

Standardized Allergen Provocation Test

Variation in Bronchial Response

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Standardized bronchial allergen provocation with the same individual allergen dose was performed 3-9 times in 20 symptomless adult asthmatic patients at intervals of 1 week or more during $\frac{1}{2}$ -22 months in order to study variations in bronchial response. The immediate type reaction was recorded by monitoring FEV₁, FVC, PEFR and MEF_{50%} for 30-40 min after provocation. FEV₁ was highly significantly correlated to the other variables.

The coefficient of variation corresponding to maximum decrease in FEV₁ was 23%. All provocations were followed by a bronchial reaction within clinically acceptable limits. There was no indication of hypo- or hypersensitization.

It is concluded that although many different factors influence the bronchial response, careful standardization of the provocation procedure leads to a moderate variation in bronchial response, making the allergen provocation test suitable for pathophysiological studies and clinical evaluation of anti-asthmatic drugs.

Key words: drug evaluation method; extrinsic bronchial asthma; standardized provocation test.

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Like other allergic diseases, bronchial asthma of allergic type is rather unique in the sense that symptoms can be induced and reversed without exposing the patient to undue risk. This has made the bronchial provocation test useful in the diagnosis of allergic asthma but the test may also be used for pathophysiological studies of the disease and for clinical evaluation of anti-asthmatic drugs.

There are many different technical and biological factors that may influence the bronchial response after allergen provocation and for the research purposes the provocation procedures have to be standardized in various respects (1, 8, 20, 26).

The reproducibility of the test has been reported to be acceptable (1, 6, 7, 12) but in few studies have the bronchial provocation

procedures been standardized and the characteristics of the patients carefully described (2, 15, 25).

The aim of this investigation was to study the variation in bronchial response when using standardized provocation procedures in a defined group of adult asthmatic patients. Only the immediate type reaction was studied.

PATIENTS AND METHODS

Patients

Twenty adult patients, 11 men and nine women, with extrinsic bronchial asthma were included in the study. The diagnosis was documented by a positive history of allergy, positive prick test, positive RAST and

bronchial challenge with one or more allergens. Relevant clinical details are given in Table 1.

Altogether 103 provocations were performed. Each patient was challenged with the same individual allergen dose on 3–9 occasions at intervals of 1 week or more during ½–22 months. All provocations were performed during symptom-free intervals and values of FEV₁ were required to be within the normal range. No provocation was performed during the pollen season in patients allergic to pollen. Before challenge the patients were instructed to avoid exercise and exposure to relevant allergens for at least 24 hours. Four of the patients were moderate smokers, but smoking was not allowed 4 hours before challenge. All patients were given placebo (orally or by inhalation) before challenge. The patients were well informed and emotionally stable. The study was approved by the Ethical Committee of the University of Gothenburg.

During the investigation period of the

present study a further 2–20 allergen challenges (mean 8) were performed in the same patients, as they were also included in studies on anti-allergic drugs. Single doses of the drugs were given orally or by inhalation 15–180 min before challenge and performed at an interval of at least 1 week (21).

Medication

None of the patients was on regular anti-asthmatic therapy. Occasionally use of inhaled terbutaline, salbutamol or disodium cromoglycate was not permitted for 12 hours before challenge. No patient was being treated with immunotherapy. Bronchodilator spray was given 30–40 min after provocation if needed.

Allergen extract

Non-dialysed allergen stock solutions were prepared in our laboratory by aqueous extraction for 2 days in a weight/volume

