Bronchodilator Effect of Ipraterol® on Methacholine-Induced Bronchoconstriction in Asthmatic Patients

Kittipong Maneecchotesuwan MD, PhD*, Tasneeya Suthamsmai MSc*, Kanokwan Ratanaanglert BSc*, Sutat Pipopsuthipaiboos BSc*

*Division of Respiratory Disease and Tuberculosis, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Background: The addition of ipratropium, a synthetic cholinergic antagonist, to β2-agonist therapy provides an additive improvement in adult with acute severe asthma and COPD because of increased vagal tone in the airways. We asked whether ipratropium in combination with fenoterol (Ipraterol®) improved pulmonary function in comparison with original Berodual®

Material and Method: In order to determine the effects of nebulized a single dose of Ipraterol®, the study was conducted in a double-blind, randomized and crossover manner by comparing the effect of nebulized a single dose of Berodual® on methacholine-induced bronchoconstriction. The study consisted of an 1-week run-in phase and two study visits separated by a washout period of 7 days.

Patients: We studied 20 patients who ranged from 18 to 80 years of age and had mild to moderate persistent asthma.

Results: Nebulized Ipraterol® provided a rapid onset of bronchodilation effect similar to nebulized Berodual® within 5 minutes by significantly increasing FEV1 from 1.19 L to 1.73 L (p < 0.001) and from 1.19 to 1.69 L (p = 0.0001), respectively. This effect of Ipraterol® lasted as long (up to 6 hours) and was similar to that of Berodual®. The absolute FEV1 values at 360 min after Ipraterol® treatment was still higher than the baseline values. We also found that there were no significant differences in the degree of improvement in FEV1 and hypokalemia following treatment with Ipraterol® and Berodual®.

Conclusion: Our data suggest that nebulized Ipraterol offers a statistically significant improvement in pulmonary function without significant systemic absorption causing hypokalemia, with the improvement being comparable to that achieved with nebulized Berodual.

Keywords: Asthma, Methacholine, Nebulized bronchodilator, Anticholinergic agent

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Inhaled ipratropium bromide, a synthetic cholinergic antagonist, is the most comprehensively studied of these agents. It has a local anticholinergic effect without significant systemic absorption. Studies of adding ipratropium to β₂-agonists in the treatment of acute asthma have shown greater statistically significant benefits than monotherapy with β₂-agonists alone. Although additional benefit for the combination approach has been shown in adult populations, the published studies have used various combinations of β₂-agonists and anticholinergics and have not always controlled concomitant interventions.

We therefore undertook the present study to compare the bronchodilator efficacy of a fixed combination of nebulized fenoterol (0.5mg) plus ipratropium bromide (0.25 mg) between Ipraterol® and Berodual®. We conducted a double-blind randomized cross-over study to determine time course effects of these bronchodilators on methacholine-induced bronchoconstriction by assessing improvement in FEV₁.

Material and Method

Subjects

Eligible patients were stable and had experienced mild to moderate persistent asthma. None had received a course of therapy with oral corticosteroids within 3 months prior to the study entry. Asthma was diagnosed by the American Thoracic Society criteria. Subjects had a baseline FEV₁ of ≥ 50% predicted and demonstrated a reversibility of FEV₁ after therapy with salbutamol (400 μg) of ≥ 12% or a provocative concentration of a substance (methacholine) causing a 20% fall in FEV₁ (PC₂₀) of < 4 mg/mL. Exclusion criteria were asthma exacerbation, a respiratory tract infection within 4 weeks before study inclusion, uncontrolled hypertension, hypokalemia, coronary artery disease, and cerebrovascular disease within 3 months before study entry, being pregnant or arrhythmia. Written informed consent was obtained from each patient, and the study was approved by the Ethics Committee of Siriraj Hospital.

Study design

This was a double-blind, randomized, and crossover study using single dose of Ipraterol® and comparative Berodual® on the day of treatment, with a 1-week washout phase between rounds of therapy. Patients entered an initial 1 week run-in period in which anti-asthmatic medications were stopped and short-acting β₂-agonist was used as needed rescue medication until the end of run-in period and throughout washout period. On the study day, patients undertook methacholine challenge test and immediately after the test was ended, the study bronchodilator was administered once via nebulizer. Pulmonary function was then evaluated to determine bronchodilator effect at different time points: 0, 5, 15, 30, 60, 120, 240 and 360 min after nebulization. In addition, serum potassium was determined at 4 hours after the inhalation. The randomized code was withheld from the investigators until completion of the study. The study medication was packed by the central pharmacy according to the randomization code.

