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Bronchodilator response to salbutamol after spontaneous recovery from nonspecific bronchial provocation tests in asthma

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ABSTRACT: Assessment of airway responsiveness by bronchoprovocation and bronchodilatation tests is important in the diagnostic work-up protocol of bronchial asthma and it would be convenient to undertake both tests on the same occasion. However, it is not known whether this can be done accurately. Therefore, this study evaluated the effect of a prior bronchial provocation test on the bronchodilator response to salbutamol after spontaneous recovery of the forced expiratory volume in one second (FEV_1) in a group of asthmatic subjects.

On two separate occasions at the same time of day, concentration-response studies with inhaled histamine or methacholine, or a sham challenge with normal saline were carried out in a blinded, randomized manner. Changes in airway calibre were followed as FEV_1 and agonist responsiveness expressed as the provocative concentration causing a 20% fall in FEV_1 (PC_{20}). After either spontaneous recovery or a fixed-duration wait of 45 min (when appropriate), the subjects received 2×100 µg of salbutamol from a metered dose inhaler with a spacer. The bronchodilator response to salbutamol was expressed as a percentage of initial FEV_1 (ΔFEV_1% init).

Bronchial challenge with both agonists failed to alter significantly the airway response to salbutamol, with the ΔFEV_1% init mean value (range) being 16.9% (9.0–31.9) and 17.5% (11.6–31.2) on the sham and histamine/methacholine challenge day respectively.

It was shown that the degree of bronchodilatation achieved after salbutamol 200 µg is not affected by prior bronchoprovocation testing when enough time is allowed for the airways to recover spontaneously to baseline forced expiratory volume in one second. Thus evaluation of airway responsiveness by both bronchial provocation tests and bronchodilator testing can be assessed reliably within a few hours in asthmatic patients.
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Evaluations of airway responsiveness to bronchodilatory drugs such as salbutamol and to spasmogenic agonists such as histamine or methacholine are routine procedures in pulmonary function laboratories. These tests may play an important role in the diagnosis and clinical assessment of bronchial asthma as well as in pulmonary research [1]. Since both reversibility of airflow obstruction and degree of airway responsiveness are known to be objective indicators of asthma severity [1, 2], it would be convenient if they could be carried out on the same visit to the pulmonary function laboratory.

However, combining a bronchodilator response and a bronchoprovocation test on the same occasion might provide inaccurate results. It is widely acknowledged that short-acting β_2-agonists administered prior to bronchoprovocation provide functional antagonism against a wide variety of bronchoconstrictor stimuli (including histamine and methacholine) for up to 4–6 h [3–5], but little is known about the effect of histamine or methacholine bronchoconstriction on a subsequent bronchodilator response to inhaled β_2-agonists. It is possible that exposure to spasmogenic agents such as histamine and methacholine may affect the subsequent response to an inhaled β_2-agonist even after airway calibre has returned to baseline. Al-though the forced expiratory volume in one second (FEV_1) recovers rapidly after exposure to histamine or methacho-line [6], these agonists may elicit changes in bronchial blood flow that may persist well beyond the recorded changes in their spirometric values [7–9]. This may therefore increase the transepithelial clearance of subsequently administered β_2-agonists and affect their efficacy.

Therefore this study was undertaken to investigate the effect of bronchial provocation testing on subsequent bronchodilator responses to inhaled salbutamol after spontaneous recovery of FEV_1 to the prechallenge level in subjects with asthma. Evaluation of airway responsiveness by combining a bronchodilator response and a bronchial provocation test on the same occasion was shown to provide accurate results.

Methods

Subjects

Twenty four patients (16 females and 8 males), mean age 29.8±1.7 yrs (±SEM), selected from chest clinics with

Table 1. – Demographic details of the subjects

Subject no.	Sex	Age yrs	Baseline FEV1 % pred	PC20 histamine mg·mL^{-1}	PC20 methacholine mg·mL^{-1}	Recovery time min
1	F	25	63	0.17	-	45
2	F	26	83	2.39	-	45
3	M	28	90	3.71	-	60
4	M	29	84	0.57	-	45
5	F	44	84	4.92	-	45
6	M	35	84	4.08	-	45
7	F	38	85	0.40	-	30
8	M	20	75	1.50	-	45
9	F	25	76	2.22	-	60
10	M	30	80	2.20	-	60
11	F	18	73	1.13	-	30
12	F	29	77	1.70	-	45
13	F	40	77	1.77	-	60
14	F	41	79	1.20	-	45
15	M	32	88	-	2.69	90
16	F	28	85	-	3.01	75
17	F	21	79	-	1.57	60
18	F	23	81	-	0.50	45
19	F	47	87	-	1.59	60
20	M	16	83	-	2.08	60
21	F	32	68	-	1.16	90
22	M	34	75	-	0.79	45
23	F	25	82	-	3.77	90
24	F	28	67	-	2.01	60
Mean		29.8	79.4	1.46*	1.65*	
SEM		±1.7	±1.4	(0.17–4.92)	(0.50–3.77)	

