

A comparative study of four different bronchial challenge tests

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The correlation between 4 commonly used tests for assessment of airway hyperresponsiveness was studied in 10 asthmatics by performing bronchial challenges with histamine, ultrasonically nebulised distilled water (UNDW), exercise, and a relevant allergen. All tests were performed within 4 weeks for each individual and at least 48 h apart. A significant correlation was found between histamine challenge and exercise challenge ($r = -0.74$). Other correlations were substantial (0.4–0.5) but did not reach statistical significance. This suggests that the challenges have different mechanisms or pathways leading to bronchoconstriction, and assessment of the pattern of individual patients' bronchial hyperresponsiveness may require application of a number of different bronchial challenge tests. For routine use histamine challenge seems the most appropriate.

Clinical aspects

A comparison between 4 commonly used bronchial challenge tests has shown few correlations between these, suggesting different mechanisms in bronchial hyperresponsiveness and the need of a number of challenge tests to assess an individual patient's pattern of bronchial hyperresponsiveness.

Key words: allergen inhalation; asthma; bronchial challenge; exercise-induced asthma; histamine inhalation; ultrasonically nebulised distilled water challenge

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Bronchial hyperresponsiveness is a characteristic of patients with bronchial asthma (8, 14). It is seen as a bronchoconstrictor response to various stimuli, such as inhalation of specific allergens, different irritants or pharmacological agents in concentrations which do not affect non-asthmatic individuals. A number of bronchial challenge tests have been developed to assess the degree of responsiveness, and efforts have been made to standardize the procedures (9, 23, 28). It is not known whether there is a simple correlation between the responsiveness obtained by different challenge tests or if patients can be characterized by their pattern of responses to different stimuli, suggesting different pathways leading to bronchoconstriction (22, 25). Earlier studies, comparing the response to 2 different challenges have shown different results (20). We here present the results of a study comparing 4 of the clinically most commonly used challenge tests in asthmatic patients. It was performed by bronchial challenge in the same individuals with histamine dihydrochloride, ultrasonically nebulised distilled water (UNDW), allergen, and exercise.

Material and methods

Patients

Included in the study were 12 adult patients with asthma according to the criteria of The American Thoracic Society (5). All patients had a history of exercise-induced asthma (EIA), and allergy towards an inhalant allergen defined as a positive clinical history, a positive skin prick test (SPT) larger than the histamine reference, and significant specific IgE (>0.35 PRU/ml measured with radio-immunosorbent-test (Phadebas RAST, Pharmacia, Sweden)) to either cat, house dust mite (*Dermatophagoides pteronyssinus*) or timothy (*Phleum pratense*). Allergen solutions were obtained from ALK, Copenhagen, and serum IgE was measured with PRIST (Pharmacia, Sweden).

None required inhaled or systemic corticosteroids to control their asthma, all patients used β_2 -agonists inhaled as needed, 2 patients used oral β_2 -agonists and one patient oral theophylline. Their forced expiratory volume in the first second (FEV₁) was more

than 75% of predicted normal at entry of the study. Predicted values for FEV₁ were according to Quanjer et al. (21). The variation in FEV₁ % predicted was <20% between each challenge (Table 2). The patients were young adults and 10 of the 12 patients had a Pharmacia RAST class ≥ 3 (≥ 3.5 PRU/ml) to the allergen used for challenge and 7 had elevated serum IgE (> 150 kU/l) (Table 1).

Study design

The challenges were performed at the same time of day for each subject, within 4 weeks and at least 2 days apart in the following sequence: histamine challenge, UNDW challenge, exercise challenge and allergen challenge. Oral medication was withheld 24 h prior to challenge and inhaled β_2 -agonists for 12 h. The challenges were performed outside the pollen and the peak house dust mite season, and none of the patients allergic to cat epithelium were exposed to cat. None of the patients had a respiratory infection within the 4 weeks before the challenges. Lung function was measured by a dry spirometer (Morgan Spiroflow 130, P.K. Morgan, Gillingham, England) connected to a Hewlett Packard 86B computer. The highest FEV₁ of 3 reproducible measurements (defined as difference <5%) was recorded and used as the parameter for changes in lung function. At all challenge tests patients wore a nose-clip to ensure oral breathing.