Table 1
Selected clinical data of the patients

Patients	Age	Sex	Allergen	Prick-test	RAST	Allergen conc.	Time of inhal. (min)	No. of chall.	Investig. period (months)
1 B.A.	41	F	dog	+	2	1:100	2	7	22
2 H.E.A.	25	M	horse	+++++	4	1:10000	1	3	4
3 L.A.	28	F	birch	+++	2	1:1000	2	9	19
4 B.B.	22	M	cat	++	2	1:100	2	3	14
5 J.Bj	30	M	rabbit	+++++	—	1:1000	2	4	8
6 J.B.	34	M	dog	+++	4	1:10	4	8	8
7 P.E.	29	M	cat	+++	2	1:100	4	3	½
8 A.F.	23	F	cat	++	4	1:100	1	4	6
9 D.G.	19	F	cat	+	2	1:100	5	3	4
10 E.J.	38	F	hamster	+++	—	1:100	5	9	15
11 B.J.	22	M	cat	+	2	1:100	5	4	3
12 H.M.	20	M	cat	++	2	1:100	5	4	4
13 L.N.	29	M	cat	+++	3	1:1000	5	9	18
14 E.R.	25	F	cat	++	3	1:10	2	5	15
15 L.R.	24	M	cat	+++	4	1:100	5	3	1
16 U.S.	30	F	birch	+++	3	1:100	2	5	15
17 U.Sp	37	M	horse	++	0	1:100	3	4	3
18 I.S.	36	F	cow	+++++	0	1:1000	1	7	19
19 J.S.	46	M	horse	+++	3	1:1000	5	4	3
20 B.T.	28	F	birch	+++++	2	1:1000	5	5	14

Mean: 5.2 9.8

proportion of 1:10. Acetone-rinsed and dehydrated skin scales were the raw materials for dog, horse and cow allergen extracts. Cat, rabbit and hamster extracts were prepared from finely cut pieces of skin with careful removal of all subcutaneous tissue and blood. All stock solutions were stored at 4°C.

Extracts for bronchial provocation tests were prepared from the stock solution by dilution in sterile saline immediately before use.

Extracts used for diagnostic prick tests were glycerinated (50%) and diluted from the stock solutions in order to obtain an allergen-induced skin reactivity equal to 10 mg/ml histamine hydrochloride (3). The activity of the dog extract after this calibration was 10 times less than the other extracts. The prick test reactions were graded in accordance with the recommendations of the Nordic Society for Allergy Research in a plus system using 10 mg/ml histamine hydrochloride as a reference (27).

RAST tests were performed and evaluated according to the Phadebas system in RAST classes using a positive reference serum pool within birch pollen allergen system. All test reagents were purchased from Pharmacia AB, Uppsala, Sweden.

The patients were allergic to one or more allergens. One allergen for each patient was selected for the study (Table 1). The same allergen batch was used for each patient.

Inhalation procedures

Diluent and allergen extract were nebulized by a Pari Inhalerboy nebulizer (Paul Ritzan Pari Werkka, Starnberg am See, Germany, particle size 0.5 μm –5.5 μm , nebulized volume 0.80 ± 0.02 ml/min, mean $\pm$ s.d.), and inhaled through the mouth by tidal volume breathing. The patients were instructed to nebulize only during inhalation. The same nebulizer was used throughout the study. The provocations were performed between 9 a.m. and 2 p.m.

Five minutes before allergen provocation

diluent was inhaled for 2 min. The nebulizer was filled with 2 ml diluent. The nebulized volume was calculated by measuring the non-nebulized diluent after inhalation.

Before the start of allergen inhalation the nebulizer was filled with 2 ml allergen extract, always a greater volume than was inhaled. The nebulized volume was calculated by measuring the non-nebulized volume at the end of the inhalation (1–5 min). During inhalation of both diluent and allergen extract the number of breaths was counted.

The individual allergen dose was determined by titration before the study and defined by means of two variables, allergen extract concentration (range 1:10–1:10,000 w/v) and inhalation time (range 1–5 min), which were to give a decrease in FEV₁ of more than 20% from prechallenge value within 40 min after provocation (Table 1).

Recording

The lung ventilation was measured by means of an electronic spirometer (Monaghan M 403, Monaghan Co., Denver, USA which was calibrated accordingly. FEV₁ (forced expiratory volume in 1s), FVC (forced expiratory vital capacity) and PEFR (peak expiratory flow rate) were registered direct from the spirometer and MEF_{50%} (maximal expiratory flow rate at 50% of vital capacity) from a recorder attached to the spirometer. Recordings were made before and after diluent and 0, 5, 10, 15, 20 and 30 min after the end of the challenge. If the values were still low at 30 min, a further recording was made at 40 min. The highest value of two technically acceptable forced exhalations was selected for the calculations.

The response to allergen was expressed as the percentage decrease from the pre-challenge value (after diluent).

Statistical procedures

Standard methods were used for calculation of means, standard deviations (s.d.) and correlation coefficients (r). The mean, s.d. and

range for maximum percentage decrease in FEV_1 , FVC, PEFR and MEF_{50} were calculated for each patient. The coefficient of variation was used for determination of the reproducibility of the method. Differences and correlations were considered statistically significant for $P < 0.05$.

