Airways Responses to Ipratropium Bromide Do Not Vary with Time in Asthmatic Subjects*

Studies of Interindividual and Intraindividual Variation of Bronchodilatation and Protection Against Histamine-Induced Bronchoconstriction

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Bronchial histamine provocation tests were performed in nine patients with nonallergic asthma on four consecutive days 45 minutes after inhalation of placebo or ipratropium bromide in a dose-response manner (40μg, 200μg, and 800μg). The drugs were administered double-blind, one dose on each day. This procedure was repeated identically after three to nine months to investigate whether the bronchial responses to ipratropium bromide are constant or change with time. Ipratropium bromide induced a significantly better bronchodilatation and protection against histamine-induced bronchoconstriction than placebo with no differences between the three doses. No correlation between bronchodilatation and protection was found. In six asthmatic patients ("responders") ipratropium bromide induced a significant protective effect against histamine-induced bronchoconstriction but no dose-response relationship was found. In three patients none or a very poor protective effect was found at all dose levels ("nonresponders"). The protective effect of ipratropium bromide against histamine-induced bronchoconstriction did not differ between the first and second occasion. Thus, the bronchoprotection differed between different asthmatic subjects but did not vary with time (three to nine months) within the same subject. This finding seems to be of clinical importance since it implicates that the effect of anticholinergic agents on the airways is predictable.

(Chest 1990; 97:46-51)

MDI = metered-dose inhaler

Antimuscarinic drugs such as atropine and ipratropium bromide have a bronchodilator effect by blocking muscarinic receptors in the airways. Consequently, these drugs have a good protective effect against bronchoconstriction induced by acetylcholine1,2 and methacholine.3-5 There is also evidence that anticholinergic drugs possess a protective effect against several bronchoconstrictor stimuli such as histamine,6-10 allergen,9,11,12 and cold air.4,13-15 however, these results are not confirmed by others.3,5,10-20 There is no clear relationship between the bronchodilator effect and the protective effect against bronchoconstrictor stimuli of anticholinergic agents. Thus, higher doses of atropine were needed to obtain a protective effect against histamine- and cold air-induced bronchoconstriction than to increase baseline airway caliber.4,10 On the other hand, in some asthmatic patients a protective effect against methacholine-induced bronchoconstriction was obtained in doses that did not influence basal bronchial tone.6 Thus, the responsiveness to antimuscarinic drugs seems to be different with regard to the bronchodilator and the protective effect. Furthermore, there is an interindividual variation of the responsiveness to antimuscarinic drugs21 indicating that there are responders and nonresponders in this respect. Possible explanations to the finding of responders and nonresponders could be fluctuations of the airway responses to anticholinergic agents in the same asthmatic subject, ie, an intraindividual fluctuation of airway responses, or different responses in different patients, ie, a "true" interindividual difference with no intraindividual variation. To our knowledge, no studies have been performed with the aim to elucidate whether the airway responses to antimuscarinic drugs are constant within an asthmatic subject or if they are varying with the time, although this item seems to be of clinical importance.

The aims of the present study were to compare the effect of different doses of ipratropium bromide on basal bronchial tone and histamine-induced bronchoconstriction and to investigate whether there is a relationship between the bronchodilator and the protective effect. We also wanted to investigate whether the bronchodilator response and the bronchoprotective effect of ipratropium bromide is a constant feature within an asthmatic subject or if there is a variation...
with time in this respect.

