ORIGINAL ARTICLE

Bronchodilator Response in Patients with Persistent Allergic Asthma Could Not Predict Airway Hyperresponsiveness

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Anticholinergics, or specific antimuscarinic agents, by inhibition of muscarinic receptors cause bronchodilatation, which might correlate with activation of these receptors by the muscarinic agonist methacholine. The aim of this study was to determine whether a positive bronchodilator response to the anticholinergic ipratropium bromide could predict airway hyperresponsiveness in patients with persistent allergic asthma. The study comprised 40 patients with mild and moderate persistent allergic asthma. Diagnosis was established by clinical and functional follow-up (skin-prick test, spirometry, bronchodilator tests with salbutamol and ipratropium bromide, and methacholine challenge testing). The bronchodilator response was positive to both bronchodilator drugs in all patients. After salbutamol inhalation, forced expiratory volume in 1 second (FEV_1) increased by 18.39 ± 6.18%, $p < .01$, whereas after ipratropium bromide, FEV_1 increased by 19.14 ± 6.74%, $p < .01$. The mean value of FEV_1 decreased by 25.75 ± 5.16%, $p < .01$ after methacholine (PC_{20} FEV_1 [provocative concentration of methacholine that results in a 20% fall in FEV_1] from 0.026 to 1.914 mg/mL). Using linear regression, between methacholine challenge testing and bronchodilator response to salbutamol, a positive, weak, and stastistically significant correlation for FEV_1 was found ($p < .05$). Correlations between methacholine challenge testing and the bronchodilator response to ipratropium bromide were positive and weak but not statistically significant. The positive bronchodilator response to ipratropium bromide could not predict airway hyperresponsiveness.

Key words: *airway hyperresponsiveness, allergic asthma, bronchodilator response, ipratropium bromide, methacholine challenge testing, salbutamol*

Airway hyperresponsiveness in asthma is characterized by an increased sensitivity and an increased maximal response to a variety of bronchoconstrictor agents.[1–4] It is known that inflammatory processes have been associated with the presence of airway hyperresponsiveness in subjects with asthma.[5,6] Airway hyperresponsiveness can be quantified by measuring the dose or concentration of inhaled methacholine or histamine that causes a 20% decrease in forced expiratory volume in 1 second (FEV_1) ($PC_{20}FEV_1$ [provocative concentration of methacholine that results in a 20% fall in FEV_1]).

Neural mechanisms have long been regarded as factors contributing to the pathogenesis of asthma and involved in airway hyperresponsivenes, a hallmark of asthma.[7] Cholinergic nerves play an important role in the regulation of airway calibre in many species, including humans, and they form the dominant constrictor mechanism in the airways. Preganglionic and postganglionic parasympathetic nerves release acethylcholine. Anticholinergics, or muscarinic antagonists, by inhibition of muscarinic receptors cause bronchodilatation, which might correlate with activation of these receptors by the muscarinic agonist methacholine.

Bronchodilator responsiveness and bronchoconstrictor responsiveness have been considered physiologic opposites in patients with obstructive airway disease. The study by Douma and colleagues suggested that bronchoconstrictor responsiveness and bronchodilator responsiveness are not highly correlated.[8]

We hypothesized whether the bronchodilator response to anticholinergic ipratropium bromide correlates better with methacholine challenge testing than the bronchodilator response to the β_2 agonist salbutamol in patients with persistent allergic asthma. If this is true, it would mean that a positive bronchodilator response to ipratropium

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bromide could predict a positive bronchoconstrictor response to methacholine in patients with persistent allergic asthma. If so, another diagnostic tool for asthma could be established that is simpler and cheaper, and thus more widely acceptable, than the procedure for methacholine challenge testing.

