

Reproducibility of Standardized Bronchial Allergen Provocation Test

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Standardized bronchial allergen provocation was performed twice (with an interval of median 14 days (range 14-44)) in 19 extrinsic, well-defined asthmatic patients to study the reproducibility of the bronchial response. Smoking and medications were strictly withheld prior to the provocation. Ten-fold increasing concentrations of allergen solution 0.9 ml were inhaled by tidal volume breathing for 5 min at 10-min intervals. The actual dose given was expressed by the concentration of the solution used. Forced expiratory volume in the first second (FEV₁) was used to determine responses, and the provocation continued until an allergen concentration (allergen PC₂₀) was reached which caused at least 20 % decrease of the post-saline FEV₁. All provocations were followed by a bronchial reaction within clinically acceptable limits. The reproducibility was high, evaluated by the Spearman rank correlation coefficient $Rho = 0.90$ ($P < 0.01$). It is concluded that bronchial provocation with allergens performed as outlined in this study represents a reproducible test which can be applied in routine allergy diagnostics, and in further investigations concerning specific bronchial reactivity.

Key words: bronchial allergen provocation; reproducibility.

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CLINICAL ASPECTS

Bronchial allergen provocation test is the only way to confirm the presence of extrinsic asthma. Confirmation is essential as allergen avoidance and immunotherapy are only effective for allergic asthma. Bronchial allergen provocation can be used in evaluation of antiasthmatic drugs and in pathophysiological studies. For these purposes the provocation test should be standardized and highly reproducible.

Bronchial asthma (26) has been divided into extrinsic and intrinsic (22) based on the clinical history, the skin prick test, and the level of specific immunoglobulin E (IgE) in peripheral blood. Only a bronchial allergen provocation, however, seems able to confirm the presence of extrinsic asthma. The importance of this distinction is stressed by the fact that allergen avoidance and immunotherapy are only effective for allergic asthma. Bronchial allergen provocation is also valuable in the evaluation of new antiasthmatic drugs as well as in pathophysiological studies. For these purposes the provocation test should be standardized and highly reproducible. The aim of the present study was to examine the reproducibility of the

immediate bronchial response to inhaled allergens defined by the allergen concentration inducing a more than 20 % fall in lung function (FEV₁) when using a standardized provocation test in a well-defined group of extrinsic asthmatic patients.

MATERIAL AND METHODS

Study population

Six female and 13 male patients participated in the study after informed consent had been obtained. Baseline antropometric data are given in Table 1.

None of the participants suffered from con-

current pulmonary diseases as indicated by normal chest roentgenograms. All patients showed a more than 15 % change in FEV₁ spontaneously or after inhaled bronchodilator. At least two of the following three criteria were fulfilled by all patients:

- 1) A positive history of breathlessness induced by exposure to allergen.
- 2) A positive skin prick test to the relevant allergen, performed on the volar side of the forearm using a Dome/Hollister-Stier needle, according to the method in the Nordic guidelines (7). The allergen extracts were used in concentrations of 1:20 w/v (mould) or in the potency of 5 HEP histamine equivalent prick (house dust mite, animal dander and pollen) purchased by Allergologisk Laboratorium A/S, Copenhagen, Denmark. The HEP potency of the extracts was found by using reference allergen extracts in five dilutions. The concentration of the extracts giving the same wheal reaction as histamine reference 1 mg/ml was 1 HEP (1). As a positive reference, histamine chloride 5 mg/ml was used according to Aas (1).
- 3) A positive radioallergosorbent test (RAST) to the relevant allergen done according to Weeke (29) and considered positive when sorbent units (SU) were measured above 9 SU. Based on the SU a clinical grading in the following allergy classes could be made (1):