**Material and Methods**

**Subjects**

Twelve asthmatic patients (two were men) with a mean age of 42 years (range, 21 to 60 years) participated in the study. All subjects had a history of nonallergic bronchial asthma and all had negative skin prick tests with the exception of one who was positive to cat dander. One subject was a smoker and two were ex-smokers. A histamine bronchial challenge was performed prior to the study to confirm the occurrence of bronchial hyperreactivity. PC<sub>20</sub>FEV<sub>1</sub>, (i.e., the dose of histamine that yields a reduction of FEV<sub>1</sub> by 20 percent from basal prechallenge value) was <2.0 mg/ml in all subjects. Treatment with β<sub>2</sub>-agonists, theophylline, and anticholinergic drugs was withdrawn 12 h prior to the trial. Treatment with inhaled steroids was continued. No patient took oral steroids, cromolyn sodium (disodium cromoglycate), or antihistaminic drugs. The patients gave their informed consent to participate in the study that had the approval of the local ethics committee.

**Procedure**

The study was divided into two equal parts with 6.8 months (three to nine months) between. In each part of the study the patients came to the laboratory on four consecutive days at the same time each day. After basal lung function measurements (FEV<sub>1</sub>, FVC, and Raw) placebo or 40 µg, 200 µg, or 600 µg of ipratropium bromide was administered in a randomized double-blind manner. Only one regimen was performed each day. Ipratropium bromide was administered with metered-dose inhalers (MDI) which gave 20 µg or 100 µg/puff, which in combination with placebo made it possible to obtain identical conditions on the four different days. Forty-five minutes after administration of the drug, lung function measurements were repeated after which a histamine challenge was performed. FEV<sub>1</sub> and Raw were measured three minutes after the start of the inhalation at each dose step. The provocation was ceased when FEV<sub>1</sub> decreased more than 20 percent from the values obtained 45 minutes after drug inhalation (postdrug value). After the last histamine dose, 1.5 mg of terbutaline was inhaled from a MDI connected to a 750-ml pear-shaped plastic tube (Nebulator, AB Draco, Lund, Sweden) and final lung function measurements were performed ten minutes later. As described above, this procedure (investigations on four consecutive days) was repeated identically after three to nine months.

**Measurements**

Forced expiratory volume in one second (FEV<sub>1</sub>) and forced vital capacity (FVC) were measured with a wedge spirometer (Vitalograph) and the best of three values was stated. Airway resistance (Raw) was measured by intermittent flow interrupter technique (AW-test, Erich Jaeger GmbH and Co., Wurzburg, FRG). The Raw value was calculated as the mean value of three measurements.

Histamine challenges were performed with inhalation of the diluent followed by inhalation of increasing doses of histamine starting at 0.125 mg/ml (0.063 mg/ml in one subject); each increment represented a doubling of the dose. The histamine challenges were performed with an Aiolas System Inhaler (Karlsdts Syrgasfabrik AB, Karlstad, Sweden) which at a driving pressure of 160 kPa has an output of 0.625 ± 0.005 ml/min and generates an aerosol with a median diameter (dry particles) of 0.8µm in which 80 percent of the mass represent particles <3.75µm. The nebulizer was connected to a metal tube with a diameter of 4 cm and a length of 70 cm to which additional air flow was connected and corrected to total air flow of 0.4 L/s. The subjects inhaled the nebulized solution through the metal tube at tidal breathing with a frequency of 0.15 Hz using a metronome to guide the breathing. At the outlet of the metal tube a back valve was connected to limit the inspiratory flow to the supplied air flow of 0.4 L/s.

**Statistical Analyses**

Results are presented as mean values ± SEM. In Table 1 median values and 25th and 75th percentiles are given. A p<0.05 is considered significant. Statistical evaluations were performed by using two-tailed Student's t test for paired and unpaired observations, analysis of variance (ANOVA), and linear regression. Statistical analyses of PC<sub>20</sub>FEV<sub>1</sub> values were performed on logarithmically transformed data.

