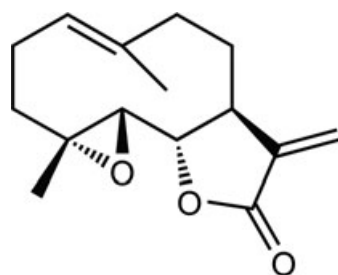
things, anti-inflammatory properties both *in vitro* and *in vivo* (5, 6). Therefore, the extract has been incorporated in moisturizers intended for use in persons with sensitive skin, manifesting as, for example, redness and tactile roughness (5).

The purpose of this study was to test, on the basis of patch test reactions, whether persons with contact allergy to feverfew could tolerate creams containing the above-mentioned feverfew extract.

Materials and Methods

Clinical study

Between August 2007 and March 2008, seven Compositae-sensitive patients who underwent supplementary patch testing were additionally tested



Parthenolide

Fig. 1. Chemical structure of the sesquiterpene lactone parthenolide present in the aerial parts of *Tanacetum parthenium* (feverfew).

with two creams containing feverfew extract. The first cream, an American product with antipruritic properties, also contained hydrocortisone 1% as an active ingredient. The second cream, a cosmetic product marketed in Denmark for hypersensitive skin, contained, among other things, a parthenolide-free feverfew extract and fragrance.

The allergens were applied in Finn chambers® on Scanpor® tape (Epitest Ltd. Oy, Tuusula, Finland) for 2 days and read after 3 and possibly 7 days, according to the standards set by the International Contact Dermatitis Research Group (7).

Chemical investigations

Aliquots of cream samples (1 g of each cream accurately weighted) were dissolved in 3 ml water and extracted successively with 3 × 3 ml dichloromethane. The dichloromethane extracts were combined and evaporated to dryness, re-dissolved in 200 µl acetonitrile for analysis using liquid chromatography with tandem mass spectrometry (LC-MS/MS). To ascertain that the described extraction procedure could be used to determine the content of parthenolide in cream samples, a cream sample was spiked with 30 µg parthenolide and used as a control.

Analysis of parthenolide was performed on a LC-MS Thermo Fisher LTQ-XL ion-trap (Thermo Fisher Scientific Inc., Waltham, MA, USA) operated with an atmospheric pressure electrospray ionization (API-ES) source as follows: electrospray ionization (ESI) mass spectra were acquired in the positive ion mode using Xcalibur 2.1 software. The ESI needle voltage was 4.0 kV, the sheath and auxiliary nitrogen gas flow rates were 45 and 15 units, respectively, and the heated capillary temperature was 220°C. The heated capillary and lens voltages were optimized in positive polarity by infusing parthenolide into the source and tuning automatically on the protonated molecule. For serial mass spectrometry, precursor ions were isolated in a window of

m/z 1.5 units and fragmented using normalized collision energy of 45 V. Sample introduction was performed by high-performance liquid chromatography (Thermo Scientific Accela™ autosampler, pump and photodiode array detector, Thermo Fisher Scientific Inc., Waltham, MA, USA) fitted with a C18 Hypersil GOLD column (1.9 µm particle size, 50 mm × 2.1 mm i.d.; Thermo Scientific). The column temperature was maintained at 35°C, and the mobile phases consisted of solvent A (aqueous 0.1% formic acid) and solvent B (0.1% formic acid in acetonitrile). Chromatographic separation of 10-µl injections was achieved using a 200-µl/min linear mobile phase gradient: 1% A to 99% B in 15 min, followed by a 10-min equilibration back to 1% A before the next injection.

Parthenolide and other sesquiterpene lactones were monitored qualitatively in full scan mode (from m/z 100 to m/z 500). In addition, parthenolide was monitored in selective ion monitoring (SIM) scan mode (ions with m/z 249 and m/z 271) and single reaction monitoring (SRM) scan mode (ion m/z 231 from m/z 249, with collision energy of 45 V) in tandem mass spectrometry (MS/MS). Quantification was performed on the ion at m/z 249 $[M + H]^+$, which gave a sixfold higher response than the sodium adduct ion at m/z 271 $[M + Na]^+$ in SIM scan mode and consequently a better signal to noise ratio. The detection limit of parthenolide was determined in SIM scan mode to be 0.15 ng/ml and the quantification limit to be 0.51 ng/ml.

Results

Clinical study

Selected patch test results are shown in Table 1. Whereas the American cream elicited doubtful positive reactions in one patient only, the European cream tested positive in four of seven patients. The majority of the patients had contact allergies to agents other than *Compositae*, most frequently to fragrance compounds (Table 2).

A total of 10 controls, five with and five without positive reactions to fragrance mix I (FM I), were patch tested with the two creams between February and May 2009: none showed positive reactions. One patient with *Compositae* allergy was inadvertently tested with the creams in February 2009, and she had a doubtful positive reaction on D3 and a doubtful positive follicular reaction on D7 to the European cream, but no reaction to the American cream.