Today, we are aware of a certain number of asthmatic patients who do not have a positive bronchodilator response to short-acting β_2 agonists, such as salbutamol, perhaps even 20% of them, owing to a β_2 agonist receptor gene polymorphism. In this light, an alternative measure of airway response, besides the bronchodilatator test with a short-acting β_2 agonist, seems more important. Because methacholine challenge testing is a costly and time-consuming procedure, and the safety of both patients and technicians should be considered in the design of the test room and the test procedures, we hypothesized that another diagnostic test, a bronchodilatator test with an anticholinergic agent, could replace it concerning the possibility of increased airway sensitivity detection as a hallmark of asthma. To test this hypothesis, we decided to perform serial lung function testing with different agents on subjects with persistent allergic asthma with the purpose of finding the best correlation between them. The simplicity and safety of the bronchodilator test with ipratropium bromide versus a methacholine challenge warrants this evaluation.

Patients and Methods

The study was performed at the Outpatient Centre for Diseases of the Respiratory System in Zagreb. The Ethics Committee approved the study.

The study comprised 40 patients with persistent allergic asthma, 23 males and 17 females, aged 16 to 65 years (34.40 $\pm$ 14.16). A diagnosis of asthma was established according to The Global Initiative on Asthma (GINA) classification[9]: 34 patients had GINA II, mild persistent asthma (peak expiratory flow (PEF) or $FEV_1 \geq 80\%$ of predicted, variability 20–30%), and 6 patients had GINA III, moderate persistent asthma (PEF or $FEV_1 \geq 70\%$ of predicted).

None of the patients had used inhaled or oral corticosteroids, long-acting bronchodilators, theophyllines, antihistamines, sodium cromoglycate, or nedocromil sodium for at least 4 weeks preceding the study. Short-acting bronchodilators were not used for at least 12 hours before pulmonary function testing. Current or ex-smokers, pregnant women, and patients with cardiovascular disease, rhinosinusitis, or respiratory tract infections during the 4 weeks before the study were excluded.

Patients with inclusion criteria were recruited into the study one after another by the time of arrival at the outpatient centre during 2 autumn months.

Each patient underwent pulmonary function testing (spirometry, bronchodilator tests with salbutamol and ipratropium bromide), methacholine challenge testing, and the skin-prick test.

Skin-prick tests were performed with standard airborne allergens[10,11]: house dust mite (*Dermatophagoides pteronyssinus*), feathers, mould, dog and cat epithelium, mixed tree pollen, mixed grass pollen, and mixed weed pollen. A mean wheal diameter $\geq$ 3 mm of control solution was considered positive.

A Pneumo Screen spirometer (Jaeger, Germany) was used for pulmonary function tests. The observed parameters were the FEV_1 and the forced vital capacity (FVC). Forced expiratory manoeuvres were repeated until three measurements of FEV_1 reproducible to 100 mL were obtained; the larger FEV_1 value was used in analysis. Reference values are those of the European Community for Coal and Steel, CECA II.[12]

On the first day, spirometry with a bronchodilator test with salbutamol was performed. After baseline spirometry, subjects inhaled 400 μg of metered dose inhaler (MDI) salbutamol (Ventolin, Pliva, Zagreb, Croatia). Spirometry was repeated after 20 minutes.

On the second day, spirometry with an ipratropium bromide test was performed. After baseline spirometry, subjects inhaled 80 μg of MDI ipratropium bromide (Atrovent, Boehringer Ingelheim, Ingelheim am Rhein, Germany). Spirometry was repeated after 45 minutes.

A reversibility test was considered positive if there was a 12% increase in FEV_1 and/or $FEV_1 = 200$ mL compared with prebronchodilator FEV_1 expressed as a percentage of predicted value.[13]

On the third day, methacholine challenge testing was performed by the standardized 2-minute tidal breathing method[14,15] using a Pneumo Screen spirometer and Pari Provocation Test I. Without a bronchodilator, the baseline FEV_1 had to be more than 70% of the predicted value.[13,16] Doubling concentrations of methacholine (acetyl-β-methacholine chloride, Janssen Pharmaceutics, Belgium) ranging between 0.03 and 8 mg/mL were given by inhalation at intervals of 5 minutes, each for a period of 2 minutes, after the first inhalation of normal saline. FEV_1 was measured after each inhalation. If methacholine did induce a drop of 20% or more, the intrapolated concentration at which a 20% drop in FEV_1 had occurred was calculated and termed the $PC_{20}FEV_1$.