**Results**

**Basal Conditions**

Nine of the 12 patients completed the study. Three of the subjects did not attend the second part of the trial, two because of social reasons and one because of an intercurrent disease. All patients were asymptomatic and in a stable phase of their disease at the time of the trials. All but two patients had basal FEV<sub>1</sub> values greater than 90 percent of predicted value<sup>22</sup> prior to all the eight bronchial challenges. The two remaining patients varied between 60 percent and 80 percent and between 75 percent and 100 percent of

Table 1—Individual, Median Values, and the 25th to 75th Percentile for PC<sub>20</sub>FEV<sub>1</sub> for Histamine in Nine Patients with Nonallergic Asthma<sup>*</sup>

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>PC&lt;sub&gt;20&lt;/sub&gt;FEV&lt;sub&gt;1&lt;/sub&gt; Placebo</th>
<th>PC&lt;sub&gt;20&lt;/sub&gt;IB 40µg</th>
<th>PC&lt;sub&gt;20&lt;/sub&gt;IB 200µg</th>
<th>PC&lt;sub&gt;20&lt;/sub&gt;IB 800µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.18</td>
<td>0.29</td>
<td>0.39</td>
<td>0.32</td>
</tr>
<tr>
<td>2</td>
<td>0.35</td>
<td>2.6</td>
<td>2.8</td>
<td>1.96</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>2.2</td>
<td>3.1</td>
<td>4.3</td>
</tr>
<tr>
<td>4</td>
<td>1.6</td>
<td>2.7</td>
<td>1.9</td>
<td>2.58</td>
</tr>
<tr>
<td>5</td>
<td>0.58</td>
<td>0.72</td>
<td>0.76</td>
<td>0.94</td>
</tr>
<tr>
<td>6</td>
<td>0.125</td>
<td>0.4</td>
<td>0.87</td>
<td>0.14</td>
</tr>
<tr>
<td>7</td>
<td>0.072&lt;0.063</td>
<td>0.063</td>
<td>0.063</td>
<td>0.063</td>
</tr>
<tr>
<td>8</td>
<td>0.23</td>
<td>0.355</td>
<td>0.3</td>
<td>0.65</td>
</tr>
<tr>
<td>9</td>
<td>0.14</td>
<td>1.42</td>
<td>0.81</td>
<td>1</td>
</tr>
<tr>
<td>Median</td>
<td>0.24</td>
<td>0.74</td>
<td>0.79</td>
<td>0.89</td>
</tr>
</tbody>
</table>

25th-75th percentile: 0.14 - 0.55 (0.34 - 2.36 0.36 - 2.20 0.29 - 2.25 0.34 - 0.96 1.18 1.12 1.18

Aspirin 1-individual, PCI, JANEUARY, 1990 47

*PC<sub>20</sub>FEV<sub>1</sub> is determined after inhalation of placebo and three doses of ipratropium bromide (IB) at separate days. I and II denote the two identical trials performed with three- to nine-month interval. Patients 1, 7, and 8 exhibit poor or no protection at all dose levels.
Protection Against Histamine-Induced Bronchoconstriction

Ipratropium bromide offered significantly better protection against histamine-induced bronchospasm than placebo. The differences in \( \text{PC}_{20}\text{FEV}_1 \) values between placebo and ipratropium bromide were statistically significant for all three doses (\( p<0.01 \)). No significant difference was found between the different doses of ipratropium bromide (\( p>0.77 \)).

The bronchial reactivity in each subject was similar when the two trials were compared (Fig 2 and Table 1) and there were highly significant correlations between the \( \text{PC}_{20} \) values of the first and second trial after inhalation of placebo and at each dose level (Fig 3). For placebo and all three doses of ipratropium the 95 percent confidence interval for the coefficient of regression included 1.0.