Chemical investigations

In November 2009, no parthenolide was detected in the European cream. Likewise, parthenolide was not

Table 1. Distribution of patch test results (maximum reactions) to FM I, feverfew, parthenolide, and a cream containing a parthenolide-free feverfew extract in seven Compositae-sensitive patients

Patient no. sex/age (years)	FM I	Cream with feverfew extract	Feverfew 1.0% pet.	Parthenolide 0.1% pet.	Parthenolide – previous test results
1 F/45	?+	++	++	+++	–
2 F/65	+	++	+++	+++	NT
3 F/42	++	++	+++	+++	+++
4 F/71	?+	++	++	++	–
5 F/38	++	–	++	?+ Foll.	NT
6 M/26	?+	–	++	NT	++
7 F/53	+++	?+	++	+D3, ++D7	–

–, negative reaction; ?+, doubtful positive; Foll., follicular reaction; NT, not done; FM I, fragrance mix I (cinnamyl alcohol, cinnamal, eugenol, alpha-amyl cinnamal, hydroxycitronellal, geraniol, isoeugenol, and *Evernia prunastri*); pet., petrolatum.

*Possibly because of sorbitan sesquioleate to which she had a 1+ reaction.

Table 2. Distribution of other contact allergies in seven Compositae-allergic patients

Patient no.	Contact allergies
1	Nickel sulfate
2	Sorbitan sesquioleate, FM I, own cream (perfumed)
3	Nickel sulfate, potassium dichromate, methylchloroisothiazolinone/methylisothiazolinone, FM I, FM II (positive hydroxyisohexyl 3-cyclohexene carboxaldehyde), cobalt chloride, thiuram mix
4	Nickel sulfate, cobalt chloride
5	FM I (positive isoeugenol), <i>p</i> -phenylenediamine
6	Nil
7	Nickel sulfate, FM I, FM II

FM I, fragrance mix I (cinnamyl alcohol, cinnamal, eugenol, alpha-amyl cinnamal, hydroxycitronellal, geraniol, isoeugenol, and *Evernia prunastri*); FM II, fragrance mix II (citral, citronellol, farnesol, coumarin, alpha-hexyl cinnamal, and hydroxyisohexyl 3-cyclohexene carboxaldehyde).

detected in a new tube of cream bought in December 2009. To determine the presence of parthenolide in the cream, a method for extracting parthenolide from the cream was developed, followed by analysis of the extracts by LC-MS. The detection limit of parthenolide was determined by LC-MS in SIM scan mode to be 0.15 ng/ml and the quantification limit to be 0.51 ng/ml, making this method even more sensitive for the detection of parthenolide in extracts than the gas chromatography-mass spectrometry method previously used to determine airborne parthenolide (8).

Discussion

Exacerbation or elicitation of dermatitis after using cosmetics containing feverfew extract has been previously reported in two women (9). The first was 45 years old and presented with a seasonal eruption on the face and scalp compatible with an allergic airborne dermatitis, which was exacerbated after a short-term use of a 'calming' moisturizer containing feverfew extract (9). Patch testing showed 1+ reactions to the moisturizer, sesquiterpene lactone

mix 0.1% in petrolatum (pet.), parthenolide 0.1% pet., *Tanacetum vulgare* (tansy) extract 1.0% pet., and other Compositae plant extracts (9). She tested negative to a moisturizer of a very similar chemical composition, but without feverfew, supplied by the same company (9). The other patient was 25 years old and developed periorbital dermatitis after using a new feverfew-containing 'eye cream' for a few weeks. She also tested positive (1+) to the cream 'as is,' sesquiterpene lactone mix 0.1% pet., and Compositae mix 6% pet. – she was not tested with parthenolide or tansy (9). These case reports suggest pre-existent Compositae sensitization, with aggravation or elicitation of dermatitis after use of the feverfew-containing products.

Parthenolide is an extremely potent contact allergen. In a Danish study documenting the occurrence of parthenolide on plant particles trapped in a high-volume air sampler and thus its role in airborne Compositae allergy, the most sensitive patient tested positive to parthenolide in a concentration of 8.1 ng per patch (8). Realizing the potential allergenicity of a topical emollient formulation containing a feverfew extract, great efforts have been put into reducing or removing α -unsaturated γ -lactones, especially parthenolide, from the extract (10). In a study by Martin et al., a parthenolide-depleted feverfew extract was stated to contain 30 times less parthenolide as a percentage of dry weight compared with whole feverfew and, furthermore, was found to be 'free of sensitization potential' (10). The latter statement was based on the presentation by Kurtz and Walczak (11): nearly 1000 individuals were tested with an allegedly parthenolide-free extract of feverfew in the topical human repeat insult patch test, and no cases of contact sensitization were recorded. Likewise, no phototoxic or photoallergic reactions were observed (11). Presumably, the subjects were healthy volunteers contrary to the two women in the above-mentioned case report (9). Although no detailed information on the products are provided in the report, it is likely that the moisturizer and

the eye cream contained a parthenolide-depleted extract of feverfew and that a very low concentration of parthenolide was the eliciting factor. It is well known that the concentration of parthenolide may vary in the raw plant material from 0.2% of dry weight in the aerial parts to 1.27% of dry weight in leaves of post-flowering feverfew plants (8, 12). Thus, even a 30-fold decrease in parthenolide concentration may leave sufficient amounts of allergen in the raw extract to elicit a reaction in pre-sensitized persons. However, the actual concentration of this extract in the cream is unknown.

