Allergen Skin Prick Test Should Be Adjusted by the Histamine Reactivity

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Key Words

Allergen · Histamine dihydrochloride · Skin prick test · Biological activity

Abstract

Background: Skin prick test results are mostly reported as mean wheal diameter obtained with one concentration of allergen. Differences in technique between personnel causes variation in wheal size. The research question was whether the influence of differences in skin prick test technique among assistants and centers can be reduced by relating the allergen wheal response to that of histamine. **Methods:** Two methods for estimating skin reactivity, the method of Nordic Guidelines using histamine as a reference and the method of Brighton et al. [Clin Allergy 1979;9:591–596] not using histamine as a reference, were applied to data from two biological standardization trials, using the same batch of freeze-dried timothy pollen preparation. **Results:** The concentration defining the Nordic biological unit, defined as a concentration of allergen eliciting a wheal of the same size as that of histamine dihydrochloride 10 mg/ml, did not differ between the centers. When not using histamine as a reference, applying the method of Brighton et al., there was a 15-fold difference in the estimate of the biological activity between the trials that was eliminated by adjusting the allergen response to that of the histamine reference. **Conclusions:** To reduce the influence of differences in test technique among assistants and centers responses to allergen-induced skin prick tests should be compared to that of histamine.

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Background

In 1973, Aas and Belin [1] proposed the area of the allergen wheal of similar size as that of histamine dihydrochloride 1 mg/ml given a relative size of +++, if double the size/area of histamine +++, and if half the size/area of histamine ++. The method that is semiquantitative gives only a rough estimate of allergen reactivity and has never been validated. Furthermore, the potency and composition of extracts used and the skin prick test technique were not considered. Since the beginning of the 1980s, standardized extracts have been marketed [2] and the recommended histamine reference recommended in Europe has been changed from 1 mg [2–4] to 10 mg histamine dihydrochloride/ml [5–7].

The EAACI position paper on allergen standardization and skin tests [6] (published in 1993) recommended using either the + system [1] without a cutoff limit or ≥ 3 mm wheal diameter as a cutoff limit for positivity. The + system [1] gives a rough estimate since the difference in reactivity of the skin differs about four magnitudes between + and ++. Both the + method and the wheal diameter are depending on many factors such as allergen potency and composition as well as the test techniques of assistants. The histamine response reflects the skin reactivity to the mediator as does the histamine

Trial Registration: The original trials were performed before registration was possible.

bronchial challenge test. The skin reactivity varies between individuals and depends on the degree of allergy.

To test the question whether relating the allergen response to the histamine response is needed or not, data from two previously published trials on biological standardization [4], using the same freeze-dried timothy pollen allergen extract, were evaluated by either using histamine HCl 1 mg/ml as a reference [3, 4] or not [8].

Methods

Patients

Totally 39 patients, 15–50 years of age, were included in two trials, one in Uppsala, Sweden, and the other in Berlin, Germany, estimating the biological activity of the freeze-dried in-house reference timothy extract from Pharmacia Diagnostics (Uppsala, Sweden) [4]. Inclusion criteria were a history of grass pollinosis and positive skin prick test and timothy-specific IgE, a wheal ≥ 4 mm in diameter using histamine HCl 1 mg/ml, no previous immunotherapy with grass allergen or cross-reacting allergens, living in an environment where grass was an adequate problem, and not taking antiallergic drugs influencing the test results [9]. Twenty-three of 31 patients in Berlin, Germany, and 8/8 in Uppsala, Sweden, fulfilled these criteria. Patients were included consecutively among patients referred to one or the other specialized allergy unit, i.e. the Department of Respiratory Medicine, Academic Hospital, Uppsala, Sweden or the Allergology Department of the Charité, Berlin, Germany [4]. The protocol was approved by the local ethics committees and all patients signed an informed consent.

Timothy Pollen Allergen Extracts

The same batch of partly purified, freeze-dried timothy allergen extract (Pharmacia, batch DI 6359) [4], 59% protein by amino acid analysis [10], was used in both centers. Reconstitution with Albumin® diluent (0.03% human serum albumin, 0.4% phenol in saline, Pharmacia) was done on the day of testing.

Skin Prick Test Procedure

The skin prick test was performed according to Pepys [11], and the principles of the EAACI position paper were applied [6].