Histamine challenge

Bronchial challenge with histamine dihydrochloride was performed according to a modification of the method described by Cockcroft et al. (11). The patients inhaled for 2 min at tidal breathing from a Pari-nebuliser (Pari Ritzau Pari Werk, Starnberg, Germany), driven by compressed air (flow 5 l/min) giving a mean output of 0.57 ml/min. The aerosol

was delivered via a facemask. Isotonic saline was first inhaled, and FEV₁ measured after 3 min was used as a baseline value. Doubling concentrations of histamine dihydrochloride were inhaled, starting with 0.03 mg/ml until a fall in FEV₁ of at least 20% had been obtained. The highest concentration used was 8 mg/ml. The concentration of histamine dihydrochloride producing a 20% fall in FEV₁ (PC₂₀ histamine) was calculated by linear interpolation on the log dose-response curve (12).

UNDW challenge

Ultrasonically nebulised distilled water was inhaled from a Mistogen-ultrasonic nebuliser (EN 143 A Schuco, London, England) (3). It was calibrated to give an output of 2.0 ml/min. Patients inhaled the mist from a facemask at tidal breathing, starting with inhalation for 1 min. FEV₁ was measured 1, 3 and 5 min after the inhalation. If this did not produce a fall in FEV₁ of 20%, it was succeeded by inhalation for 3 and as next step for 5 min, eventually repeating the last dose until a 20% fall in FEV₁ occurred. The cumulated dose of delivered nebulised water producing a 20% fall in FEV₁ (PD₂₀ UNDW) was calculated by linear interpolation on the dose-response curve.

Allergen challenge

The allergen extracts used were partly purified, standardized extracts (Aquagen®, SQ-U, ALK, Hørsholm, Denmark). The challenge was a modification of a procedure previously described (15), starting with the concentration of allergen extract giving a 2–3 mm weal at skin prick test. The same Pari-inhaler as used for histamine challenge nebulised the extract. Inhalations were taken via a facemask at tidal breathing. Five inhalations of diluent were taken and FEV₁ measured after 10 min. The allergen challenge was started by giving 2 inhalations of the starting concentration and FEV₁ was recorded after 5 and 10 min. If there was a fall in FEV₁ of <10%, 4 inhalations were taken. If FEV₁ fell between 10 and 20%, the last dose was repeated and if after 4 inhalations the fall was <10% then 8 inhalations were taken. The next step was a 10-fold increment in allergen concentration repeating the same procedure until a fall in FEV₁ $\geq 20\%$ occurred. The cumulated dose of allergen (SQ-U $\times 10^5$) giving a 20% fall in FEV₁ (PD₂₀ allergen) was calculated by linear interpolation on the log dose-response curve.

Exercise challenge

Exercise challenge was performed on an ergometer-cycle at submaximal workload for 6 min according

Table 1. Patient characteristics

Sex	Age (years)	Allergen	RAST PRU/ml	Serum IgE kU/l	No. positive skin prick tests
M	20	timothy	23.8	895	2
F	32	<i>D. pteronyssinus</i>	20.5	162	2
F	35	timothy	1.4	18	1
F	21	cat	3.6	29	3
M	26	cat	26.4	199	3
F	18	<i>D. pteronyssinus</i>	29.8	678	1
M	34	cat	6.9	64	3
F	16	cat	19.7	51	2
M	37	<i>D. pteronyssinus</i>	16.6	63	3
M	42	<i>D. pteronyssinus</i>	3.5	111	2
F	23	timothy	3.2	368	4
F	21	cat	15.6	> 1000	6

Table 2. Challenge results and baseline FEV₁ % predicted value measured before each challenge