Table 1

Pa-tients	Sex	Age	Height (cm)	FEV ₁ % pre-dicted	RAST (SU)	Pricktest + + + / -	Allergic history	PC ₂₀ histamine (mg/ml)	Allergen	PC ₂₀ allergen I	PC ₂₀ allergen II
1	M	44	168	60	4	+++	+	0.5	house-dust mite ¹	100,000 SQ	10,000 SQ
2	M	19	188	86	14	+++	+	0.48	house-dust mite ¹	10,000 SQ	10,000 SQ
3	M	34	173	55	9	+++	+	0.028	house-dust mite ¹	1,000 SQ	1,000 SQ
4	M	58	182	84	6	+++	+	0.2	house-dust mite ¹	negative	negative
										100,000 SQ	100,000 SQ
5	M	18	178	65	313	+++	+	0.074	house-dust mite ¹	10,000 SQ	10,000 SQ
6	M	53	181	92	35	+++	+	0.25	rabbit	1:10 ³ w/v	1:10 ³ w/v
7	M	58	192	94	12	+++	+	0.2	dog	100,000 SQ	100,000 SQ
8	F	24	172	68	73	+++	+	0.028	dog	1,000 SQ	1,000 SQ
9	M	43	175	100	4	+++	+	1.00	dog	100,000 SQ	100,000 SQ
10	F	27	170	80	55	+++	+	1.00	dog	1,000 SQ	1,000 SQ
11	M	35	185	55	1	+++	+	0.20	dog	negative	negative
										100,000 SQ	100,000 SQ
12	M	43	175	84	40	+++	+	0.20	dog	100,000 SQ	100,000 SQ
13	F	42	191	100	2	+++	+	2.00	timothy ²	1:10 ² w/v	1:10 ² w/v
14	M	74	178	68	193	+++	+	0.25	cat	1:10 ³ w/v	1:10 ² w/v
15	M	24	170	64	3	+++	+	0.125	mugwort ³	1:10 ³ w/v	1:10 ⁴ w/v
16	F	27	170	76	6	+++	+	1.00	horse	1:10 ⁶ w/v	1:10 ⁶ w/v
17	F	14	163	90	35	+++	+	0.50	Scotch pine ⁴	negative	negative
										1:10 ² w/v	1:10 ² w/v
18	M	15	178	82	92	+++ (Phleum pratense)	+	0.19	Scotch pine ⁴	negative	negative
						+++ (Phleum pratense)				1:10 ² w/v	1:10 ² w/v
19	F	30	164	69	76	+++ (Phleum pratense)	+	1.00	Scotch pine ⁴	negative	negative
										1:10 ² w/v	1:10 ² w/v
Median		34	175	82	10.5			0.25			
Range		14-58	163-192	55-100	1-313			0.028-2.00			

¹ *Dermatophagoides pteronyssinus*; ² *Phleum pratense*; ³ *Artemisia vulgaris*; ⁴ *Pinus sylvestris*.

- Allergy class 0: < 2 SU
- Allergy class 1: < 4 SU
- Allergy class 2: < 20 SU
- Allergy class 3: < 100 SU
- Allergy class 4: $\geq$ 100 SU

All patients were studied at a time when there had been no known exposure within the last 4 weeks to the allergen used for provocation (except house dust mite: *Dermatophagoides pteronyssinus*). No patients had respiratory tract infections or immunotherapy vaccinations within the last 4 weeks, or had smoked 4 h prior to the provocation. Antihistaminics were withheld for 72 h before provocation ($n = 0$), sustained release methylxanthines for 48 h ($n = 7$), plain methylxanthines, Ipratropin and oral beta-2-adrenergic agonists for 12 h ($n = 1$), and nebulized beta-2-adrenergic agonists for 6 h ($n = 19$). Local ($n = 10$) and systemic ($n = 0$) steroids were continued unchanged at the same dose.

The non-specific hyperreactivity was assessed by performing a histamine challenge carried out as a non-cumulative dose-response study according to Cockcroft (4). After an initial saline inhalation, histamine chloride was given starting at 0.03 mg/ml increasing with increments of a factor 2 every 5 min. FEV₁ was measured 60 sec after inhalation and the challenge was interrupted when a decrease of at least 20 % of the post-saline FEV₁ was observed. PC₂₀-histamine was calculated by interpolation on the log-dose response curve. This test took place within 1 month of the allergen provocation.

