

Skin Prick Testing and the Use of Histamine References

H.-J. MALLING

Laboratory of Clinical Allergology, Medical Dept. TA, Rigshospital, University of Copenhagen, Denmark

The skin prick test is a fundamental test in biological allergen standardization and in evaluation of changes in skin sensitivity due to treatment. The allergen concentration eliciting a wheal equal to that produced by histamine 1 mg/ml is generally accepted as the skin sensitivity. Using a standardized quantitative skin prick test, 25 mould allergic patients were tested with quadruplicate determinations of five 10-fold allergen concentrations of highly purified and standardized extracts. Histamine 1 and 10 mg/ml were used as positive references. The 10-fold increase of histamine resulted in a doubling of the histamine reaction and increased the mean wheal diameter from 4 to 7 mm. The correlation between skin sensitivity estimated by histamine 1 and 10 mg/ml is significant, but with a dissociation between the two ways of estimating the sensitivity of 0.25 log step in the low sensitivity range and 1.8 log step in the high sensitivity range (the difference at median sensitivity is 1 log step). No correlation was found between histamine- and allergen-induced wheal area increase, and the discrepancy might be caused by a difference in the endogenous histamine release and/or difference in the number of histamine receptors at different degrees of sensitivity. With the use of median values it is possible to perform biological standardization with histamine 10 mg/ml and interpolate to histamine 1 mg/ml. However, the response in individual patients varies, and because of the small wheal area and the low reproducibility with histamine 1 mg/ml we recommend the exchange of histamine 1 mg/ml to histamine 10 mg/ml as an international positive reference.

Key words: Skin prick test, histamine reference, biological standardization, quantitative skin test, skin sensitivity.

Accepted for publication 2 June 1984

CLINICAL ASPECTS

The skin test reaction to allergen extracts is an expression of the degree of allergy (the skin sensitivity). The result of the skin test might be expressed as the allergen concentration eliciting a wheal equal to a reference. In Scandinavia histamine chloride 1 mg/ml (the HEP system) is used. Due to very small and hardly reproducible wheal reactions with this reference it is often substituted by histamine 10 mg/ml and the result is interpolated to histamine 1 mg/ml. In order to improve the biological standardization of allergens and thereby the safety and precision of diagnostic procedures the use of histamine 10 mg/ml as reference is recommended.

Skin testing of allergic patients is valuable, both as a diagnostic tool and as a quantitative technique for measuring allergen potency and stability. Furthermore, the quantitative skin test may be used to evaluate the change in sensitivity during a course of immunotherapy.

The skin prick test has almost the same reproducibility as the intracutaneous test (5) and

has the further advantages of being technically easy to handle, involving a reduced risk of anaphylaxis, and being more convenient for the patients.

The result of the skin test (skin sensitivity) might be expressed as end-point (i.e. a trace reaction) or as reference equivalent reaction (the allergen concentration eliciting a wheal

equal to the one produced by a reference, i.e. histamine, compound 48/80, etc.). A histamine reference of 1 mg/ml has been recommended by the Northern Society of Allergology (7). However, the use of histamine 1 mg/ml might result in very small wheal reactions difficult to interpret, thus some authors use histamine 10 mg/ml as reference and then interpolate the observed result to histamine 1 mg/ml (the HEP system) (1).

The present study is an evaluation of the legality of performing this transformation both in biological standardization and when monitoring the individual patient response during treatment.

MATERIAL AND METHODS

Patients

Twenty-five adult asthmatics (9 females and 16 males) aged 17–45 years were tested. Thirteen patients were allergic to *Cladosporium* and 12 to *Alternaria* based on symptom score, skin prick test, RAST and bronchial challenge. Excluded from the study were patients with eczema on the test area, patients treated with anti-histamines, steroid ointments and oral steroids > 10 mg prednisone per day, and, finally, patients who had been on immunotherapy with the involved allergens within the last 12 months.

