

Assessment of balsam of Peru patch tests

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To find an ideal test technique for as low a dose of balsam of Peru (*Myroxylon Pereirae*) as possible, subjects testing positive to balsam of Peru are re-tested with a 25% concentration of balsam of Peru in petrolatum. Applications are with Finn Chambers[®] for 6 different application times, and directly by foils for 96 h (4 days (D)). The goals are to confirm which subjects are positive and which are not, and, using that information, to see if it is possible to distinguish between these 2 groups, tested concomitantly at much lower serial dose levels, in terms of perfusion or by visual assessments. 5 different serial doses are applied with strips for 3–96 h (4D) and with foils for 96 h (4D). The Finn Chamber[®] tests allow a distinction between visually positive and negative subjects supported by perfusion assessments. With the foils, a 24× lower serial dose level than with the 25% test substance is sufficient to distinguish between positive and negative subjects in terms of perfusion values. This approach requires readings up to 9 days. With this test, the visual approach yields only 3 of 10 positive subjects. This study demonstrates that a lower test dose is possible with perfusion assessments compared to visual ones.

Key words: test technique; test procedure; laser Doppler perfusion imaging; bioengineering methods; dose level; application time; reading time; sensitivity; specificity; balsam of Peru; *Myroxylon Pereirae*. © Munksgaard, 2000.

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Balsam of Peru (*Myroxylon Pereirae*) is a marker of fragrance sensitivity (1). It is a mixture obtained from *Myroxylon pereirae*, a tree mainly found in Central America (2). Variable ingredients have been defined (2–5), but the allergens are still poorly defined (6). Many perfume allergens are ingredients of balsam of Peru or closely related chemicals (1). Aside from contact dermatitis, there have been reports of contact urticarial reactions (3, 7, 8) and phototoxic reactions (9) due to the balsam.

Patch tests are traditionally assessed visually, and thus subjectively. An alternative to such assessments are non-invasive objective assessments of perfusion of patch tests by the laser Doppler imaging technology used in current study (10–18).

Goals

For a study population followed over time, we first determine allergy status of subjects. We test them with 25% balsam of Peru in petrolatum (approximately 6.6 mg/cm² balsam of Peru) applied with Finn Chambers[®] for 6 different time intervals and directly by foils. We follow reactions up to 216 h

(9 days (D)) at certain time intervals. Then we use the information of allergy status of subjects to determine if it is possible to distinguish between positive and negative subjects by using much lower concomitantly tested serial dose levels with either of 2 reading techniques – the conventional visual assessment approach and the laser Doppler perfusion imaging approach.

Materials and Methods

This study uses the same subjects, materials and methods as used in a recent study investigating the test technique of balsam of Peru and problems associated with the interpretation of perfusion with pigmented test substances. For a detailed description, the reader is referred back to that study (19). In summary, 13 balsam of Peru patch-test positive subjects are re-tested with tapes, each containing approximately 20 µl of 25% balsam of Peru in 9-mm Finn Chambers[®], and balsam of Peru in 5 serial doses printed on polyester squares (0.88, 0.28, 0.091, 0.03 and 0.01 mg/cm² balsam of Peru resin in polyvidone). All strips contain control

patches. Additionally, all tests are applied with transparent foils with the exception of direct applications of the 25% test substance and petrolatum controls to foils. Application times are 3, 6, 24 (1D), 48 (2D), 72 (3D) and 96 h (4D) on each subject with the tapes and 96 h (4D) with the foils. Readings are performed at 3, 6, 24 (1D), 48 (2D), 72 (3D), 96 (4D), 168 (7D) and 216 h (9D) following applications regardless of the time when applications are detached. Tests applied with foils were read twice at the session when foils were detached, both prior to and following the detachment. All readings are performed with a laser Doppler perfusion imaging technique with the laser Doppler perfusion imager® (LDPI), and visually according to a standard accepted by the North American Contact Dermatitis Group (20). Approximately 10,000 readings were performed. Approximately 5% of planned readings were not performed, mostly explained by subjects who were allowed to skip 3- and 6-h applications. In order to obtain perfusion caused by the active test agent, mean perfusion of control patches is subtracted from allergen containing patches. The resulting value is termed "adjusted perfusion value" (APV), indicated in V.

Results

With the Finn Chambers®, 10 of 13 subjects became visually positive. All 10 became positive with 48-h (2D) applications. Table 1 shows test results at that application time with required reading times for detection purposes. The testing in the table reflects the most sensitive way of testing in the study when visual assessments were used. Perfusion in the table relates well to visual assessments. No additional subjects were detected visually with the 25% test substance applied with the foils.