Lung function measurement

FEV₁ and FVC were measured using a dry wedge spirometer (Vitalograph, Buckingham, UK). Values are expressed as the percent of predicted normal values. Baseline values were measured after 15 min of rest and were taken as the highest of three readings. Single readings only were taken at other times. Bronchial provocation test results were measured at the study visits. The level of bronchial reactivity was assessed by methacholine challenge, which was performed according to a standardized technique.

To further assess the change in lung function with taking into account the baseline lung function in relation to the patient’s optimal lung function (i.e., the potential increase), we used the relative potential improvement (RPI) with some modification as previously described(14). The change in FEV₁ (FEV₁ at 60 min minus the baseline FEV₁) divided by the potential improvement in FEV₁ (predicted value based on age, sex, height, and race, minus baseline FEV₁):

\[ \text{RPI} = \frac{\text{FEV₁ at } t_{60} - \text{FEV₁ at } t_{0}}{\text{FEV₁ (predicted)} - \text{FEV₁ at } t_{0}} \]

We then computed the proportion of patients achieving their potential improvement (RPI greater than 20%), and computed the differences in proportion between treatment groups.

Statistical analysis

The results are expressed as mean (SD). Changes in FEV₁ after treatment within group were compared using Wilcoxon signed-rank test. Response to Ipraterol (FEV₁) versus Berodual was assessed by unpaired t-test. Statistical significance was assumed for \( p < 0.05 \). All statistical testing was performed by
using a two-sided 5% level of significance (GraphPad Prism software; GraphPad Software Inc; San Diego, CA).

Sample size estimation is computed as a non-inferiority study. The FEV₁ after methacholine is 2.7. The FEV₁ at 60 minutes after receiving nebulized Ipraterol® is 3.3 (a difference of 0.6 from baseline FEV₁ after methacholine) and the FEV₁ at 60 minutes after receiving nebulized Berodual® is claimed to be non-inferior to nebulized Ipraterol® when a difference of ≥0.5 from baseline FEV₁ after methacholine is observed. We accept type I error of 5%, type II error of 20% and a common standard deviation of 0.1. Therefore the number of subject is 14 according to nQuery Advisor 3.0. A total of 24 patients were recruited to ensure that 14 patients completed the study.

**Results**

Twenty-four patients with asthma were recruited in the present study. 4 patients were excluded because their lung functions were unacceptable. 4 of 20 patients had been treated with β₂ agonists only before study entry. The remaining patients were treated with ICS in the absence or presence of LABA. Demographic data was shown in Table 1. The mean (SD) of baseline FEV₁ after methacholine challenge in both groups was not significantly different with a value of 1.19 L (0.28) in the Ipraterol® therapy group vs. 1.19 (0.28) in the Berodual® group. There was no significant difference in FEV₁ at the initiation of treatment between the groups including severity of bronchial hyperreactivity (Table 2). There was significant improvement with the mean FEV₁ in the Ipraterol® group being 1.72, 1.77, 1.83,

<table>
<thead>
<tr>
<th>Variable</th>
<th>n = 20</th>
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<tbody>
<tr>
<td>Male sex, n (%)</td>
<td>5 (20)</td>
</tr>
<tr>
<td>Mean age (years) (SD)</td>
<td>49.3 (12.48)</td>
</tr>
<tr>
<td>Median equivalent</td>
<td>360.0 (200-725)</td>
</tr>
<tr>
<td>beclomethasone daily dose (μg) (IQR)</td>
<td></td>
</tr>
<tr>
<td>Mean FEV₁ (% predicted) (SD)</td>
<td>75.05 (13.36)</td>
</tr>
<tr>
<td>Mean FVC (% predicted) (SD)</td>
<td>93.10 (11.79)</td>
</tr>
</tbody>
</table>