Test extracts

Pharmalgen®, *Cladosporium* and *Alternaria* (Pharmacia, Uppsala, Sweden), highly purified and standardized freeze-dried extracts, were reconstituted in albumin diluent to a concentration of 1:20, 1:100 and 10-fold to 1:1,000,000 w/v. Reconstitution and dilution were performed on the day of skin testing to assure maximum potency. Histamine chloride 1 and 10 mg/ml (5.43 and 54.3 mmol/l) was used as positive control and albumin diluent as negative.

Skin test procedure

Skin prick test was carried out on both forearms of the patients' in quadruplicate determinations

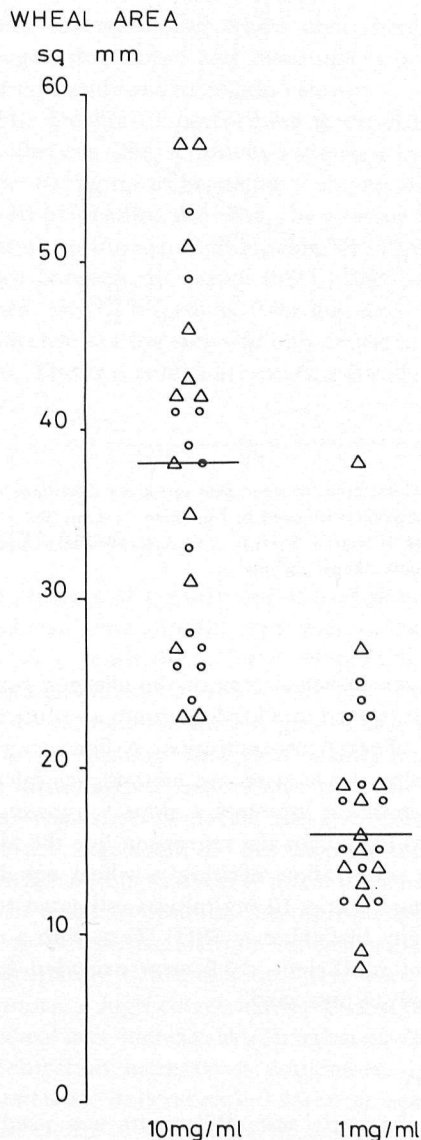


Fig. 1. Skin prick test wheal area using histamine HCl 1 and 10 mg/ml. The median values are marked. △ represents patients primarily allergic to *Cladosporium* and ○ to *Alternaria*.

of allergen, diluent and histamine 1 and 10 mg/ml. A 1 mm precision needle was used for testing, as previously described (4). The wheal reactions were recorded after 12 min and the contour outlined and transferred to a record sheet. The wheal area was calculated in square mm by a Hewlett Packard digitizer.

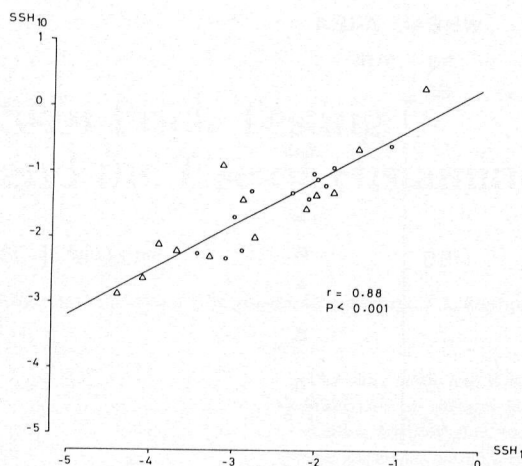


Fig. 2. Correlation between skin sensitivity (histamine equivalent reaction) estimated by histamine 1 mg/ml (SSH_1) and histamine 10 mg/ml (SSH_{10}). Δ *Cladosporium*-allergic patients. $\circ$ *Alternaria*-allergic patients.

The mean wheal area of the allergen reactions was plotted in a log-log system as a function of the allergen concentration. A linear regression using the least square method was calculated ($\log \text{ area} = \text{intercept} + \text{slope} + \log \text{ concentration}$) (6). From the regression line the allergen concentration eliciting a wheal equal to histamine 1 and 10 mg/ml was estimated (skin sensitivity histamine = SSH). Tests with a correlation coefficient < 0.9 were excluded from the study as non-valid.