Statistical analysis of the data was performed by using a paired *t*-test. The results are expressed as mean ± SD. Methacholine PC_{20} was calculated from the log concentration-response curves by linear interpolation of the two adjacent data points. All PC_{20} values were log-transformed before analysis. Analyses were performed using linear regression for correlations between the log-transformed PC_{20} values and both bronchodilator test values.

Results

Forty patients with persistent allergic asthma were included in the study. In all patients, the skin-prick test was positive. *Dermatophagoides pteronyssinus* was the most positive single allergen (37 of 40).

Table 1 shows the baseline characteristics of patients with persistent allergic asthma (age, gender, baseline FEV_1 and FVC, and atopic status).

Our results showed a positive bronchodilator response to salbutamol in all patients with persistent allergic asthma. After salbutamol inhalation, the mean value of FEV_1 increased by 18.39 ± 6.18% (from 12.40 to 35.60%). The mean value of FVC increased by 7.96 ± 6.96% Both spirometric values (FEV_1 and FVC) were significantly higher after the bronchodilator test ($p < .01$).

The bronchodilator response to ipratropium bromide was also positive in all patients with persistent allergic asthma. The mean value of FEV_1 increased by 19.14 ± 6.74% (from 12.10 to 37.20%). The mean value of FVC increased by 8.74 ± 6.98%. Spirometric values for FVC and FEV_1 were significantly higher after ipratropium bromide inhalation ($p < .01$).

All patients with persistent allergic asthma had positive methacholine challenge testing. The mean value of FEV_1 decreased by 25.75 ± 5.16% (from 20.20 to 40.40%) after inhalation concentrations between 0.03 and 2.0 mg/mL of methacholine ($PC_{20}FEV_1$ from 0.026 to 1.914 mg/mL). Spirometric parameters were significantly lower ($p < .01$) after methacholine challenge testing.

Table 1. Baseline Characteristics of Patients with Persistent Allergic Asthma

Number	*40*
Age (yr)	34.40 (16–65)
Male/female	23/17
Baseline FEV_1 %	94.75%
Baseline FVC %	110.61%
Sensitization to 1 allergen	47.5%
Sensitization to 2 to 5 allergens	52.5%

FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity.

Table 2 shows the percentage of increase for parameter FEV_1 after the bronchodilator test with salbutamol and ipratropium bromide and $PC_{20}FEV_1$ after methacholine challenge testing.

Correlations between methacholine challenge testing and bronchodilator response to salbutamol and ipratropium bromide for FVC were positive, weak, and statistically not significant. The correlation between methacholine challenge testing and the bronchodilator response to salbutamol for FEV_1 was positive, weak, and statistically significant ($p < .05$). The correlation between methacholine challenge testing and the bronchodilator response to ipratropium bromide for FEV_1 was positive, very weak, and statistically not significant.

Figure 1 shows a scatterplot diagrams of the percent change in FEV_1 after inhalation of ipratropium bromide versus the natural logarithm of $PC_{20}FEV_1$ for methacholine challenge ($r = .169$; $p > .05$) and in FEV_1 after inhalation of salbutamol versus the natural logarithm of $PC_{20}FEV_1$ for methacholine challenge ($r = .314$, $p = .049$).

Discussion

This study has measured the acute bronchodilator effect of an inhaled anticholinergic agent (ipratropium bromide) in 40 subjects with persistent allergic asthma to determine if a positive bronchodilator response predicts the presence of airway hyperresponsiveness. Methacholine chloride is a synthetic derivate of acetylcholine, which is a parasympathetic neurotransmitter that is very important for airway smooth muscle tone. As anticholinergic agents (such as ipratropium) act through the same neural pathway as methacholine but in opposite directions, we were interested in whether there is a correlation between bronchoconstrictor and bronchodilator response in airways.