Allergen		Histamine		UNDW		Exercise	
Baseline FEV ₁ %	PD ₂₀ (10 ⁵ SQ)	Baseline FEV ₁ %	PC ₂₀ (mg/ml)	Baseline FEV ₁ %	PD ₂₀ (ml water)	Baseline FEV ₁ %	EIA % fall
76	3.71	96	0.64	80	10.99	76	25
103	0.26	94	0.03	87	0.93	105	49
57	2.72	85	0.07	83	3.50	62	69
93	0.48	83	0.51	92	29.85	86	9
127	3.51	130	0.79	122	4.57	123	6
87	1.10	102	0.39	96	2.19	93	22
78	0.53	79	7.07	78	19.23	77	20
87	0.34	81	0.05	75	2.58	92	38
79	0.69	89	0.10	84	14.18	89	52
87	0.11	85	0.09	78	6.00	76	42
86	10.39	94	0.54	89	33.33	85	29
93	0.86	134	0.19	90	5.01	82	2

to Anderson et al. (2). The mean pulse rate during the last min of exercise was 180/min, range 160–200/min. Ambient temperature was mean 22.7°C, range 21–26°C and relative humidity was mean 36.5%, range 26–50%. The maximum % fall in FEV₁ (EIA % fall) compared with a pre-test value was used as the measure of the degree of reactivity to exercise.

It was calculated as:

$$\frac{(\text{baseline FEV}_1 - \text{minimum post-exercise FEV}_1) \times 100}{\text{baseline FEV}_1}$$

A positive test was considered present when the EIA % fall was $\geq 20\%$.

Consent was given by all patients after written and oral information, and the study was approved by the Ethics Committee of Aarhus County.

Statistics

Statistical analyses were performed using the SPSS package (19). Logarithmic transformation of PC₂₀ histamine and PD₂₀ allergen was used, and after this challenge results were found normally distributed by the Lilliefors test (19) allowing parametric Pearson correlation analysis. Differences in baseline FEV₁ on the 4 test days were examined by one-way analysis of variance.

Results

There was no significant difference between baseline FEV₁ on the 4 test days ($p > 0.70$). The patients were highly sensitive to histamine with a median PC₂₀ histamine of 0.45 mg/ml (range, 0.03 mg/ml–7.07 mg/ml). Three patients had a negative exercise test (EIA % fall $< 20\%$), and the median EIA % fall was 26.7% (range, 2%–69%). The median PD₂₀

allergen was 0.61×10^5 SQ-U (range, 0.26×10^5 SQ-U to 10.39×10^5 SQ-U) and the median PD₂₀ UNDW was 8.5 ml water (range, 0.93 ml–33.33 ml). The results of the challenge tests for each individual and baseline FEV₁ % predicted before the tests are shown in Table 2. Two patients (patients 3 and 12) had a variation in baseline FEV₁ % predicted $> 20\%$, and were excluded from the statistical analyses. To study the correlations between challenge tests, a Pearson correlation analysis was used (Table 3) and a significant correlation was found between histamine and exercise challenge test ($r = -0.74$) while all other correlations were statistically non-significant.

Discussion

In the present study we have performed bronchial challenge using 4 different stimuli in the same individuals, making a comparison between these tests possible. The strong association between sensitivity to histamine and exercise which has been found in the present study is in accordance with other studies. A good correlation between exercise and histamine challenge (4), and between exercise and methacholine challenge (16) has previously been observed.

Table 3. Pearson correlation matrix. Upper figure indicates the correlation coefficient, lower figure indicates the significance level (2-tailed significance).

Variable	1	2	3
1. log PC ₂₀ histamine (g/ml)			
2. log PD ₂₀ allergen (SQ $\times 10^5$)	0.463 0.178		
3. PD ₂₀ UNDW (ml)	0.488 0.158	0.401 0.251	
4. EIA % fall	-0.739 0.015	-0.449 0.194	-0.303 0.394