Statistics

Non-parametric test (Wilcoxon) was used to compare paired data. Linear regression was performed with the least square method.

RESULTS

The reproducibility of the presented way of estimating skin sensitivity using linear regression was within 0.5 log step (data not shown).

The wheal area increase using 10-fold allergen concentration was median a doubling (slope 2, range 1.5–3). Similarly, increasing the histamine concentration from 1 to 10 mg/ml resulted in a doubling of the wheal area (me-

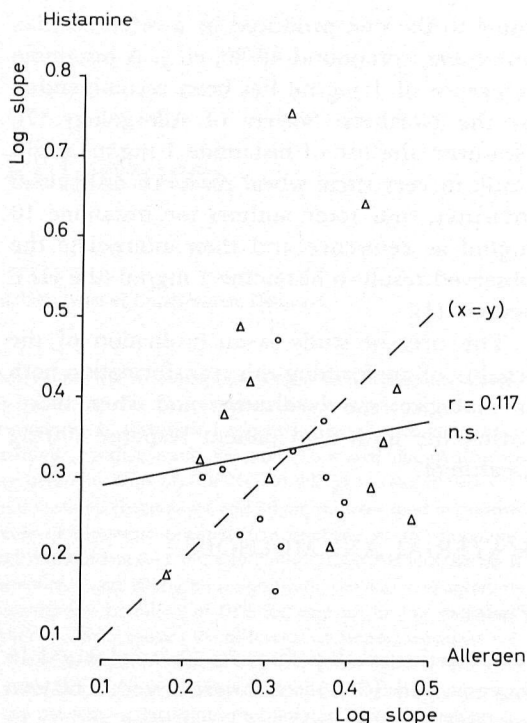


Fig. 3. Correlation between corresponding values of histamine- and allergen-induced wheal area increase (log slope of the regression curve of individual patients).

The line of identity ($x = y$) in the log-log system is indicated. This corresponds to an identical wheal area increase using 10-fold dilution of histamine and allergen (identical log slope of histamine and allergen regression curve). Δ *Cladosporium*-allergic patients. $\circ$ *Alternaria*-allergic patients.

dian) (Fig. 1). As is also evident from the figure, the median wheal area of histamine 1 mg/ml is 16 sq mm (8–38 sq mm) corresponding to a diameter of 4.5 mm (3.2–7 mm). Using histamine 10 mg/ml the results are median 38 sq mm (23–57 sq mm), diameter 7 mm (5.4–8.5 mm).

The relation between the histamine equivalent reaction estimated by 1 and 10 mg/ml is evident from Fig. 2. The correlation between SSH_1 and SSH_{10} (corresponding to histamine 1 and 10 mg respectively) is highly significant ($P < 0.001$). The slope of the regression line is 0.7, indicating a non-constant relation between the two ways of estimating the sensitivity at different degrees of sensitivity. The difference between SSH_1 and SSH_{10} in the low sensitivity

Log allergen conc.