The forced expiratory volume in the 1st sec (FEV₁) was measured on a dry wedge spirometer (Vitalograph Ltd., Buckingham, England), and at least two technically correct forced expiratory manoeuvres with a variation < 5 % in FEV₁ were obtained, and the highest value was used for further calculations. Results were reported at ambient temperature, atmospheric pressure and saturated with water vapour (ATPS).

Aerosols were delivered by a Pari nebulizer No. 16 (Paul Ritzau, Pariwerk, Stanber am See, W. Germany) with a flow of 5 l/min, a

particle size of 3.5 μ m (range 0.5–5.5) and an output of 0.221 ml/min. The output was determined as the mean output of five 2 min nebulization periods. The nose was clipped and aerosols were inhaled through a mouthpiece. Only one nebulizer was used throughout the study. Bronchial allergen provocation was performed twice with an interval of 2 weeks. The tests were performed at the same time of the day, each patient beginning at 09 a.m. or 01 p.m. Inhalations were performed for 5 min with intervals of at least 10 min.

Before the allergen provocation, the FEV₁ was measured and the patient was excluded if the FEV₁ was < 60 % of the predicted value. The provocation began with an inhalation of isotonic saline (diluent), whereafter inhalation of allergen extracts was performed using 10-fold increasing concentrations beginning with the lowest allergen concentration at each provocation. FEV₁ was recorded 0, 5, 10 min after termination of each inhalation period, and the provocation was continued until an allergen concentration was reached which caused at least 20 % decrease of the mean post-saline FEV₁ (allergen PC₂₀).

The following modification was used: if a decrease between 15 % and 20 % of the mean post-saline FEV₁ was obtained, FEV₁ was measured with intervals of 5 min until no further decrease in FEV₁ was observed, then the next inhalation was given. The provocation was considered negative if a < 20 % decrease in FEV₁ was obtained 30 min after the strongest concentration of allergen extract was inhaled. To normalize the pulmonary function inhalation of salbutamol (Ventoline® 5 mg) was given. The patient stayed at the clinic for 1 h of observation. Every 30 min, FEV₁ was measured until a FEV₁ of at least 85 % of preprovocation value was obtained before the patient left the clinic. After each provocation the patients were instructed to use their regular daily medication. In order to record any late bronchoconstriction after provocation (late response), which can develop 3–8 h after exposure to allergen (5), the patients were asked to determine peak expiratory flow (PEF) every hour for the following 24 h. If breathlessness developed the patients were

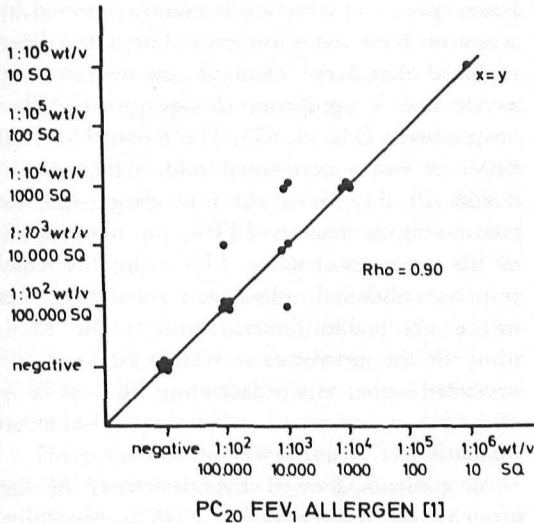
PC₂₀ FEV₁ ALLERGEN [2]

Fig. 1. Second provocation (2) versus first provocation (1). Line of identity: $x = y$ ($n = 19$), $Rho = 0.90$, $P < 0.001$ ($n = 15$).

instructed to inhale 0.2 mg salbutamol (Ventoline) and if no release of breathlessness was induced to contact the clinic at once. Allergen stock solutions (Allergologisk Laboratorium A/S, Copenhagen, Denmark) weight/volume (w/v) were used except for the provocations with dog and house dust mite, for which standard quality (SQ) stock solutions have recently been produced. The SQ-extracts have been characterized and standardized according to the knowledge of the concentration of major allergens as well as the HEP-potency (1, 12, 18). Extracts for bronchial provocation tests were prepared immediately before use from the stock solutions (1:10 w/v or 10⁶ SQ) by dilution with sterile isotonic saline in concentrations of 1:10⁶–1:10² w/v or 10 SQ–100,000 SQ.