In a study of 15 asthmatics (10) no significant correlation between histamine sensitivity and exercise ($r = 0.44$) was found, while the correlation between methacholine sensitivity and exercise was significant ($r = 0.54$). In our study, we found a stronger ($r = -0.74$) correlation between histamine and exercise. Our patients were selected on the basis of a positive history of EIA. Yet 3 of 12 had a negative test (EIA % fall $< 20\%$), and as these were the only negative tests, exercise may be the least sensitive way of demonstrating bronchial hyperresponsiveness. A comparison of the response to exercise and UNDW challenge was performed by Bascom & Bleeker (6) and a good correlation was found between these 2 tests ($r = 0.81$). Only 7 of the 15 asthmatics examined reacted to both UNDW and exercise, while all had a positive methacholine test. We found it possible to establish a PD_{20} UNDW in all patients, although inhalation of a large amount of water was necessary in some cases in accordance with previous reports (3). Our results are in accordance with Anderson & Schoeffel (1), who found a poor correlation between the response to UNDW challenge and the response to histamine and methacholine challenge. The association found between allergen and UNDW challenge was new to us, but the correlation ($r = 0.40$) was not significant. The correlation coefficients were, however, substantial (0.4-0.5) from a biological point of view and probably would have reached statistical significance with a larger number of persons. In other studies a significant correlation between PC_{20} histamine and PD_{20} allergen has been found, which we have not been able to reproduce in the present study (13, 18). An understanding of the mechanisms involved in bronchial hyperresponsiveness would be of major importance in the diagnosis, treatment and, hopefully, prophylaxis of bronchial asthma. The possible mechanisms (20) include direct stimulation of effector cells and indirect neural reflex-mediated stimulation. Also immunological and non-immunological activation of inflammatory cells and release of mediators may be important.

Mediators of potential importance for generating bronchial obstruction have been found after challenges with allergen (26), UNDW (7, 24) and exercise (17, 26), although the pathways leading to their release may be different (27). As a common mechanism of eliciting bronchoconstriction should be expected to be followed by a high degree of correlation between the response to different challenges, our results suggest that these challenges may have different mechanisms or pathways leading to bronchoconstriction. Bronchial challenge with histamine in daily routine examination of patients suspected of bronchial hyperresponsiveness is useful, and in groups of patients a correlation between the response to histamine and clinical data has been found (11).

It would be of value if a challenge test could be developed where the response more clearly reflected clinical disease, and it is possible that a laboratory assessment of responsiveness should include a number of challenge procedures to reflect clinical disease because mechanisms may operate in various combinations.

References

1. ANDERSON SA, SCHOEFFEL RE. The inhalation of ultrasonically nebulized aerosols as a provocation test for asthma. In: HARGREAVE FE, WOOLCOCK AJ (eds.): *Airway responsiveness: measurement and interpretation*, Astra, Mississauga, Ontario, 1985: 39-48.
2. ANDERSON SA, SCHOEFFEL RE. Standardization of exercise testing in the asthmatic patient: a challenge in itself. In: HARGREAVE FE, WOOLCOCK AJ (eds.): *Airway responsiveness: measurement and interpretation*, Astra, Mississauga, Ontario, 1985: 51-60.
3. ANDERSON SA, SCHOEFFEL RE, FINNEY M. Evaluation of ultrasonically nebulised solutions for provocation testing in patients with asthma. *Thorax* 1983; 38: 284-91.
4. ANDERTON RC, CUFF MT, FRITH PA, et al. Bronchial responsiveness to inhaled histamine and exercise. *J Allergy Clin Immunol* 1979; 63: 315-20.
5. American Thoracic Society: Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987; 135: 225-43.
6. BASCOM R, BLEECKER ER. Bronchoconstriction induced by distilled water. *Am Rev Respir Dis* 1986; 134: 248-53.
7. BELCHER NG, MURDOCH RD, DALTON N, et al. A comparison of mediator and catecholamine release between exercise and hypertonic saline-induced asthma. *Am Rev Respir Dis* 1988; 137: 1026-32.
8. BOUSHEY HA, HOLTZMANN MJ, SHELLER JR, NADEL JA. Bronchial hyperreactivity. *Am Rev Respir Dis* 1980; 121: 389-413.
9. CHAI H, FARR S, FROELICH LA, et al. Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975; 56: 323-7.
10. CHATHAM M, BLEECKER ER, SMITH PL, ROSENTHAL RR, MASON P, NORMAN PS. A comparison of histamine, methacholine and exercise airway reactivity in normal and asthmatic subjects. *Am Rev Respir Dis* 1982; 126: 235-40.
11. COCKCROFT DW, KILLIAN DN, MELLON JJA, HARGREAVE FE. Bronchial reactivity to histamine: a method and clinical survey. *Clin Allergy* 1977; 7: 235-43.
12. COCKCROFT DW, MURDOCK KY, MINK JT. Determination of histamine PC_{20} : comparison of linear and logarithmic interpolation. *Chest* 1983; 84: 505-6.
13. COCKCROFT DW, RUFFIN RE, FRITH PA, et al. Determinants of allergen-induced asthma: dose of allergen, circulating IgE antibody concentration and bronchial responsiveness to inhaled histamine. *Am Rev Respir Dis* 1979; 120: 507-15.
14. EISER NM. Assessment of bronchial hyperresponsiveness. Bronchial provocation tests. In: NADEL JA, PAUWELS R, SNASHALL PD (eds.): *Bronchial hyperresponsiveness. Normal and abnormal control, assessment and therapy*. Blackwell, Oxford, 1987: 173-254.
15. KILLIAN D, COCKCROFT DW, HARGREAVE FE, DOLOVICH J. Factors in allergen-induced asthma: relevance of the intensity of the airways allergic reaction and non-specific bronchial reactivity. *Clin Allergy* 1976; 6: 219-25.
16. KIVILOOG J. Bronchial reactivity to exercise and methacholine in bronchial asthma. *Scand J Respir Dis* 1973; 54: 347-58.
17. LEE TH, NAGY L, NAGAKURA T, WALPORT MJ, KAY AB.