Abbreviations: FEV₁, force expiratory volume in second; FVC, force vital capacity; PC₂₀, provocative concentration of a methacholine causing a 20% fall in FEV₁; IQR, interquartile range.
1.89, 1.88, 1.83 and 1.77 L at 5, 15, 30, 60, 120, 240, 360 min, respectively (p-values as shown in Table 2) (Fig. 1) when compared with baseline FEV₁ after methacholine challenge at time -10 min. Similarly, there was significant change in mean FEV₁ from baseline in the Berodual® group being 1.69, 1.75, 1.82, 1.86, 1.88, 1.81 and 1.78 L at 5, 15, 30, 60, 120, 240, 360 min, respectively (p-values as shown in Table 2) (Fig. 2). However, delta changes in FEV₁ at each time point were not statistically significant when compared between groups (Table 3).

Comparing differences in proportion defined by RPI showed a benefit of Ipraterol® and Berodual® was 85% and 85%, respectively (p = 1.0) using McNemar test.

**Discussion**

In this study, we evaluated the short-term efficacy and safety of nebulized Ipraterol® compared with Berodual® for the treatment of methacholine-induced bronchoconstriction mimicking acute exacerbation. Our study demonstrates the efficacy of inhaled Ipraterol® in the treatment of asthmatic patients with methacholine-induced bronchoconstriction. The efficacy of nebulized Ipraterol® and Berodual® for the improvement of airflow rates in a 6 hr period after bronchoprovocation with methacholine was comparable (Fig. 3). The magnitude of improvement in post-bronchodilator FEV₁ after Ipraterol® treatment was comparable to that found in Berodual treatment. Similar to Berodual®, nebulized Ipraterol® had no effect on potassium levels.

Although pathogenetic mechanisms of asthma exacerbation are associated with exaggerated airway inflammation and airway wall edema, luminal obstruction is a consequence of mucus hypersecretion,

![Fig. 1](image1.png)  
**Fig. 1** The time-course effects of Ipraterol on FEV₁. Results 20 patients are expressed as the mean ± SD, * p < 0.05.

![Fig. 2](image2.png)  
**Fig. 2** The time-course effects of Berodual on FEV₁. Results 20 patients are expressed as the mean ± SD, * p < 0.05.

**Table 3.** The magnitude of changes in FEV₁ at each time point after Ipraterol® and Berodual®

<table>
<thead>
<tr>
<th>Drugs</th>
<th>0</th>
<th>5</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>240</th>
<th>360</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipraterol®</td>
<td>0.46 (0.25)</td>
<td>0.53 (0.21)</td>
<td>0.58 (0.21)</td>
<td>0.63 (0.20)</td>
<td>0.70 (0.22)</td>
<td>0.69 (0.18)</td>
<td>0.64 (0.21)</td>
<td>0.58 (0.17)</td>
</tr>
<tr>
<td>Berodual®</td>
<td>0.42 (0.22)</td>
<td>0.50 (0.22)</td>
<td>0.56 (0.23)</td>
<td>0.63 (0.22)</td>
<td>0.67 (0.22)</td>
<td>0.69 (0.24)</td>
<td>0.62 (0.21)</td>
<td>0.59 (0.22)</td>
</tr>
<tr>
<td>p-value*</td>
<td>0.51 (0.22)</td>
<td>0.61 (0.23)</td>
<td>0.83 (0.22)</td>
<td>0.91 (0.22)</td>
<td>0.65 (0.24)</td>
<td>0.95 (0.21)</td>
<td>0.83 (0.22)</td>
<td>0.83 (0.22)</td>
</tr>
</tbody>
</table>

Abbreviations: FEV₁, force expiratory volume in second; PC₂₀, provocative concentration of a methacholine causing a 20% fall in FEV₁.  
* The comparison of FEV₁ at each time point after indicated treatments between the groups using unpaired t-test.
and premature airway closure\(^{(12,9)}\). ICS had been withdrawn for 1 week, possibly leading to increased airway inflammation. Our patients were challenged with methacholine to induce bronchoconstriction as shown by evidence that there was a significant decline in FEV\(_1\). This might mimic the pathophysiology of asthma exacerbation in clinical practice. The combination of ipratropium with short-acting \(\beta_2\) agonist could rapidly reverse methacholine-induced bronchoconstriction and the time-course of Berodual\(^{\circ}\) and Ipraterol\(^{\circ}\) was comparable, suggesting that if Ipraterol\(^{\circ}\) was used in asthmatic patients with an exacerbation, it should provide bronchodilating effect to a similar extent as with Berodual\(^{\circ}\). We excluded the possibility that differences in bronchial hyperreactivity between Ipraterol\(^{\circ}\) and Berodual\(^{\circ}\) groups were involved in response to these two combination bronchodilator because there was no significant difference in PC\(_{20}\) in both groups. We also found no difference in serum potassium levels at 4 hours after treatment with either Berodual\(^{\circ}\) or Ipraterol\(^{\circ}\).