In our patients with allergic asthma, FEV_1 increased by 18.39% after salbutamol inhalation, whereas after ipratropium, FEV_1 increased by 19.14%. Our results correspond to the results of other authors who have proven reversibility to bronchodilators in patients with asthma.[8,13,14] A positive bronchodilator response to ipratropium bromide indicates that patients with persistent allergic asthma have increased cholinergic tone.[17]

Change in FEV_1 is the primary outcome measure for methacholine challenge testing.[18] All asthmatics had positive methacholine challenge testing. After inhalation concentrations between 0.03 and 2.0 mg/mL of methacholine ($PC_{20}FEV_1$ from 0.026 to 1.914 mg/mL), the mean

Table 2. Percentage of Increase for Parameter FEV_1 after the Bronchodilator Test with Salbutamol and Ipratropium Bromide and $PC_{20}FEV_1$ after Methacholine Challenge Testing

	Salbutamol % of Increase FEV_1	*Ipratropium % of Increase FEV_1*	*$PC_{20}FEV_1$ mg/mL*
Mean	18.39	19.14	0.645
SD	6.18	6.74	0.540
Minimum	12.40	12.10	0.026
Maximum	35.60	37.20	1.914

FEV_1 = forced expiratory volume in 1 second; $PC_{20}FEV_1$ = provocative concentration of methacholine that results in a 20% fall in FEV_1.

value of FEV_1 decreased by 25.75% (from 20.20 to 40.40%).

In this study, the correlation between a pre- and a postbronchodilator response to salbutamol and methacholine challenge testing for parameter FEV_1 ($p < .05$) was proven significant. There was no significant correlation between the pre- and postbronchodilator response to ipratropium bromide and methacholine challenge testing for FEV_1. Our main finding was that a positive bronchodilator response to ipratropium bromide did not predict a positive methacholine hyperresponsiveness test.

The study by Douma and colleagues showed a lack of correlation between bronchoconstrictor response and bronchodilator response in a population-based study.[8] Bronchoconstrictor responsiveness and bronchodilator responsiveness are two different phenotypic markers that are not interchangeable in epidemiologic studies. The absence of bronchodilator responsiveness does not imply the absence of bronchoconstrictor responsiveness, even in individuals with airway obstruction.

It is not surprising that application of an anticholinergic drug to predict methacholine-induced bronchoconstriction in this study has failed given the complexity of chronic airway inflammation and remodelling in asthma, even if the activity of allergy-related airway inflammation is known.

In this study, responses to salbutamol are better correlated with the methacholine response than responses to ipratropium. Anticholinergic therapy may prevent only the bronchoconstrictor component resulting from a cholinergic reflex, not the direct effects of bronchoconstrictor mediators, in contrast to β-adrenergic agonists, which reverse bronchoconstriction irrespective of mechanism because they are functional antagonists. Ipratropium bromide is a non-selective anticholinergic drug because it blocks not only M_3 receptors but also the prejunctional M_2 receptors.[19] Inhibition of the M_2 receptors leads to more acetylcholine release during cholinergic nerve stimulation, which may overcome postjunctional blockade. Thus, the non-selective cholinergic antagonists may be less efficient