In three patients the provocation was performed with an innocent allergen, pine (*Pinus sylvestris*), an allergen to which the patients had no known allergy. Interdependence was tested by means of the Spearman rank correlation coefficient Rho (8). Median values followed by the range in brackets are indicated in the text. The 5% level was used as significant for correlation.

RESULTS

The interval between the two provocations was median 14 days (14–44 days). FEV₁ determined prior to the first provocation was 2.9 l/min (2.0–4.1) and prior to the second provocation 3.0 l/min (1.9–4.0). The post-saline FEV₁ was 3.0 l/min (1.8–4.1) at the first provocation and 3.0 l/min (2.0–4.1) at the second. All patients inhaled the 0.9 ml allergen solution nebulized. A 20% or more decline in FEV₁ was reached by 14 of 19 patients. Only four of 14 of these patients differed with regard to the PC₂₀ allergen value obtained at the two provocations. Three of the four patients obtained a 10-fold decrease in PC₂₀ at the second provocation compared with the first provocation, the fourth obtained a tenfold increase in the PC₂₀ allergen at the second provocation. The median reduction of the FEV₁ at the first provocation was 26% (21–42%) and at the second provocation 25% (20–50%). Five patients did not obtain a PC₂₀ allergen value, three of five patients in this group were provoked with pine (*Pinus sylvestris*).

No systemic reaction was observed, or other complications reported, and all patients left the clinic after the 1 h control period, none of the patients developed any late response indicated by a more than 15% fall in PEF. Ten patients, however, developed a feeling of breathlessness and indicated in the diary card the use of extra beta-2-adrenergic agonist spray 6 h (6–12 h) after termination of the provocation. The reproducibility of the allergen PC₂₀ obtained in 19 patients is shown in Fig. 1, and the Spearman correlation coefficient Rho was 0.90 ($P < 0.001$). The Spearman correlation coefficient – Rho – was only calculated for the 15 patients who obtained a PC₂₀ allergen, but as shown in Fig. 1 also the negative provocations were reproducible.

DISCUSSION

Reports of the reproducibility of the bronchial allergen provocation have been few (17). The reproducibility of the method ought to be high if the method is to be used in diagnostic procedures, in the follow-up during immuno-

therapy, and if the results should be comparable between different clinics.

The allergen provocation procedure as previously described has shown to be highly reproducible (Fig. 1). The three major problems in the standardization of bronchial allergen provocation are the mode of administration, the standardization of the allergens, and the determination of the bronchial response. At present, two techniques for bronchial provocations have been used. The use of a dosimeter allows the delivery of a timed bolus of aerosol at the beginning of a deep inspiration from functional residual capacity to total lung capacity. The number of breaths taken by the patient can be varied, as well as the concentration of the allergen, so that the amount given can be expressed as cumulative breath units (3). In the other technique a continuous inhalation of allergen (aerosol) is given with different concentrations by tidal volume breathing, and the dose given is then expressed by the concentration of the solution used (4). In both techniques described it is a problem to determine the exact amount of allergen reaching the bronchi. The generated dose will be lost in the equipment, partly exhaled, partly metabolized. The particles may be removed from their site of deposition, and the presence of airway obstruction may influence the site of deposition. The dose might be better controlled by a dosimeter (3, 24), by which a constant amount of extract is delivered during inspiration, but the dose reaching the bronchi as well as the pattern of deposition of the aerosols in the lungs are still influenced by the factors described above (10, 13, 16, 20, 21, 23). However, it has been shown that a comparison of the two methods displays no significant difference in the bronchial response to histamine obtained and the amount deposited in the bronchi (2, 25).