Based on test results with the 25% test substance as an indicator of subjects' allergy, the lowest serial dose levels that distinguished between positive and negative subjects with the LDPI were either 0.03 or 0.28 mg/cm² balsam of Peru applied with foils. Table 2 shows optimal testing with the 0.28 mg/cm² dose level. In the table, 6 subjects, positive to the 25% test substance, are visually negative and 1 has a questionable reaction. For the 0.03 mg/cm² dose level, a 6-h reading is required to detect 1 of the positive subjects. Since that reading time was performed on only a limited number of subjects, a comparison between positive and negative subjects at that dose level is unreliable. For visual assessments, no serial dose was usable to distinguish between the positive and the negative subjects and no subject was visually positive with only the serial doses.

Table 1. Application of 25% balsam of Peru in Finn Chambers® with tape for 48 h; readings are taken at 72, 96 and 168 h following the application

Subject no.	Highest APV assessment (V)	Highest visual assessment
7 ^{a)}	4.87	+
8	4.75	+++
13	3.71	++
11	1.76	+
1	1.47	+
6	1.41	+
4	1.10	+
10	1.01	+
5	0.96	+
12	0.20	+
3	0.11	—
9	0.11	—
2	0.10	—

a) Patch-test area partly covered by a compound nevus.

Table 2. Test results of a 96 h application of 0.28 mg/cm² balsam of Peru polyester patches applied with Tegaderms™; readings are performed at 24, 72, 168 and 216 h following the application

Subject no.	Highest APV assessment (V)	Highest visual assessment
8 ^{a)}	3.17	++
4	1.79	+
12	0.87	?
10	0.59	—
6	0.56	—
7	0.52	+
13	0.21	—
1	0.20	—
11	0.20	—
5	0.17	—
9	0.11	—
2	0.03	—
3	0.00	—

a) Foil detached between 24 and 48 h.

Discussion

Ideally, an allergic patch test should use as low bio-availability of allergens as possible yet still achieve both 100% sensitivity and 100% specificity. This requires a sensitive test that is without irritant properties. This may be difficult to achieve (21, 22). The current study uses the 25% concentration of balsam of Peru in pet. applied with Finn Chambers® for different time intervals on each subject and directly by foils to detect as many positive subjects as possible. The reason for this selection is a study that recommends the 25% concentration be used for testing based on experiments with 1% to 25% balsam of Peru in laceranum anhydricum (23). The 25% concentration had even been used before by

Hjorth in his famous study (21) and has been suggested for children (24).

One problem with interpreting APVs for a specific test concerns the decision about which perfusion value divides subjects who are allergic from subjects who are not. One way to solve this problem is to rely on some other method as a sensor of subjects' allergy status at the time of testing. Obviously, patient histories may serve that purpose even though their reliability may sometimes be questioned and they may not represent allergy status at the time of testing. This study relies on a good relationship between visual assessments and perfusion assessments with the Finn Chambers® to assess those subjects who are positive and those who are not (Table 1). Such a good relationship between assessments of 2 different aspects of patch-test responses increases the reliability of visual assessments as a comparative method. Final allergy assessment may be complicated in that convincing clinical relevance of positive test results may be lacking or suspicion of allergy exist despite negative patch tests.

If the 25% test substance is used to confirm the allergy state of tested subjects in the current study (Table 1), different test techniques may be used if assessments are made with the LDPI to distinguish between positive subjects and those who are not. One possible way is the one in Table 1. However, one of the aims with patch testing is to keep the bioavailability of active test agents as low as possible by applying agents in appropriate vehicles for as short a period of time as possible, while sensitivity and specificity are kept as high as possible. While the favorable results with the LDPI at a dose which is $24 \times$ lower than the 25% test substance are felt to be promising (Table 2), real bioavailability with these tests is unknown. The same comment applies to the comparison between the visual test results with the 25% test substance and with the serial doses.

When balsam of Peru patch tests fail to confirm a clinically suspected contact allergy, other methods become alternatives. Oral challenge with balsam of Peru has been suggested as a diagnostic measure for cases with eczema of an obscure nature and where patch tests are negative (25). One disadvantage when interpreting such oral tests relates to the question of whether immunological or non-immunological mechanisms are responsible for flares seen in subjects with negative patch tests (26).

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