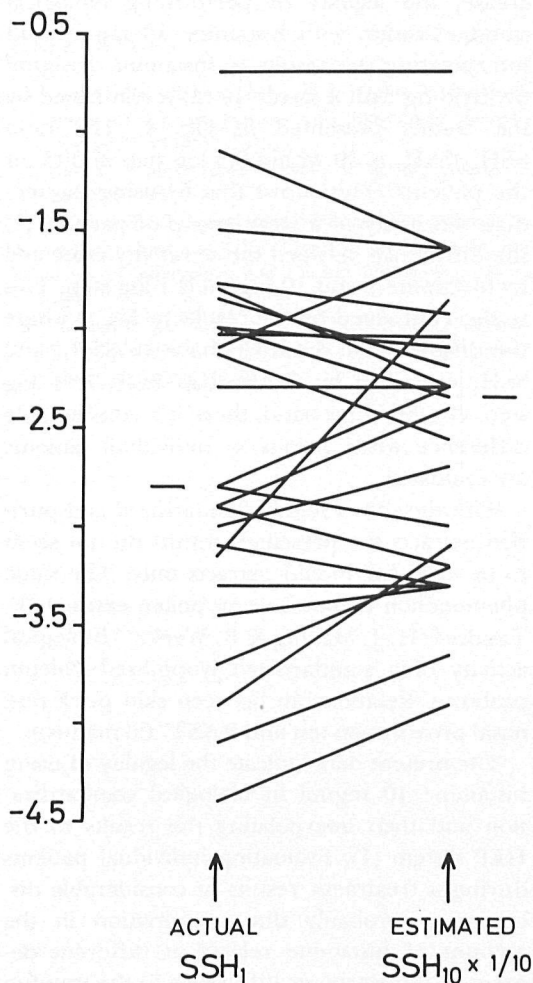


Fig. 4. Corresponding values of skin sensitivity evaluated by histamine 1 mg/ml (actual SSH_1) and interpolated from histamine 10 mg/ml (SSH_{10}) divided by a factor 10 (estimated). The median values are marked.

range (log allergen concentration = 0) is 0.25 log step contrary to 1.8 log step in the high sensitivity range (log allergen concentration = -5).

In the case of a constant relationship between the allergen skin sensitivity estimated by histamine 1 and 10 mg/ml, a constant relation between wheal area increase using 10-fold allergen concentration and the two histamine concentrations should be expected. Fig. 3

shows corresponding wheal area increase of allergen (log slope) and histamine (log slope), and no significant correlation exists.

The legality of performing interpolation of the allergen skin sensitivity estimated by histamine 10 mg/ml to histamine 1 mg/ml was evaluated by dividing the SSH_{10} by a factor 10 and relating to the actual SSH_1 (Fig. 4). The difference between the actual (SSH_1) and the estimated ($SSH_{10} \times 1/10$) is 0.40 log step, and a difference > 1 log step was only found in about 10%. The two results are not statistically different.

DISCUSSION

The purpose of performing titrated skin test is to achieve a meaningful expression of the patients' skin sensitivity. This is valuable both in individual patients in order to evaluate the efficacy of a treatment and in groups of patients when performing biological standardization. The advantage of quantitative skin test using linear regression is that all allergen concentrations are equivalent in determining the best fitted line and in that way to assess the sensitivity (6). In order to optimize the reproducibility of skin prick test five allergen concentrations and quadruplicate determinations were used. Furthermore, a high correlation coefficient (identical wheal area increase as a function of allergen concentration increase) is mandatory. In the present study only values > 0.9 were accepted.

The choice of a definition of skin sensitivity is arbitrary and the most important point is the reproducibility. The advantage of using histamine equivalent reactions contrary to end-point titration is that the relatively large reactions are more easy to reproduce (low standard deviation) (2).

The recommended histamine concentration of 1 mg/ml (1, 6) seems in our hands to give rather small reactions. Especially with the histamine reference it is important to have a high reproducibility and we therefore prefer a wheal area > 20 sq mm. Smaller reactions tend to give a high variability. In order to compensate for

this some authors prefer the use of histamine 10 mg/ml as reference (3). The wheal area of histamine 10 mg/ml is approximately the double of histamine 1 mg/ml and in all cases >20 sq mm. In the present study no reactions exceeded a diameter of 10 mm and no case of irregular wheals with pseudopodium was found.

The correlation between the skin sensitivity estimated by histamine 1 and 10 mg/ml (Fig. 2) is interesting. A slope of 0.7 indicates that a reduced sensitivity of 1 log step estimated by histamine 1 mg/ml only results in a reduction of 0.7 log step estimated by histamine 10 mg/ml. This is caused by the difference between the two estimates of sensitivity in the high and low sensitivity range. In the case of a constant relationship between the sensitivity estimated by the two different histamine concentrations the slope would be expected to be 1.0 with a difference of 1 log step. The size of the histamine wheal is the primary factor in determining skin sensitivity. The slope of allergen-induced wheal area is only of secondary importance. The data show a clear-cut tendency towards a very small wheal area increase by elevating the histamine concentration from 1 to 10 mg/ml in the low sensitivity range and vice versa. The cause of the discrepancy might be the more than 10-fold increase of skin mast cell histamine release in low sensitive patients and the contrary in highly sensitive patients.