RESULTS

Respiratory rate

The individual difference between highest and lowest number of breaths during diluent and allergen inhalation was on the average 1.8 (range 0–3) breaths/min.

Nebulized volume

The mean nebulized volume of diluent and allergen extract during an inhalation time of 2 min was 0.57 ml. The individual difference between greatest and smallest nebulized volume of allergen extract was on the average 0.15 ml (range 0–0.40 ml).

Lung ventilation tests

Highly significant correlations were found between FEV_1 and FVC, PEFR and MEF_{50} , respectively ($P < 0.001$). The greatest percentage changes were seen in MEF_{50} .

Before and after diluent inhalation

There was no significant difference in FEV_1 , FVC, PEFR and MEF_{50} before and after diluent inhalation. After diluent (before allergen provocation) the individual difference between highest and lowest value of FEV_1 was on the average 0.37 l (range 0.09–0.72 l). Corresponding difference expressed as percentage of the highest value was on the average 10% (range 3–17%).

After allergen provocation

The mean decrease in FEV_1 after allergen provocation was 17% at 0 min, 27% at 5 min, 31% at 10 min, 32% at 15 min, 31% at 20 min

and 27% at 30 min. The mean maximum decrease in FEV_1 (independent of time) was 35% (range 20–58%).

The mean maximum decrease in FVC (independent of time) was 26% (range 10–44%). The mean maximum decrease in PEFR was 35% (range 12–55%) and the mean maximum decrease in MEF_{50} was 65% (range 40–91%).

Reproducibility of the allergen provocation procedure

The individual difference between highest and lowest value of FEV_1 at maximum decrease was on the average 0.66 l (range 0.26–1.29 l). Corresponding difference expressed as percentage of pre-challenge value was on the average 18% (range 4–30%).

There was a positive correlation between the individual mean per cent decrease in FEV_1 and the corresponding s.d. during 0–30 min after challenge. The coefficient of variation for the maximal per cent decrease was 23% for FEV_1 , 32% for FVC, 23% for PEFR and 19% for MEF_{50} .

The maximal per cent decrease in FEV_1 in six patients challenged seven times or more is illustrated in Fig. 1.

Systematic variation during repeated allergen provocations

The maximal per cent decrease in FEV_1 after the first and last challenge in all patients is illustrated in Fig. 2. The mean values were 38% and 33%, respectively. The difference was not significant.

There was a positive but not significant correlation between the investigation period (months) and the individual s.d. (corresponding to maximum decrease in FEV_1).

During the investigation period a further 2–20 allergen provocations (mean 8) were performed in the same patients. The number of additional provocations was not significantly correlated to the change in bronchial response (maximal percentage decrease in FEV_1 , first challenge minus last

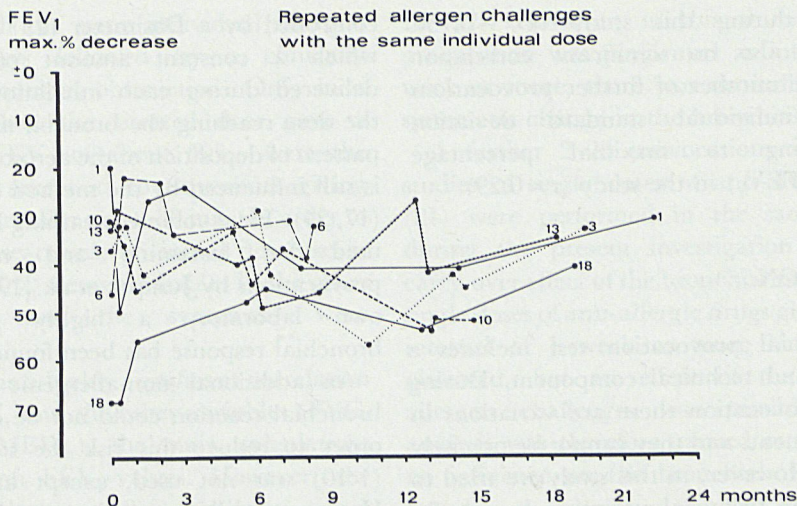


Fig. 1. Six patients (Nos. 1, 3, 6, 10, 13 and 18 in Table 1) challenged with the same individual allergen dose 7–9 times at intervals of 1 week to 8 months. The bronchial responses (dots) are expressed as percentage decrease in FEV₁ from baseline.