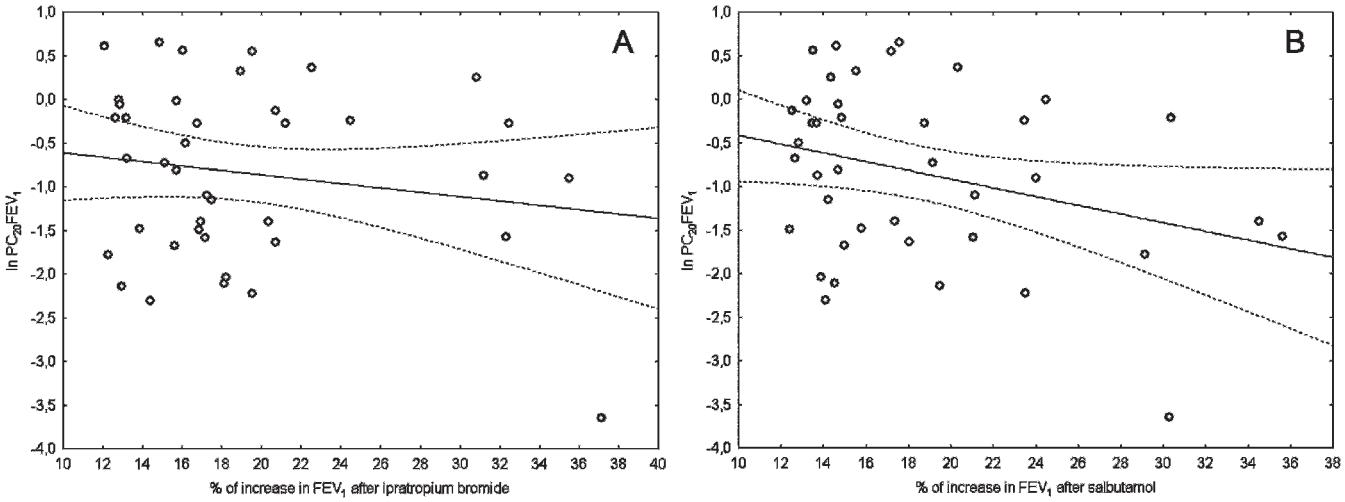

Figure 1. *A*, Scatterplot diagram of the percent change in forced expiratory volume in 1 second (FEV_1) after inhalation of ipratropium bromide versus the natural logarithm of $PC_{20}FEV_1$ (provocative concentration of methacholine that results in a 20% fall in FEV_1) for methacholine challenge ($r = .169$, $p > .05$). *B*, Scatterplot diagram of the percent change in FEV_1 after inhalation of salbutamol versus the natural logarithm of $PC_{20}FEV_1$ for methacholine challenge ($r = .314$, $p = .049$). *Dashed lines* represent the 95% confidence interval for linear regression.

than selective M_3 receptor antagonists. Recent data show that the long-acting muscarinic antagonist tiotropium bromide could inhibit smooth muscle–specific myosin expression, which also inhibits the increase in and contractility of airway smooth muscle mass and remodelling, and prevent airway hyperresponsiveness.[20]

The β_2 agonists produce bronchodilatation by direct stimulation of β_2 receptors on airway smooth muscle, leading to relaxation. But β_2 adrenoceptors, through which salbutamol acts, are desensitized in asthma, in part owing to the inflammatory effect of cysteinyl leukotrienes.[21] In addition, there are over 100 different inflammatory mediators that modulate airway smooth muscle tone in humans. It is well known that mediators of allergic reaction, such as cysteinyl leukotrienes and endothelin, could increase bronchial muscle hypertrophy.[22] It is also known that salbutamol could prevent bronchoconstriction owing to cysteinyl leukotrienes, whereas this is not the case with anticholinergics.[23] Salbutamol is a potent bronchodilator, but it could also inhibit cysteinyl leukotriene synthesis by the airway cells.[24]

Asthma is a complex disorder with a different disease pattern and a different response to therapy, depending on the combination of asthma genotype and phenotype in each patient. It is worth the effort to perform the study with a larger number of asthma patients to determine what happens in those asthmatics with a negative bronchodilator response to salbutamol. Futher studies must investigate if ipratropium bronchodilator testing results in asthmatics with a negative salbutamol test (and probably with a gene polymorphism for the β_2 agonist receptor) could produce more useful data concerning asthma diagnosis, therapy benefit, and prognostic value.

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