This hypothesis is supported by the finding of no correlation between the wheal area increase using allergen and histamine (Fig. 3). In a constant relationship between histamine- and allergen-induced wheal area increase the observations would be expected to be grouped along the line of identity. The release of different amounts of histamine from skin mast cells in different patients after allergen challenge seems a reasonable explanation. Difference in the number of histamine receptors would not result in a dissociation between the wheals induced by either endogenous or exogenous histamine, but a reduced number of histamine receptors in low sensitive patients would explain the very small wheal area increase between the two 10-fold histamine concentrations.

In spite of the obvious discrepancy between histamine- and allergen-induced wheal area increase, the legality of performing biological standardization with histamine 10 mg/ml and interpolating the results to histamine 1 mg/ml by dividing with a factor 10 (3) is confirmed by the results presented in Fig. 4. The ratio SSH_{10}/SSH_1 is 10 within 0.5 log step in 90% of the patients. This shows that by using the median sensitivity of a large group of patients (1) the difference between the sensitivity estimated by histamine 1 and 10 mg/ml is 1 log step. This is also confirmed by the results in Fig. 2 where the difference in sensitivity between SSH_{10} and SSH_1 estimated by the median SSH_1 is 1 log step. On the other hand, there is a considerable difference when results in individual patients are evaluated.

With the use of highly standardized and purified extracts the presented results do not seem to be valid for mould extracts only. The same phenomenon is found using pollen extracts (E. Taudorf, H.-J. Malling & B. Weeke: "Biological activity of a standardized lyophilized *Phleum pratense*. Relationship between skin prick test, nasal provocation test and RAST" (in manus)).

The present data indicate the legality of using histamine 10 mg/ml in biological standardization and then interpolating the results to the HEP system (1). Evaluating individual patients during a treatment results in considerable discrepancy, probably due to variation in the amount of histamine release at different degrees of sensitivity, or difference in the number of histamine receptors. The mentioned limitations compared with the problem of very small and hardly reproducible wheals using histamine 1 mg/ml, might imply a reevaluation of the recommended histamine reference. An international agreement on substituting histamine 1 mg/ml with histamine 10 mg/ml would be a consequent way of solving the problem, and is therefore recommended.

ACKNOWLEDGEMENTS

This study was supported by grants from Fortsættelsessygkassen *Danmark's Sundhedsfond*. Mould extracts and hista-

mine used for skin test were kindly supplied by Pharmacia Diagnostics AB, Uppsala, Sweden.

REFERENCES

1. Aas, K., Backman, A., Belin, L. & Weeke, B.: Standardization of allergen extracts with appropriate methods. *Allergy* 33, 130-137, 1978.
2. Aas, K.: Some variables in skin prick testing. *Allergy* 35, 250-252, 1980.
3. Dreborg, S.: Biological standardization. The aims and the methods. Abstract. XII Congress of the European Academy of Allergology and Clinical Immunology, Rome 1983.
4. Malling, H.-J., Andersen, C. E., Boas, M.-B., Holgersen, F., Munch, E. P. & Weeke, B.: The allergy pricker. *Allergy* 37, 563-657, 1982.
5. Nierop, G., Voorhorst, R. & Temmerman-van de Vijver, R. L.: Atopic skin test re-evaluated. X. Comparison of a perfected technique of intracutaneous skin testing and the prick test performed with a modified Morrow Brown needle. *Ann. Allergy* 46, 105-109, 1981.
6. Turkeltaub, P. C., Rastogi, S. C., Baer, H., Anderson, M. C. & Norman, P. S.: A standardized quantitative skin-test assay of allergen potency and stability: studies on the allergen dose-response curve and effect of wheal, erythema, and patient selection on assay results. *J. Allergy Clin. Immunol.* 70, 343-352, 1982.
7. Northern Society of Allergology: Standardization of allergen extracts. *Acta Allergol* 28, 219-220, 1973.

Address:

Hans-Jørgen Malling, M.D.
Laboratory of Clinical Allergology
Medical Department TA 7511
Rigshospital
Tagensvej 20
DK-2200 Copenhagen N
Denmark

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.