Standardization of House Dust Mite and Cockroach Extracts: Current Status of Allergen Standardization in Korea

Department of Internal Medicine and Institute of Allergy, Yonsei University College of Medicine

Kyoung Yong Jeong

Introduction

Standardization of allergen extracts is essential for the manufacturing diagnostic and immunotherapeutic reagents for allergic diseases. Currently, allergens of importance in Korea are being standardized by in vitro and in vivo methods. House dust mites, Dermatophagoides farinae and D. pteronyssinus, are the most important source of indoor allergen, followed by cockroach. Allergen extracts from Korean isolates of house dust mites and German cockroach are prepared and characterized. In vitro standardization of allergen extracts are mainly based on the overall allergenicity of the extracts. Allergenicity of the extracts is known to be proportional to the concentration of major allergen in the extracts, and characterization and quantification of major allergen is a major concern for the standardization.

The commercial extracts have been standardized by comparison with a reference standard produced at the Center for Biologics Evaluation and Research (CBER) at the Food and Drug Administration (FDA) in the US since 1988. However, in-house reference (IHR) preparations from European companies are utilized for standardization. In Korea, commercial allergen extracts are mostly imported from European countries. Commercial mite and cockroach extracts shows considerable variability in protein and major allergen content.

We will discuss the current status and future directions of allergen standardization. Currently, allergen extracts, Korean reference preparations, of house dust mites, Dermatophagoides farinae and D. pteronyssinus, and German cockroach were prepared at the Research Center for the Standardization of Allergic Disease, Department of Internal Medicine and Institute of Allergy, Yonsei University College of Medicine, Seoul, Korea. At present, allergen extract and recombinant proteins which are thought to be importance in Korea including Japanese hop, Humulus japonicus, and Asian needle ant, Pachycondyla chinensis, are being prepared and characterized.
Major allergen content in the extracts

The consistency of allergen preparations, not only the potency but also the composition, is important for the standardization. However, concentration of major allergens even in the standardized skin test reagents of house dust mite is variable, ratios of Der p 1 (12 ~ 30 μg/mL)/Der p 2 (3 ~ 15 μg/mL) ranging from 1.1/1 to 6/1. In the preparations of allergen extracts from Korean house dust mite isolates, 5.0 μg/mg of Der f 1, 12.0 μg/mg, 11.6 μg/mg and 12.4 μg/mg were determined by two-site ELISA. Measurement of major allergen content in the house dust mite extract is found to be hampered by the sequence polymorphism. Furthermore, mite extracts rich in fecal material (most of European companies) contain higher ratios of group 1/group 2 allergens than those rich in mite bodies.

The content of Bla g 1 and Bla g 2 in commercial glycerinated German cockroach extracts were 2,218 ~ 4,854 U/mL and 8 ~ 66 μg/mL, respectively. Furthermore, concentrations of Bla g 1 and Bla g 2 were not found to be correlated with the subjects’ skin test responses. Bla g 1 and Bla g 2 contents were determined to be 405 U/mg and 273 ng/mL in the extracts from Korean German cockroach isolate, where as 187 U/mg of Bla g 1 and 56 ng/mg of Bla g 2 was detected from US extract (HollisterStier). Sequence polymorphism of cockroach allergens does not seem to influence on the measurement of allergen concentration by two-site ELISA since no sequence variation was found at the monoclonal antibody recognition site. Allergenicity of cockroach extracts between Korean and US preparations was shown to be similar, despite of different concentration of Bla g 1 and Bla g 2. This observation may indicate that the concentrations of Bla g 1 and Bla g 2 do not proportional to the allergenicity of the extracts. It was also reported that Bla g 1 and Bla g 2 are not important in Korean atopic asthmatic children. It is necessary to identify new allergens which is valuable for the standardization of cockroach extract.

No cockroach extracts on market is strictly standardized until now. Weight to volume (w/v) is the most frequently expressed unit for the concentration of cockroach extract supplied by the manufacturers. A 1:10 w/v indicates that the extract contains the extractable material from one g of raw material added to 10 mL of extraction buffer. Biological potency of commercial German cockroach extracts were estimated to be 10 ~ 8,570 bioequivalent allergy unit (BAU)/mL in USA.

Stability of allergen extracts

Glycerol and human serum albumin are common used to enhance the stability of allergen extract, especially for the dilution. The amount of group 1 and 2 allergen in mite extracts is known to decrease rapidly after 1 year especially at elevated temperature.

Human serum albumin was found to increase the stability of Der p 1 in the extract about 13% when kept at 4°C. However, it could not play protective role when placed at room temperature. Addition of 50%
glycerol retained the allergenicity of *D. pteronyssinus* extract when placed at room temperature for one year. Addition of 0.03% human serum albumin increase the allergenicity of the extract to 93.1%. Temperature is the most important factor for the storage of mite extract for 94.3% of the allergenicity was kept for one year even no additive was added to the buffer.

It is natural that proteins in the extract degrade over time because mite group 1, 3, 6 and 9 allergens have proteolytic activities. There are believed that some more proteases which are not allergens, in the extract. Cockroach group 10 allergen is a serine protease. Protease activities of cockroach extracts are known to be much stronger than those of mite extracts. Protease inhibitors may be helpful to enhance the shelf life of the extracts.

**Concluding remark**

It is essential to standardize the allergen extracts allergy diagnosis and immunotherapy. However, there are some limitations to standardize the crude extracts. For example, allergenicity of house dust mite extract determined by in vivo method showed 3~4 stronger, compared the value determined by in vitro method. Standardization based on the concentration of allergenic components is a global trend. It is necessary to produce recombinant allergens in order to overcome the problems of crude extract. However, both crude extracts and the recombinant allergens are believed to be produced and complement each limitations. Production of recombinant allergens will also allow the component-resolved diagnosis and immunotherapeutic approach with genetically engineered molecules.

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