In summary, Ipraterol\(^{\circ}\) is as effective to treat methacholine-induced bronchoconstriction as Berodual\(^{\circ}\), without significant changes in potassium levels.

Acknowledgements
We would like to thank Professor Visanu Thamlikitkul for his excellent advice. We thank all patients in this study for their participation.

Potential conflicts of interest
Pharma Innova Co., Ltd Thailand.

References
ฤทธิ์ขยายหลอดลมของ Ipraterol® ต่อหลอดลมตีบที่เกิดจากการกระตุ้นด้วยสาร methacholine ในผู้ป่วยโรคหืด

ภูมิหลัง: ยาขยายหลอดลมชนิดผสมระหว่าง fenoterol และ ipratropium เช่น Berodual® เป็นยาที่จำเป็นในการรักษาผู้ป่วยโรคหืดทั้งในระยะที่โรคกำเริบเฉียบพลันและรุนแรง อย่างไรก็ตามยังไม่มีการศึกษารูทิลของ Ipraterol® ซึ่งเป็นยาขยายหลอดลมชนิดผสมแบบเดียวกับ Berodual® ดังกล่าวในประเทศไทย

วัสดุและวิธีการ: เพื่อศึกษาผลของการรักษาด้วย Ipraterol® ชนิดพ่นผ่านหน้ากาก 1 ครั้ง การศึกษาเป็นลักษณะ double-blind, randomized, cross-over โดยการเปรียบเทียบผลของยาที่เปรียบเทียบกับยา Berodual® ชนิดพ่นผ่านหน้ากาก 1 ครั้ง ตลอดระยะเวลาที่เกิดจากการกระตุ้นด้วย methacholine การศึกษาประกอบด้วย run-in phase เป็นเวลา 1 สัปดาห์ และการมาพ่นยาศึกษา 2 ครั้ง ซึ่งถูกคั่นด้วย washout period เป็นเวลา 7 วัน กลุ่มผู้ป่วยที่นำมาศึกษา: ผู้ป่วยโรคหืดที่นิพนธ์ศึกษาคัดเลือกผู้มีอาการเฉียบพลัน จำนวน 20 ราย ที่มีอายุอยู่ระหว่าง 18-80 ปี

ผลการศึกษา: ยา Ipraterol® ออกฤทธิ์เร็วภายในเวลา 5 นาทีในการขยายหลอดลมตีบที่เกิดจากการกระตุ้นด้วย methacholine เมื่อเทียบกับ Berodual® โดยทำให้ค่า FEV1 เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติจาก 1.19 L เป็น 1.73 L (p < 0.001) และจาก 1.19 เป็น 1.69 L (p = 0.0001) ตามลำดับ โดยพบว่าค่า Ipraterol® ยังคงเพิ่มขึ้นไปได้ 6 ชั่วโมง ซึ่งมากกว่าค่า FEV1 ในกลุ่มผู้ป่วยที่รับยา Berodual® ที่มีค่า FEV1 เพิ่มขึ้น 360 นาทีหลังการรักษาด้วย Ipraterol® หลังจากกระตุ้นด้วย methacholine การศึกษาแสดงว่ายา Ipraterol® มีผลกระตุ้นดีขึ้นจากการกระตุ้นด้วย methacholine

สรุป: ข้อมูลของการศึกษาแนะนำว่า nebulized Ipraterol® ช่วยให้สมรรถภาพปอดดีขึ้นอย่างมีนัยสำคัญทางสถิติโดยปราศจากการคัดกรองข้อมูลการสูญเสียสมรรถภาพในการทำให้สมรรถภาพปอดดีขึ้นเทียบกับ nebulized Berodual®