In this study, the strongly positive reactions to the cream, labelled as containing parthenolide-free feverfew extract, were not associated with feverfew allergy as such as all patients tested strongly positive to feverfew extracts (Table 1). Likewise, there was no consistency between patch test results with FM I and those obtained with the cream (Table 1). However, there may be an association between reactivity to parthenolide and the cream when they are applied simultaneously: patients nos. 5 and 6, who tested negative to the cream, were either not tested or doubtful positive to parthenolide on D7. Patient no. 7 had a weak positive reaction to parthenolide on D3, which was stronger on D7, and at the same time a doubtful positive reaction to the cream. In comparison, the four patients who tested positive to the cream had either strongly positive (2+) or extreme (3+) reactions to parthenolide on D3. Table 3 shows the association between the strength of patch test reactivity to the cream and that to parthenolide.

Jin et al. studied the stability of parthenolide in different states (13). Parthenolide in feverfew solution fitted a first-order reaction, whereas parthenolide in powdered feverfew (solid state) did not react according to any particular model, but was influenced by temperature and moisture: both high temperature and an increase in moisture would accelerate degradation of the sesquiterpene lactone (13).

It is, however, difficult to predict the degradation of this compound in the complex composition of a cream. The chemical investigations in November 2009 could not detect parthenolide, indicating that the content of parthenolide in the cream, if present, was <0.15 ng/g, which is below the concentration of parthenolide that has previously been shown to elicit an allergic reaction in highly sensitive patients tested positive to parthenolide (8). However, this does not rule out the occurrence of the compound previously. A gradual decomposition of the preservative-free cream, and especially of parthenolide, may offer another explanation of the patch test results. The patients were numbered consecutively, that is patient no. 1 was tested in August 2007 and patient no. 7 in March 2008. If the concentration of parthenolide decreased in this period, this would explain the weaker reactions in the last three patients. However, the cream elicited doubtful positive reactions in February 2009 in a parthenolide-positive patient, and although this supports the view that decomposition had taken place, it does not explain the negative reactions in patients nos. 5 and 6 a year earlier: the requirement of simultaneous testing with parthenolide would.

Single application of a small amount of cream containing traces of parthenolide – even extended to 2D under occlusion – may not suffice to elicit a positive patch test reaction, but the simultaneous application of parthenolide may cause an immunological response to a degree that will make even minute amounts of parthenolide on the skin detectable for the specific T-cells. On the other hand, application of such a cream on a daily basis will probably be sufficient to cause allergic contact dermatitis, as exemplified by the two cases reported previously (9). This reaction may be worsened by simultaneous exposure to airborne parthenolide in the surroundings: in a Danish study, one feverfew plant released 100 ng of parthenolide per hour, meaning that a herbaceous border full of these

Table 3. Association between patch test reactivity to parthenolide and cream 2 containing a parthenolide-free feverfew extract when tested simultaneously (the numbers refer to the patients in Table 1)

Cream 2	Parthenolide										
	NT	–D3	–D7	?+D3	?+D7	+D3	+D7	++D3	++D7	+++D3	+++D7
–D3	6	5				7					
–D7	6				5						
?+D3											
?+D7									7		
+D3								4			
+D7											
++D3											
++D7									4	1, 2, 3	1, 2
+++D3											
+++D7											

?+, doubtful positive; –, negative reaction; NT, not done.

plants would release considerable amounts and certainly enough to induce contact dermatitis in a Compositae-sensitive person (8).

The fact that parthenolide was not detectable in a new tube of cream, bought in December 2009, may be as a result of batch to batch variability in parthenolide content: it is well known that different batches of feverfew powdered extracts from the same manufacturer may show a significantly different parthenolide content (14). This may also apply to the American cream that did not elicit any positive reactions; another possible explanation is that the hydrocortisone of this preparation may have diminished any reactivity.

In conclusion, topical formulations containing parthenolide-depleted feverfew extracts may elicit positive patch tests in feverfew-allergic persons. The clinical results suggest that this may be caused by traces of parthenolide in the product, as even a 30-fold parthenolide-depleted extract may contain enough allergen to elicit a response in pre-sensitized persons. The patch test reactivity seems to be enhanced by simultaneous testing with parthenolide. Clinically, the reactivity is lost over time, probably because of degradation of parthenolide, and in accordance with this, chemical studies, performed more than 2 years after the first patient was tested, could not detect parthenolide in the cream.

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