In this study the same batch of allergen extract as stock solution was used in both provocations, and since the interval between the two provocations was median 14 days no change in the potency of the allergen extracts could be expected.

The bronchial response to an allergen

provocation can be determined in several ways. Large airway obstruction is mainly detected by sGaw or Raw measurements, but it has been reported that large changes are necessary to assure that a significant change in air caliber has occurred (11, 14, 27). The measurement of FEV₁ is easily performed and highly reproducible (9, 11). Since the provocation did not continue if the decline of FEV₁ was above 15 % of the mean post-saline FEV₁, the bronchial response obtained after each inhalation was within acceptable limits. Further, the FEV₁ prior to the provocation was > 60 % of the predicted value, as a reduction in FEV₁ of 50 % of the mean post-saline value obtained after an inhalation of allergen was reported.

All patients showed hyperreactivity of the bronchi, demonstrated by a PC₂₀ histamine value of < 1.00 mg/ml. In three patients the provocation was performed with an allergen to which they showed no known allergy (*Pinus sylvestris*) in order to eliminate that the induction of the method released a bronchial response found in bronchial hyperreactivity. These three patients showed non-specific hyperreactivity as demonstrated by a PC₂₀-histamine of 0.50 mg/ml, 0.19 mg/ml and 1.00 mg/ml, respectively, and they also presented an FEV₁ > 60 % of predicted value before provocation. It can be claimed that allergen provocation only reflects a non-specific bronchial response, although the method is widely accepted (19). In this study, however, an innocent allergen for provocation did not induce a bronchoconstriction, as would have been the case if the response was due to a merely reflex-induced bronchoconstriction. The time between the two provocations was a minimum of 14 days in order to eliminate the increasing non-allergic bronchial reactivity demonstrated after allergen exposure (5). Since no significant correlation was found between allergen reactivity and initial FEV₁, expressed as percent of predicted value, as has been reported concerning histamine, we began with the lowest concentration in each provocation test. However, it has been shown that it is possible to estimate the dose of allergen required by inhalation to trigger an asthmatic reaction by a knowledge of the skin responsive-

ness to allergen and the bronchial reaction to histamine (6), whereas a relatively poor correlation between history, skin test, and bronchial provocation test has been reported (15, 28).

A late response indicated by $> 15\%$ fall in PEF within 3–8 h after provocation was not seen, but 10 patients developed a subjective feeling of breathlessness. This discrepancy may be due to frequent use of extra beta-2-adrenergic agonist spray and the instructions that all patients should continue their daily medication, thus any fall in PEF might have been "masked", and late response would have been reflected in PEF if the patients had not taken their daily medication after the provocation.

Three patients showed a 10-fold increase in the specific bronchial reactivity and one patient a 10-fold decrease. All four patients fulfilled the inclusion criteria (Table 1) as well as showing a non-specific hyperreactivity with a PC_{20} -histamine below 1 mg/ml. The prechallenge FEV_1 was within a 10% difference at the two provocations and the same batch of allergen extracts was used at both provocations. A correlation coefficient of 0.90 is satisfactory for diagnostic purposes, but this may be a reflection of the 10-fold increase in allergen dose. The PC_{20} allergen was evenly distributed between $1:10^5$ – $1:10^2$ w/v. To determine a more "sensitive" PC_{20} allergen value, a slighter increase in concentration of allergen solution than the one used in this study could be used.

If bronchial allergen provocation is going to be used in the diagnostic procedures in routine allergy diagnostics, or if further investigations are to be performed to interpret the bronchial response to an allergen provocation, a need for the standardization of bronchial allergen provocation procedures has been emphasized in order to compare results from different clinics (17). Bronchial provocation with allergens performed as outlined in this study represents a reproducible test which can be applied in future investigations as well as in routine allergy diagnostics.

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