

Collaborative clinical assay of the biological potency of allergen skin-test extracts

J. CARREIRA*, M. RUBIO†, T. HERRERO†, F. HORAK‡, L. BUSINCO§, B. WÜTHRICH¶, V. BEARZI DE PRADA¶, J. B. BERRY**, G. LISCHKA†† and W. D. BRIGHTON‡‡

**Department of Biology, Research Centre, Abello SA, Madrid, Spain, †Hospital Provincial, Madrid, Spain, ‡1st ENT Clinic, University of Vienna, Vienna, Austria, §1 Clinical Pediatrica Università di Roma, Rome, Italy, ¶Allergiestation, University Hospital, Zurich, Switzerland, ** Merton Chest Clinic, Mitcham, Surrey, U.K., †† Universitätsklinik, Tübingen, West Germany, and ‡‡ Allergy Technical Consultancy, Havant, Hampshire, U.K.*

(Received 15 March 1983; accepted for publication 5 April 1983)

Summary

A multicentre collaborative clinical trial has been made at one centre in each of six countries to decide if the potency of allergen extracts can be determined satisfactorily by skin-prick tests in man.

Although there was considerable variation in potency of antigen when determined in different patients, if a sufficiently large group ($n = 54$) of persons were tested, it was shown to be feasible to compare potencies of sequential batches of the same specificity, and also of antigens of different specificities.

It was shown that batches of mite extract were weaker than that of a grass pollen, but it proved to be a simple matter to adjust the concentration to give the same biological potency.

Introduction

When allergen extracts are to be used for skin tests as an aid to diagnosis, it is essential that physicians can be sure that the potency of the extracts is constant and does not vary between batches of the same specificity. It is also preferable that extracts of different specificities should have the same activity. There is the additional complication of the wide variations in skin response in different patients and indeed in the same patient at different times.

If one of the factors—either potency of reagents or variation of patients—can be

Correspondence: W. D. Brighton, Allergy Technical Consultancy, 33 Bedhampton Hill, Havant, Hampshire, U.K.

controlled and standardized, it becomes easier to assess the significance of the results of the other. It is therefore clearly desirable that the skin-test reagents should be of constant potency between specificities, and reproducible between batches.

Assay of biological potency in man has often been unsatisfactory because the tests were based on numbers that were inadequate to overcome the limitation of variation in the patients tested, but the report of Brighton, Topping and Henocq (1979) showed that, if thirty patients were tested, straight regression lines could be achieved.

We report here the results of a collaborative clinical trial, done to decide whether this technique could be applied to the measurement of biological potency of a manufacturer's 'in-house' reference extract, and thereby to the potency control of sequential production batches.

Materials and methods

Skin-test extracts

These were from a new Abello SA range, made in glycerol-saline (50%) pH 7.2. The extracts are dialysed to remove irritants and other low-molecular weight substances and are then controlled in concentration by RAST inhibition and qualitatively by iso-electric focussing. When needed 2-dimensional electrophoresis is also done.

The participant physicians were asked to make duplicate prick tests on appropriately sensitive patients, with three different batches each of three different specificities: *Phleum pratense*, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*. The reasons for this choice were as follows: (i) to prove reproducibility of successive batches of extracts of the same specificity; (ii) pollen and mites may prove to be of different potencies; and (iii) it is known that the two mite species share some common allergens, whilst others are different. We wished to know whether there is sufficient difference between commercially prepared extracts to justify marketing extracts of separate specificities, or whether the effects of the cross-reacting allergens outweighs that of the differences.

Patients

The six physicians each examined four groups of nine patients, each of whom was of an appropriate sensitivity: Group 1 was tested with *D. pteronyssinus*; Group 2 was tested with *D. farinae*; Group 3 was tested with *Phleum pratense*; and Group 4 was tested with *D. pteronyssinus* and *D. farinae*.

Patients were of either sex, aged between 16 and 50, with a clinical history that showed sensitivity to the allergen and a positive skin-prick test. No patient was receiving medication or therapy at the time of the test. The following were excluded from the trial: pregnant women, patients suffering an acute allergic reaction, and patients receiving antihistamines or corticosteroids.

Tests

Each patient was tested twice, at the same time, with a randomized block pattern of prick test that included the original extract and two serial dilutions, all prepared in a central laboratory. Two pricks of the routine Abello production of histamine hydrochloride were included as positive controls. A total of twenty prick tests per patient.

Recording of results

Each weal was outlined by the physician with a fine felt-tipped pen, and the outline was transferred by means of adhesive cellulose tape to a record sheet. The completed sheets were collected together and the weal areas, in mm² were measured centrally with a transparent mm² grid.