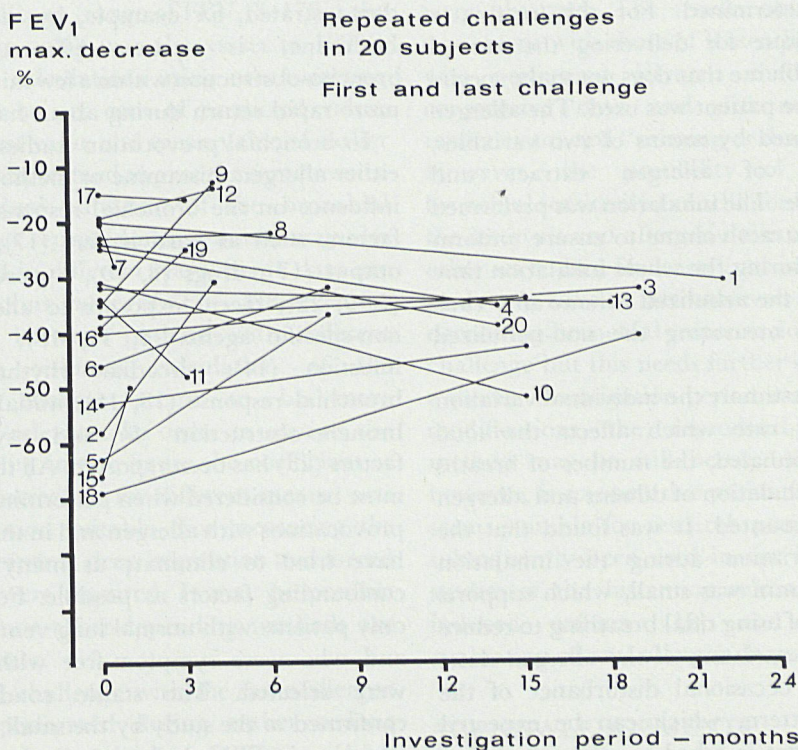


Fig. 2. Bronchial response, expressed as maximal percentage decrease in FEV₁ from pre-challenge value, after the first and last challenge (dots combined by lines) in 20 patients (Patients Nos. 1–20 in Table 1) provoked 3–9 times with the same individual allergen dose during an investigation period of $\frac{1}{2}$ –22 months.

challenge) during this study ($r = -0.13$). There was also no significant correlation between the number of further provocations and the individual standard deviation (corresponding to maximal percentage decrease in FEV_1) in the study ($r = 0.29$).

DISCUSSION

The bronchial provocation test includes a biological and technical component. During repeated provocation there are variations in both components and they cannot be precisely separated. However, in this study we tried to minimize the technical variation by careful standardization in order to obtain information on the biological variation.

Special attention was paid to the administration of the allergen extract as the exact amount of allergen reaching the bronchi cannot be determined. For this reason, a simple technique for delivering the aerosol during tidal volume that does not make special demands of the patient was used. The allergen dose was defined by means of two variables, concentration of allergen extract and inhalation time. The inhalation was performed in excess of extract volume to ensure uniform nebulization during the whole inhalation time and to control the nebulized volume after each inhalation by measuring the non-nebulized volume.

In order to estimate the individual variation in respiratory rate which affects the total allergen dose inhaled, the number of breaths during both inhalation of diluent and allergen extract were counted. It was found that the individual variation during the inhalation period of 1–5 min was small, which supports the relevance of using tidal breathing to reduce the individual variation of the allergen dose. The effect of occasional disturbance of the respiratory pattern, which can be expected when the patient is asked to start inhalation from a nebulizer, may be assumed to be eliminated during such a long period of time.

The volume of extract inhaled may be better

controlled by a Dosimeter (25) by means of which a constant amount of extract is delivered during each inhalation. However, the dose reaching the bronchi, as well as the pattern of deposition of the aerosol in the lung, is still influenced by the method of inhalation (17, 24). Tidal volume breathing has also been used for histamine and methacholine provocations by Juniper et al. (17) and in our own laboratory a highly reproducible bronchial response has been found.