Methods for Estimation of the Biological Activity of the Extract

The method of Brighton et al. [8], estimating the concentration of allergen causing a 75-mm² wheal area, and that described by Dreborg and Grimmer [3], estimating the concentration eliciting a wheal of the same size as that of histamine dihydrochloride, 1 mg/ml, were used.

Original Method and Data. The original trials [4] used six threefold dilutions of the stock solution and histamine dihydrochloride, 1 mg/ml, i.e. 5.34 mmol/l, tested in duplicate on the back of the patient.

Evaluation Methods

Method of Brighton et al. [8]. Originally, four tenfold predetermined dilutions were tested in duplicate [8]. The geometric mean of all areas obtained in all patients with each concentration was used, i.e. the regression line was based on only four geometric

means, summarizing the response in the cohort. The model $\log(\text{area}) = a + b \log(\text{concentration})$ was employed to estimate the concentration eliciting a wheal with an area of 75 mm², i.e. about 10 mm in diameter (fig. 1a) that defined the unit. Since six threefold concentrations had been used in our original trial, these six concentrations were utilized (fig. 1b).

Method of Dreborg and Grimmer [3]. Six threefold predetermined dilutions of allergen preparation and histamine HCl 1 mg/ml were tested in duplicate [3, 4]. The mean diameter of each concentration was used and the concentration eliciting a wheal of the same size as that induced by histamine HCl 1 mg/ml in the individual patient (C_{h1}) was calculated according to the model best fitted to the dose response of allergen, i.e. $\log D = a + b \log(\text{concentration})$ [12, 13]. The mean of the C_{h1} concentrations defined the unit (fig. 1c). Thus, C_{h1} was determined for each patient and the mean C_{h1} determined the unit.

Results

Data were accepted from 23 patients in Berlin and from 8 patients in Uppsala. The histamine wheal sizes were significantly larger ($p < 0.05$) in Uppsala than in Berlin. The same basic data, i.e. the sizes of skin prick wheals, were used for evaluation using both methods.

As measured by the method of Brighton et al. [8], without adjustment to the histamine reference, the estimated amount of allergen in dry weight per milliliter of the extract calculated to induce a 75-mm² wheal response in the patient sample was 15 times higher in Berlin than in Uppsala.

Correcting for the smaller wheals in Berlin, by using the histamine reference for equilibration between the centers [3, 4], i.e. reducing the influence of differences in technique, the biological activity of the timothy extract as evaluated based on data from Berlin was 0.8 of that based on data from Uppsala (n.s.). Thus, the total difference between the methods was 19 times.

Discussion

The + method of Aas and Belin [1] was proposed in 1973, i.e. before any standardized extracts had been launched. Thus, the total allergenic potency of extracts of the same source material could differ as much as 1,000 times in potency between batches [14]. The + method has been widely used since then, but never validated. The method gives a very rough estimate of skin sensitivity, since half the diameter of a wheal 6 mm in diameter (++) means about a 30-fold lower sensitivity and double the diameter means a much higher reactivity, i.e. a difference between ++ and ++++ of about 1,000 times.

Fig. 1. a The method of Brighton et al. [8], the original method, using duplicate tests with four tenfold dilutions of allergen and no histamine reference. Circles show the geometric mean of the mean size of two wheals with the same allergen concentration in all included patients. **b** The method of Brighton et al. [8] applied to our original data [4] using six threefold dilutions. Circles show the geometric mean of the mean size of two wheals with the same allergen concentration in all included patients. **c** The method of Dreborg and Grimmer [3] using duplicate tests with six threefold dilutions per patient [4]. Circles show the mean size of two wheals in 3 individual patients. Modified after Dreborg [15].

The technique varying between technicians and centers causes differences in wheal size, making it difficult and not evidence-based to compare skin test results between assistants and centers as is often done in e.g. epidemiological trials.

This study shows that by adjusting the allergen skin response to that of histamine, the difference in technique within and between testing personnel and centers can be minimized. Simple methods for equilibration of skin test results using histamine reference will be evaluated.

Conclusions

Adjusting the allergen wheal size to that of the directly acting histamine reference corrects for differences in technique. Therefore, the histamine reference should be used for equilibration of results among testing personnel in the same unit, and among centers and regions, both in clinical trials and in clinical practice.

Acknowledgments

My sincere thanks to Prof. Olle Zetterström, Uppsala, and Prof. Gerhard Kunkel, Berlin, and their skin testing nurses who did the original skin testing, as well as Margareta Holgersson[†], PhD, who gave statistical advice and Anders Hansson, BSc, statistician, who did the computer work.

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