Comparison of irritatant reactions between using lyophilized and commercial food allergen extracts in atopy patch tests in a normal population

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Summary

Background: **Several authors have investigated the use of the atopy patch tests (APT) for the diagnosis of non–IgE mediated food allergy, primarily in patients with atopic dermatitis and digestive disorders. However, one of the difficulties in atopy patch testing is the lack of standardization. Several commercial APTs containing freeze-dried food extracts are now available, but their diagnostic accuracy is still largely undefined. The objective of this study is to evaluate the irritant reactions and safety of atopy patch tests in healthy subjects by using lyophilized and commercial food allergen extracts.**

Methods: **A cross-sectional study was carried out in healthy volunteers. Atopy patches using lyophilized and commercial allergens, including cow's milk, egg, wheat, soy and shrimp, were assessed. Additionally, commercial extracts of house dust mite (*D. pteronyssinus* 10,000 AU/ml, *D. farinae* 10,000 AU/ml) and American cockroach were also evaluated.**

Results: **Eighteen healthy volunteers (13 women, median age 26 years) were enrolled. All APT results, both from using lyophilized and commercial allergen extracts, showed no reactions. There were no systemic allergic reactions or irritant reactions observed.**

Conclusion: **APTs using lyophilized and commercial food allergen extracts and commercial extracts of house dust mite and American cockroach showed no irritant reactions in Thai non-atopic subjects.** ***(Asian Pac J Allergy Immunol 2012;30:158-61)***

Key words: *atopy patch test, food allergen, aeroallergen, normal population*

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Abbreviations:

AD = Atopic dermatitis
APT = Atopy patch test

Introduction

Since it was recognized that IgE-mediated allergic diseases are caused by exposure to allergens, it has been a common practice to establish the presence or absence of sensitization by re-exposing the individual to the allergen. Avoidance is a therapeutic strategy available upon the diagnosis of the allergy. Therefore, an early identification of patients who would profit from strict avoidance of allergens is important. Skin prick tests or determination of specific IgE to allergen represent the primary tools for investigation in allergic patients. However, there are many limitations to these tests because they can only detect IgE-mediated allergic response and some allergic diseases, such as atopic dermatitis (AD) and food allergy, may be mediated either by IgE (immediate type, type I) or by non-IgE factors, as seen in delayed type reactions mediated by T- cells (type IV). Atopy patch tests have become increasingly recognized in AD pathogenesis in the last few decades.[1-3] The T-cell response seems to play a role in producing skin lesions in AD patients and in skin reactions from atopy patch tests. Patch testing of aeroallergens especially in patients with atopic dermatitis was published as early as in 1937 by

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Rostenberg and Sulzberger[4] and in 1982 by Mitchell et al.[1]

In atopy patch tests (APT), the allergen is applied to skin under occlusion. The allergen penetrates the epidermis and is captured by IgE molecules which then bind to IgE receptors on Langerhans' cells.[5] Allergen–specific T-cells are thereby activated and initiate an eczematous reaction. The immunocyto-chemical findings of APT are the same as those found in atopic dermatitis lesions.[6] Previous studies have demonstrated that eczematous skin lesions could be induced in patients with atopic dermatitis by patch testing with aeroallergens and foods.[7-14] Food allergy may also involve both IgE-mediated and non IgE-mediated reactions. One study suggested that, compared to the use of skin prick testing alone, an additional evaluation of food protein sensitization by skin patch testing increased the effectiveness of identification of food allergy.[15] However, the major problems of APT are the variations in reproducibility, concentration and standardization of allergen extracts. While fresh food APT is preferred over commercial extract APT,[16] it can become putrid during a test time which spans 48 hours. Therefore, food allergens extracted under lyophilization are a better alternative to other types of allergen extracts used in atopy patch tests.

Nonetheless, before lyophilized allergen extracts-based APT can be applied to atopic patients, we should demonstrate that the test preparation is nonirritant and harmless in healthy control subjects. The purpose of this study was to evaluate the outcome of atopy patch test reactions in non-atopic subjects when lyophilized food and commercial allergen extracts are used. If the test proves non-reactive, nonirritant and harmless to healthy control subjects, it could be used further in the investigation in atopic patients.

Methods

Study population

This cross-sectional study was carried out in 18 healthy adult volunteers. Subjects with chronic diseases such as autoimmune diseases, immune deficiencies, cancer or allergic diseases and pregnant women were excluded. Approval for the study was obtained from the Institutional Ethics Committee and written informed consent was obtained from the subjects before enrollment into the study.

Figure 1. Demonstration of atopy patch testing on the back of a normal volunteer.