*: geometric mean (range); FEV1: forced expiratory volume in one second; PC20: provocative concentration producing a 20% fall in FEV1; M: male; F: female.

stable asthma as defined by the American Thoracic Society [1], participated in the study (table 1). All subjects had a history of dyspnoea with wheezing or chest tightness and were nonsmokers with positive skin-prick tests (>3 mm weal response) to one or more of six common aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Parietaria sp*. pollens, mixed grass pollens, cat fur and dog hair). At the beginning of the study the baseline FEV1 of all subjects was at least 60% of the predicted values. None had been receiving steroids, theophylline or antihistamines within the preceding 4 weeks. Inhaled bronchodilators were discontinued for at least 8 h before each visit to the laboratory. Subjects were not studied within 4 weeks of an upper respiratory tract infection or exacerbation of their asthma and all visits to the laboratory were carried out at the same time of day and outside the pollen season. Additional criteria for inclusion were a provocative concentration of histamine or methacholine reducing FEV1 by 20% (PC20) of <8 mg·mL^{-1} and a bronchodilator response to standard salbutamol of at least 12% from initial FEV1 [10]. The study was approved by the Ethical Committee of the University of Catania, and all subjects gave their informed consent.

Bronchial provocation

Airway calibre was recorded as the FEV1 using a dry wedge spirometer (Vitalograph, Buckinghamshire, UK), with the better of two consecutive measurements being recorded.

Histamine acid phosphate (Sigma Chemical Co., St Louis, MO, USA) was dissolved in 0.9% (w/v) sodium chloride to produce a stock solution of 16 mg·mL^{-1}. In eight of the subjects studied, methacholine instead of histamine was used. Methacholine (Sigma Chemical Co.,) was freshly prepared in 0.9% (w/v) sodium chloride to produce stock solutions of 32 mg·mL^{-1}. Each stock solution was then diluted with 0.9% (w/v) sodium chloride to produce a concentration range of 0.03–8 mg·mL^{-1} for histamine and 0.03–16 mg·mL^{-1} for methacholine.

The solutions were administered as aerosols generated from a starting volume of 3 mL in a disposable Inspiron Mini-nebulizer (C.R. Bard International, Sunderland, UK) driven by compressed air at 8 L·min^{-1}. Under these conditions the nebulizer had an output of 0.48 mL·min^{-1} and generated an aerosol with a mass median particle diameter of 4.7 μm [11]. Subjects inhaled the aerosolized solutions in five breaths from end-tidal volume to full inspiratory capacity *via* a mouthpiece, as described by CHAI *et al*. [12]. Subjects were trained to take 3 s to reach full inspiratory capacity.

Bronchodilator response

FEV1 was measured with a dry wedge spirometer (Vitalograph) connected to an Apple microcomputer. The best of three technically satisfactory FEV1 measurements was recorded, expressed as a percentage of the predicted value and used for subsequent analysis. The bronchodilation test

was carried out by administering inhaled salbutamol according to a standardized protocol [10]. Two puffs of salbutamol (100 μg·puff^{-1}) were given from a metered dose inhaler with a spacer device (Aerochamber). Salbutamol was inhaled during a single, slow inspiration from functional residual capacity to total lung capacity immediately after each actuation. The breath was then held for about 10 s before exhalation. A second actuation was then repeated and FEV_1 recorded 20 min later. The bronchodilator res-ponse to salbutamol was expressed as a percentage of initial FEV_1 (ΔFEV_1% init) [10].

Study design

The subjects were selected on two separate days. On the first occasion skin testing, spirometry and bronchial challenge were performed. On the second visit they underwent a standardized bronchodilatation test at the lung function laboratory. After inclusion, the subjects entered a single-blind, randomized, cross-over study consisting of two separate visits 3–7 days apart, during which a bronchodilator test with salbutamol was preceded by a bronchial provocation test with inhaled histamine or methacholine, or normal saline (sham challenge). To minimize bias, statistical analysis was carried out by an independent investigator.