Complete protocol

A copy of the detailed protocol is available on request. This includes the randomized block prick-test patterns.

Mathematical processing of the results

Geometric means were calculated according to the formula:

$$GM = (x_1 \cdot x_2 \cdot x_3 \cdot \dots \cdot x_n)^{\frac{1}{n}}$$

When this had been done for the original solution and the dilutions, the results gave a regression line for each batch of each specificity.

The coefficient *a* (intercept) was then calculated as a method of using all the results, dilutions as well as undiluted, to determine the corrected value of the mean potency of the undiluted extract. In this way 324 single tests were used to measure the mean potency of each extract.

$$\text{Coefficient } a = \frac{1}{n} (\Sigma y - b \Sigma x),$$

and the slope of the line, *b*, was

$$\text{Coefficient } b = \frac{\Sigma xy - \bar{x} \Sigma y}{\Sigma x^2 - \bar{x} \Sigma x}.$$

Results

The Geometric mean results are shown in Tables 1–4. Such differences as are shown in the tables are well within the limits recommended by Brighton (1980) and Nordic Council on Medicines (1982).

Table 1. Group-I patients, treated with *D. pteronyssinus* (geometric mean weal mm²)

Extract	Undiluted	1/3	1/10	Coefficient <i>a</i>
A	43.08	31.88	19.1	43.34
B	39.33	28.2	17.1	39.23
C	41.05	26.81	19.41	39.90

There was no significant difference between the batches.

Table 2. Group-2 patients, treated with *D. farinae* (geometric mean weal mm²)

Extract	Undiluted	1/3	1/10	Coefficient <i>a</i>
E	36.7	23.5	16.3	35.8
F	42.3	23.7	16.5	40.4
G	40.3	24.9	14.6	39.5

There was no significant difference between the batches.

Table 3. Group-3 patients treated with *Phleum pratense* (geometric mean weal mm²)

Extract	Undiluted	1/3	1/10	Coefficient <i>a</i>
H	67.6	48.5	30.5	67.4
I	63.4	41.6	29.4	61.4
J	69.9	47.6	32.0	68.8

There was no significant difference between the batches.

Table 4. Group-4 patients, treated with both *D. pteronyssinus* and *D. farinae* (geometric mean weal mm²)

Extract	Undiluted	1/3	1/10
<i>D.pt.</i>			
A	48.2	35.3	19.9
B	49.1	33.5	19.8
C	46.3	33.4	22.0
<i>D.far.</i>			
E	43.1	28.4	22.0
F	53.8	33.9	20.0
G	40.9	25.9	19.6

Table 5. Results of second assay of mite batches, after adjustment of strength, following first assay (geometric mean weals mm²)

<i>D. pteronyssinus</i>	63
<i>D. farinae</i>	69

Discussion

The concept of this method of assay was given by Brighton *et al.* (1979). For 75 mm² these workers assigned a potency of 10 BU, but we believe that when these extracts are to be made commercially and may be needed as weaker solutions for intradermal tests or therapy it is better that the unit should be smaller to avoid possible confusion with decimal points.

We have therefore assigned a value as follows: 100 Abello biological units (ABU) to that concentration of extract that produces a geometric-mean weal of around 75 mm² when tested in at least thirty sensitive patients.

The results of this trial showed without doubt that, whilst the production methods used for the extracts made batches of very close potency, the mite batches were of lower potency than those of the grass pollen.

When applying the regression lines produced by the trial, it was simple to alter the concentration of allergen in the mite extracts so as to induce weals of about the same size as those of the grass pollen, for materials intended for subsequent supply to physicians.

A second check was made after the changes in 44 patients, using only the undiluted material, to prove that the desired effect had been achieved. The results (Table 5), show that the revised products were of very close potency not only from sequential batches of the same specificity but also between specificities.

We have used these results to calibrate our *in vitro* RAST control assays, as recommended by the IUIS Joint Steering Committee on Allergen Standardisation (1977).

References

- BRIGHTON, W.D. (1980) Possibilities and limitations of potency determination. 'Regulatory control and standardisation of allergenic extracts. *Arbeiten aus dem Paul-Ehrlich Institut*, **75**, 113.
- BRIGHTON, W.D., TOPPING, M.D. & HENOCQ, E. (1979) Activity units for allergen extracts. *Clinical Allergy*, **9**, 591.
- IUIS JOINT STEERING COMMITTEE ON ALLERGEN STANDARDISATION (1977) *Report No. 1*. IUIS Standardization Committee, London.
- NORDIC COUNCIL ON MEDICINES (1982) *Registration of Allergen Preparations. Nordic Guidelines*, p. 13. N.L.N. Publication No. 7, Uppsala, Sweden.

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.