The protection of ipratropium bromide is considered to be significant if the \( \text{PC}_{20}\text{FEV}_1 \) is doubled when compared with placebo. In three subjects (No. 1, 7, and 8) ipratropium bromide offered a very poor or no bronchoprotection at all dose levels. In subject 7 the exact \( \text{PC}_{20}\text{FEV}_1 \) could not be calculated in most tests since he had a significant reduction of \( \text{FEV}_1 \) at the lowest dose which was 0.063 mg/ml. However, the \( \text{PC}_{20}\text{FEV}_1 \) values were close to 0.063 mg/ml at all occasions. Regarding the protective effect against histamine-induced bronchoconstriction there was no clear dose-response relationship in any of the nine patients. The subjects could thus be divided into a

**FIGURE 1.** \( \text{FEV}_1 \) (mean values ± SEM) before and 45 minutes after inhalation of placebo and ipratropium bromide (40, 200, and 800 \( \mu \)g) in the first (closed circles) and the second (open circles) trial.

Predicted values, respectively. There was no significant difference in basal (prechallenge) lung function (\( \text{FEV}_1 \) and Raw) when values obtained on the eight occasions were compared by means of ANOVA (for \( \text{FEV}_1 \) [\( F=2.04 \)] \( p=0.07 \), and for Raw [\( F=1.66 \)] \( p=0.14 \)).

**Bronchodilatation**

The bronchodilator response is shown in Figure 1. Significant bronchodilatation (increase in mean \( \text{FEV}_1 \) and Raw) was obtained by all three doses of ipratropium bromide (\( p<0.01 \)) but not by placebo. There was no statistically significant difference between the bronchodilator effect of the three doses of ipratropium bromide.

No significant correlations were found with regard to the bronchodilator effect when the same regimens (ie, placebo or either of the three doses of ipratropium) of the first and the second part of the trial (ie, with an interval of three to nine months) were compared. Mean values of \( \text{FEV}_1 \) were significantly higher (\( p<0.001 \)) after inhalation of ipratropium bromide than after terbutaline (given after the histamine provocations). No significant difference was found when mean \( \text{FEV}_1 \) after inhalation of placebo was compared with values after terbutaline (\( p=0.67 \)). Calculating data on Raw values did not add further information.

**FIGURE 2.** \( \text{PC}_{20}\text{FEV}_1 \) (mean values ± SEM) for histamine in a bronchial provocation test started 45 minutes after inhalation of placebo and ipratropium bromide.
responder group (ie, subjects who responded with good protection against histamine-induced bronchoconstriction) with no difference between the different doses and a nonresponder group (see Table 1).

Relation Between Bronchodilatation and Protection

No correlation between the protective effect and the bronchodilator effect was found (r = 0.08, Fig 4).

**DISCUSSION**

The PC values on the placebo days were similar on the two parts of the trial indicating that the “basal” bronchial reactivity was not significantly changed during the time of the study. For each dose of ipratropium bromide there was a good correlation between the bronchial reactivity on the first and second trial. Furthermore, the coefficient of regression for the PC values of the first and second trial were approaching 1.0 implicating that in each subject the protection of ipratropium bromide against histamine-induced bronchoconstriction was similar in the two parts of the trials. With respect to the protective effect of ipratropium bromide against histamine-induced bronchoconstriction, the patients could be divided into two groups — responders and nonresponders. This responsivenss was similar during the three to nine months during which the study was performed. Hence, the interindividual variation in the airway response to an anticholinergic agent, ie, being a responder or a nonresponder, does not seem to be explained by changes in muscarinic receptor sensitivity from time to time in the same subject but does

![Figure 3](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21605/)

**Figure 3.** Correlation between the $10\log PC_{20} FEV_1$ values of the first (x-axis) and the second (y-axis) trial for placebo and each of the ipratropium bromide doses.

![Figure 4](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21605/)

**Figure 4.** The protection of ipratropium bromide against histamine-induced bronchoconstriction as a function of the bronchodilatation obtained 45 minutes after inhalation of ipratropium bromide (IB). The protection is expressed as $PC_{20}$ values for inhaled histamine after inhalation of ipratropium bromide divided by $PC_{20}$ values after inhalation of placebo and the bronchodilator effect is expressed as the percentage of increase in FEV$_1$ after inhalation of ipratropium bromide. Each point is based on the mean value of the first and second parts of the trial.
rather seem to be a constant quality within a subject. Thus, our results indicate that asthmatic subjects do not turn from being a responder to being a nonresponder implicating a possibility to predict the bronchodilator and bronchoprotective effect, a finding that should be of clinical importance.