- Identification and partial characterisation of an exercise-induced neutrophil chemotactic factor in bronchial asthma. *J Clin Invest* 1982; 69: 889-99.
18. NIEJENS HJ, DEGENHART HJ, RAATGEER HC, KERREBIJN K. Study on the significance of bronchial hyperreactivity in the bronchus obstruction after inhalation of cat dander allergen. *J Allergy Clin Immunol* 1979; 64: 507-15.
19. NORUSIS MJ. SPSS/PC+ V 3.0 Update manual. Chicago, 1988.
20. PAUWELS R, JOOS G, VAN DER STRAETEN M. Bronchial hyperresponsiveness is not bronchial asthma. *Clin Allergy* 1988; 18: 317-21.
21. QUANJER PH. (ed.): Standardized lung function testing (Report working party "Standardization of lung function tests"). *Bull Eur Physiopath Respir* 1983; 19: Suppl. 5, 45-51.
22. REED CE, BUSSE WW. Asthma: definition and pathogenesis. In: MIDDLETON E Jr, REED CE, ELLIS CF, ADKINSON NF Jr, YUNGINGER JW (eds.): *Allergy: principles and practice*. C.V. Mosby, St. Louis, 1988: 969-98.
23. SEPCR Working Group "Bronchial hyperreactivity". Guidelines for standardization of bronchial challenges with (non-specific) bronchoconstricting agents. *Bull Eur Physiopath Respir* 1983; 19: 495-514.
24. SHAW RJ, ANDERSON SD, DURHAM SR, et al. Mediators of hypersensitivity and "fog"-induced asthma. *Allergy* 1985; 40: 48-57.
25. VAN DER STRAETEN M. Introduction. In: PAUWELS R, VAN DER STRAETEN M (eds.): *Bronchial hyperreactivity*. *Eur J Respir Dis* 1982; 63: Suppl. 117, 1-7.
26. WASSERMAN SI. Mediators of immediate hypersensitivity. *J Allergy Clin Immunol* 1983; 72: 101-15.
27. WEILER-RAVELL D, GODFREY S. Do exercise and antigen-induced asthma utilize the same pathways? *J Allergy Clin Immunol* 1981; 67: 391-7.
28. WOOLCOCK AJ. Tests of airway responsiveness in epidemiology. In: HARGREAVE FE, WOOLCOCK AJ (eds.): *Airway responsiveness: measurement and interpretation*. Astra, Mississauga, Ontario, 1985: 136-140.

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