Intervention

Non-pregnant subjects were asked to discontinue antihistamines, systemic corticosteroids and topical corticosteroids at least 7 days prior to the test. The APT was performed using lyophilized food and commercial allergen extracts from both common foods and aeroallergens. Lyophilized food was prepared in lamina flow as 1 g/10 ml of lyophilized cow's milk, egg white, egg yolk, wheat, and soy bean in water and 1g/10 ml of lyophilized shrimp in Coca's solution (Coca's solution: 0.9% NaCl 5g, 0.4% phenol 4 g and $NaHCO_3$ 2.5g in sterile water 1000 ml., pH7.0). Commercially available allergen extracts included those from cow's milk, egg white, egg yolk, wheat, soy, shrimp, *D. pteronyssinus* 10,000 AU/ml, *D. farinae* 10,000 AU/ml and American cockroach 1:20 w/v (ALK-Abello, Port Washington, NY 11050). Isotonic saline solution was used as the negative control. All of the solutions were kept in 4°C. One drop (50 µL) of each allergen was dropped on filter papers which were contained in 12-mm aluminum cups (Finn Chambers on Scan pore; Epitest Ltd Oy, Tuusula, Finland). Then, the Finn Chambers on the Scan pore were taped with adhesive tape on the unaffected skin of the volunteers' backs. The occlusion time was 48 hours. The results were recorded twice at 20 minutes and 24 hours after removal of the cups (48 and 72 hours after starting the test). The reactions were classified according to the European Task Force on Atopic Dermatitis (ETFAD) guidelines[17] as: negative (no reaction), ± doubtful reaction (only erythema, questionable), + weak reaction (erythema, infiltration), ++ moderate reaction (erythema, few

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papules), +++ strong reaction (erythema, many papules) and ++++ extreme reaction (erythema, vesicles). Erythema alone without infiltration was regarded negative, as it can be the result of local irritation.

Statistical analysis

The descriptive values of continuous variables were expressed as median (interquartile range). A *p* value of 0.05 or less was considered significant. The statistical analysis was performed using the SPSS software package for Windows (release 13.0; SPSS Inc., Chicago, IL, USA).

Results

Eighteen healthy volunteers (13 women and 5 men) participated in the study. The median age was 26 years, with a range of 22 to 34 years. None of them had a history of atopic diseases or chronic or severe illnesses. All APT results showed negative reactions with both lyophilized food (cow's milk, egg white, egg yolk, wheat, soy and shrimp) and when commercial allergen extracts (cow's milk, egg white, egg yolk, wheat, soy, shrimp, *D. pteronyssinus*, *D. farinae* and American cockroach) were used. The only adverse reaction was mild pruritus in the area around atopy patch application which was found in 12 of 18 volunteers (66.7%). Six of those with pruritus had erythematous rashes at the micropore area of APT. This adverse reaction occurs similarly with both lyophilized food and when commercial allergens were used. No systemic or irritant reactions, such as sharply demarcated confluent erythema, were observed in the APT area.

Discussion

Many reports suggest that APT is useful in identifying the cause of allergic contact dermatitis. It involves the placement of various concentrations of contact allergens in aluminum (Finn) chambers onto the normal skin, held against the skin using a hypoallergenic paper tape.[8,18] It has been estimated that approximately 16% of all chronic eczema patients would benefit from contact allergy patch testing.[19]

The APT reaction is histologically characterized by acanthosis, spongiosis, and a dermal infiltrate consisting predominantly of CD1+ cells, CD4 + T-cells and activated eosinophils.[20] The macroscopic and microscopic findings were similar between the specimens from APT sites and skin with lesions of patients with atopic eczema.[20] Positive APT with cow's milk was found in about 50% of aeroallergen-sensitized patients with atopic eczema.[21]

In 1996, Isolauri and Turjanmaa[21] first reported that a combined skin prick and patch test enhanced the diagnosis of food allergy in AD infants. The effectiveness of APT over SPT in the diagnosis of flood allergy in AD is controversial.[7,22-24] These controversial data might be explained by the fact that the APT is sometimes difficult to interpret. Moreover, APT with foods is not well-standardized and different methods in preparing test materials are likely to produce inconsistent results.

A study in 2007 demonstrated that the diagnostic accuracy of APT is higher with fresh food than with commercial food extracts.[16] However, using fresh food as the allergen creates some difficulties, as the food may become putrid during the 48-hour long application and as it is not practical to prepare many kinds of fresh food in each session.

This study has shown that using lyophilized food and commercial allergen extracts, including extracts of house dust mite and American cockroach, in APT is a safe technique. Positive APT responses did not occur in normal subjects. There was only an erythematous rash around the Finn chambers in 6 volunteers as a result of irritation from adhesive tape. Mild pruritus was found in about 67%. We conclude that using lyophilized food and commercial allergen extracts, including extracts of house dust mite and American cockroach in APT, in these concentrations show no irritant reaction in healthy subjects and that the method may be further applied in atopic patients

Conclusions

No irritant reaction in Thai non-atopic subjects has been shown when using lyophilized and commercial food allergen extracts and commercial extracts of house dust mite and American cockroach in APT.

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