On the first visit, subjects underwent concentration-response studies with inhaled histamine (except for subjects no. 15–24, who were given methacholine). After 15 min rest, three baseline measurements of FEV_1 were made at intervals of 3 min followed by inhalation of 0.9% (w/v) sodium chloride and further FEV_1 measurements repeated at 1 and 3 min. Provided FEV_1 had not fallen by >10% of the baseline value, a histamine or methacholine concentration-response study was carried out. After administration of each concentration of the agonist, FEV_1 was measured at 1 and 3 min. Increasing doubled concentrations of histamine or methacholine were inhaled at intervals of 5 min until FEV_1 had fallen by >20% of the post-saline baseline value and the corresponding PC_{20} values had been derived. Following the bronchoprovocation test, the airways were allowed to recover spontaneously, until FEV_1 had returned to within 5% of the post-saline baseline value. On achieving this, after approximately 30–90 min, a bronchodilator test with inhaled salbutamol (200 μg) was then undertaken, and the FEV_1 value recorded 20 min later.

On the second visit, all the subjects studied underwent a sham challenge with inhaled normal saline to maintain blindness. In brief, three solutions of normal saline were nebulized at intervals of approximately 5 min and the subsequent bronchodilator test with inhaled salbutamol was carried out after 45 min in the manner described above.

Data analyses

Values refer to the mean±SEM unless otherwise stated, and a p-value of <0.05 was accepted as significant. Pre- and post-challenge baseline values of FEV_1 prior to the bronchodilator test were compared between and within study days by two-factor analysis of variance (ANOVA) followed by the Neuman-Keuls test where appropriate.

Concentration-response curves were constructed by plotting the percentage change in FEV_1 from the post-saline baseline value against the cumulative concentration of the agonist administered on a logarithmic scale and the PC_{20} determined by linear interpolation. PC_{20} values were log transformed prior to analysis.

Repeatability of bronchodilatation testing was assessed according to the method of ALTMAN and BLAND [13] by deriving the standard deviation of the differences between the ΔFEV_1% init values obtained at the inclusion in the study and those obtained on the sham challenge day. The coefficient of repeatability (CR) is twice this standard de-viation.

The response to the bronchodilator obtained on the two study days was expressed as ΔFEV_1% init and compared using the Student's t-test for paired data. Any relationship between ΔFEV_1% init and airway responses to histamine or methacholine (log PC_{20}) was examined by least-squares linear regression analysis. The bronchodilator response in subjects challenged with histamine was compared with that in subjects challenged with methacholine using the Mann-Whitney U-test.

The probability of a Type II error (β) was assessed, that is, the risk of wrongly accepting the null hypothesis of absence of difference in bronchodilation between the two study days. Thus, the power of the test to detect change was calculated. Power calculations, based on the assumption that a significant change in the ΔFEV_1% init is approximately 3%, indicated for the 24 subjects studied that there was a 85% chance of detecting a significant difference with a significance level of <5% (two-sided).

Results

Of the 30 consecutive subjects who entered the study, five did not complete the study because FEV_1 recordings on the second study day differed by more than 10% from those on the first day and one because he repeatedly failed to attend his appointment. Thus, a total of 16 women and eight men completed the study (table 1).

There was no statistically significant change between baseline FEV_1 values obtained on the two study days. After bronchoprovocation, FEV_1 had spontaneously returned to within 5% of the baseline values in all of the subjects studied in 90 min (table 1).

The bronchodilator response to salbutamol in this group of subjects was found to be repeatable, with a CR of 6.4%. The differences between the ΔFEV_1% init values obtained at the inclusion in the study and those obtained on the sham challenge day were within 6.4% in 23 out of 24 subjects receiving salbutamol.

Twenty min after the administration of 200 μg salbutamol, FEV_1 obtained on the histamine or methacholine challenge day was not significantly different from that obtained on the control challenge day. ΔFEV_1% init after 200 μg salbutamol on the agonist study day was 17.5%, while that on the control challenge day was 16.9% (table 2).

There was no statistically significant relationship between ΔFEV_1% init and airway responses to histamine or methacholine (log PC_{20}). Finally, no significant difference was detected when ΔFEV_1% init values were analysed separately for histamine and methacholine.