The similarity in basal lung function on the eight trial days (four days in the first trial session and four days in the second) makes comparisons between the days possible. All the subjects were in a free interval of their disease at the time of the trials. This makes the interpretation of the bronchodilator response difficult since the lung function (measured as FEV₁ and Raw) was close to 100 percent of predicted values in almost all subjects providing a very small space for bronchodilatation. This finding may explain the lack of difference between the three doses of ipratropium bromide and the lack of correlation between the bronchodilator response of the same ipratropium dose of the first and second part of the trial. The bronchodilator response to ipratropium bromide was good and probably near maximum since no further bronchodilatation was obtained after inhalation of terbutaline.

In earlier studies higher doses of atropine were needed to protect against histamine-induced bronchoconstriction than to induce maximal bronchodilatation. In the study by Sheppard et al³ all patients responded with a protective effect against histamine-induced bronchoconstriction in a dose-response manner. In the present study we found no relationship between bronchodilatation and protection and no dose-response relation of the protective effect. Furthermore, we found asthmatic patients who do not benefit from muscarinic blockade with regard to the protection against inhaled histamine. The most probable explanation to the discrepancies of our findings in comparison to those of Sheppard et al³ is that all patients in that study were responders.

In the study by Sheppard et al³ there was a dose-response relation with regard to the protective effect but not to the bronchodilator effect of atropine. From these data it was concluded that the protection of atropine against histamine-induced bronchoconstriction is not merely depending on the bronchodilatation. Thus, it seems that the bronchoconstrictor effect of histamine, at least partly, is mediated through cholinergic mechanisms. The lack of correlation between bronchodilatation and protection in the present study supports this hypothesis. In three subjects ipratropium bromide completely failed to inhibit histamine-induced bronchoconstriction in both parts of the trial in five or all six bronchial challenges (in patient 8 there was a slight protection of 800µg at the first occasion). This existence of responders and nonresponders may explain the earlier findings where some authors found no protection of antimuscarinic drugs against histamine-induced bronchoconstriction³,⁵,¹⁷,¹⁹ while others found variable degrees of protection.⁸,⁹,¹⁸

The explanation for this nonresponsiveness is not clear. One explanation could be that the cholinergic contribution in histamine-induced bronchoconstriction may differ between asthmatic subjects. To our knowledge, there are no data available that can elucidate this question. It is also possible that the ability of the antimuscarinic drug to reach the receptor varies between individuals. Hence, even if histamine acts through cholinergic mechanisms it is not certain that the inhaled muscarinic antagonist reaches the vagally stimulated cholinergic receptor. There are data from animal experiments supporting such a hypothesis. Thus, Holtzman et al²³ observed that intravenously administered atropine possessed a better inhibitory effect than inhaled atropine on bronchoconstriction induced by vagal stimulation. Another possibility could be an uneven distribution of muscarinic receptors, i.e., an interindividual variation of the density of muscarinic receptors on cells other than those of the airway smooth muscle. However, Howarth et al¹⁴ showed that ipratropium bromide does not inhibit the increase in plasma levels of histamine and neutrophil chemotactic factor that normally is observed in association with bronchial allergen challenges. This indicates that ipratropium bromide has no effect on mast cell degranulation and to our knowledge there is no convincing evidence for the occurrence of muscarinic receptors on mast cells. This is also in accordance with the finding that anticholinergic agents offered none or a very poor protection against allergen-induced bronchoconstriction¹¹,¹⁸,²⁰ in most studies, indicating that the protection of ipratropium bromide against histamine-induced bronchospasm may not be clinically relevant for patients with allergic asthma.

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