An additional non-allergenic (unspecific) bronchial reaction could not be excluded. In order to reduce this risk the stock solution (1:10) was not used, except in two cases. However, in all provocations the time course of the changes in lung ventilation tests was typical for an immediate type reaction—maximal broncho-obstruction after 10–20 min and slow return within a few hours. The time course of the unspecific reaction, which can be demonstrated, for example, by inhalation of histamine, is quite different—maximal broncho-obstruction within a few minutes and a more rapid return during about half an hour.

In bronchial provocation studies, including either allergen, histamine or methacholine, an influence on the bronchial response by other factors, such as particle size (17), nebulizer output (17), drugs (8, 10), hyposensitization (2, 9, 22), recent exposure to allergens and non-specific agents (4, 11, 18), respiratory infection (14), circadian rhythm of the bronchial response (13, 15), initial degree of broncho-obstruction (5) and psychological factors (23) has been reported. All these factors must be considered when performing repeated provocations with allergen and in this study we have tried to eliminate as many suspected confounding factors as possible. Furthermore only patients with normal lung ventilation test and who were symptom-free without drugs were selected. The stable condition was confirmed in the study by the small individual variation in FEV_1 before challenge.

With these precautions, and the initial criterion of more than a 20% decrease in FEV_1 , this study has shown that repeated

challenge with the same individual allergen dose is always followed by a clinically significant bronchial obstruction. Only in a few cases, when the titrated dose gave a decrease in FEV_1 of about 50%, were the symptoms so severe as to necessitate giving bronchodilators after about 30 min. In order to avoid severe reactions, an initial decrease in FEV_1 of 30–40% seems advisable. In the other provocation tests the symptoms were moderate.

As there was a highly significant correlation between FEV_1 and the other variables (FVC, PEFR and MEF_{50}), the main calculations were based on FEV_1 -values. However, the percentage changes after challenge were different in the four variables, MEF_{50} always giving the greatest percentage change. The bronchial response after allergen challenge may be expressed in different ways. As there was only small individual variation before allergen challenge (range of FEV_1 3–17%) the pre-challenge value was always set to 100%. Preliminary calculations using other starting points, such as per cent of predicted value, earlier highest value and mean value of earlier measurements, seemed to give the same results. The individual variation of the bronchial response after repeated provocations may also be expressed in different ways. Some data are given in absolute values of FEV_1 . However, for comparison between patients with different basal lung volumes calculation of the percentage changes seems to be more biologically relevant. In order to express the reproducibility in one figure the coefficient of variation (correction for different means) was considered most suitable. For measuring the bronchial response deep inspiration and forced exhalation were required. It may be possible that other non-effort-depending methods are more adequate.

Repeated challenge with the same allergen dose was performed during rather a long period in order to obtain information about any systematic variation indicating development of either hypo- or hypersensitization, which has been reported in earlier studies (9,

16, 22). However, in this study in which provocation was performed at intervals of 1 week or more, there was no indication of a systematic change in the bronchial response.

A further 2–20 provocations included in studies of single doses of anti-allergic drugs (21) were performed in the same patients during the present investigation period. A carry-over effect of the bronchial response after single doses of anti-allergic drugs given with an interval of 1 week or more has not been observed, but an effect of the additional allergen challenges was possible and an analysis was therefore carried out. However, no influence on the bronchial response was found.

In conclusion, a model for standardized allergen provocations has been outlined. The reproducibility when used in symptom-free asthmatic patients seems to be acceptable and no indications of hypo- or hypersensitization were observed. The results obtained are in agreement with those of Rosenthal et al. (25), who performed allergen challenges on 4 consecutive days and did not find any tendency toward "priming or desensitization". However, the possibility of changes in bronchial sensitivity can still not be excluded if the intervals and allergen doses are selected in other ways. In a few cases (not included in this study) we have in fact observed indications of decreasing bronchial response during repeated challenge but this needs further study. During a shorter investigation period and with further modifications the reproducibility of the method may be still better. A prerequisite, however, is co-operative patients. The method seems suitable for research purposes and has subsequently been used in our laboratory for studies of the histamine release in allergen-induced asthma (19) and for clinical evaluation of anti-allergic drugs (21).

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