Table 2. – Effect of previous bronchoprovocation test on bronchodilator response

Subject no.	ΔFEV1% init		
	Baseline	Post-control	Post-agonists*
1	25.0	31.9	26.6
2	19.7	16.3	14.3
3	13.3	12.7	12.4
4	12.5	17.7	13.3
5	14.9	9.0	12.3
6	15.1	12.1	11.6
7	16.3	12.0	13.2
8	12.0	13.5	14.2
9	14.4	17.6	21.2
10	12.0	14.5	12.0
11	18.6	17.8	30.0
12	15.1	17.4	18.9
13	14.0	15.7	14.7
14	14.9	18.8	18.4
15	21.0	24.6	20.7
16	18.8	18.7	22.4
17	15.5	17.7	13.0
18	12.2	13.1	16.2
19	12.0	13.5	14.2
20	17.7	14.9	12.5
21	22.2	18.0	20.0
22	17.0	14.5	18.6
23	18.0	19.5	18.0
24	25.7	24.0	31.2
Mean	16.6	16.9	17.5
SEM	±0.7	±1.0	±1.2

*: subjects 1–14, bronchoprovocation test with histamine; 15–24, bronchoprovocation test with methacholine. ΔFEV1% init: percentage of the initial forced expiratory volume in one second.

Discussion

Although it has been suggested that pulmonary function tests can be accurately performed after a histamine challenge when FEV1 has returned to 95% of the baseline value [14, 15], the effect of a prior bronchial provocation test with nonspecific agonists on subsequent bronchodilator response to salbutamol has not been thoroughly elucidated. This is important in order to assess the validity of performing the two tests on the same occasion. The findings of the present study have shown that, when compared to sham challenge, the bronchodilator response to salbutamol is unaffected by prior bronchoprovocation test with histamine and/or methacholine when spontaneous recovery to baseline spirometry is allowed. This result is independent of the agonist used in bronchoprovocation testing. Thus, the evaluation of airway responsiveness by combining a bronchodilator response and a bronchial provocation test on the same occasion provides accurate results. Similar conclusions have been obtained in children with stable asthma [16].

Airway obstruction caused by spasmogenic stimuli is the result of a complex process in which airway smooth muscle shortening and oedema of the airway wall due to increased post-capillary venular leakage are thought to be predominant [7–9]. In theory, a thickened mucosa and submucosa and altered volume and properties of airway secretions may affect the availability of the β_2-agonists to their related receptors, limiting or delaying the bronchodilator response even in presence of restored baseline respiratory function. The present findings that bronchodilatation with β_2-agonists remains unaffected by prior bronchoprovocation tests with nonspecific agonists clearly indicate that the above-mentioned mechanisms are not important. However, timing of administration of the β_2-agonist after challenge and its dosage may have influenced the bronchodilator response. MERKUS *et al.* [16] have shown that this may be a possibility. Indeed, when 800 μg salbutamol was administered instead of 400 μg, no effect of prior bronchoprovocation with histamine challenge was observed.

No significant difference was detected when ΔFEV1% init values were analysed separately for histamine and methacholine. When results were examined separately for histamine and methacholine, similar falls in FEV1 values from baseline were reported, thus excluding any potential agonist-related effect (or lack of effect). This again confirms the view that even if the action of agonists is perpetuated on the airway structures for longer than their action on the calibre of the large airways, this does not affect subsequent changes in airway calibre after inhaled salbutamol when assessed as FEV1.

Bronchial hyperresponsiveness (BHR) to nonspecific stimuli and the increase in FEV1 in response to bronchodilators are both important hallmarks of asthma [1]. Therefore, it is tempting to regard the two phenomena as highly correlated, and several studies have used the response to bronchodilators as an indicator of BHR [17, 18]. We have found that there was no statistically significant relationship between ΔFEV1% init and airway responses to histamine or methacholine. This is in keeping with a number of studies which failed to show a correlation between BHR and the bronchodilator response to salbutamol [19, 20]. It is likely that important differences are present in the mechanisms underlying both phenomena. Patients with irreversible airway obstruction exhibit significant bronchoconstrictor responses, whilst healthy subjects without BHR may reveal large bronchodilator responses [21]. Moreover, the observation that the bronchodilator response persists longer than the protective effect against bronchoconstrictor stimuli [3] adds further evidence to the view that diverse pathophysiological mechanisms are involved. Thus, in asthmatic subjects, the bronchodilator response should not be directly related to BHR.

In conclusion, in asthmatic subjects the degree of bronchodilatation achieved after a single 200 μg dose of salbutamol is not affected by prior bronchoprovocation testing when enough time is allowed for the airways to recover spontaneously to prechallenge levels of forced expiratory volume in one second (FEV1). Thus evaluation of airway responsiveness by both bronchial provocation tests and bronchodilator testing can be assessed reliably within a few hours in asthmatic patients.

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