Tolerance to the Protective Effect of Salmeterol on Allergen Challenge Can Be Partially Restored by the Withdrawal of Salmeterol Regular Treatment*

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Study objective: To assess whether the withdrawal of salmeterol treatment for 3 days (72 h) can restore its bronchoprotective ability on specific bronchial provocative test (sBPT) with allergen, which was completely lost after 1 week of regular treatment with salmeterol.

Study design: Single-blind design.

Patients and methods: We investigated 10 nonsmoking subjects (8 men and 2 women; mean ± SD age, 24 ± 8 years) with mild intermittent allergic asthma in the stable phase of the disease, who were never previously treated with regular β₂-agonists. Subjects with a previous positive early airway response (EAR) to a screening allergen challenge were considered. They underwent sBPT with allergen after a single dose of inhaled salmeterol, 50 μg (T₁), and then underwent sBPT after 1 week of regular treatment with inhaled salmeterol, 50 μg bid (T₂); after that, they continued inhaled salmeterol treatment for 4 days, and then changed to inhaled salmeterol with placebo (two puffs bid) for 3 days (72 h) and underwent sBPT with allergen after a single dose of salmeterol, 50 μg (T₃).

Results: EAR to allergen (ΔFEV₁ ≥ 20% with respect to postdiluent value) was completely abolished by a single dose of salmeterol (T₁; protection index [PI] > 50% in all subjects), but it was still present after 1 week of regular treatment with salmeterol (T₂; PI < 50% in all subjects). The maximum FEV₁ percentage fall during sBPT with allergen was significantly lower after withdrawal of regular inhaled salmeterol (T₃) than after regular treatment with salmeterol (T₂) (mean, 23% vs 29.5%; range, 4 to 41% vs 18 to 49%, respectively; p < 0.05); a similar result was obtained considering the PI of salmeterol on sBPT with allergen (mean, 44% vs 20%; range, 2 to 86% vs −11 to 49%, respectively; p < 0.05). However, the maximum FEV₁ percentage fall and PI were significantly different in T₃ than after T₁, and only 4 of 10 patients showed in T₃ a PI ≥ 50%.

Conclusions: The bronchoprotective effect of salmeterol on allergen-induced EAR, completely lost after 1 week of regular treatment with salmeterol, may be partially restored by the withdrawal of salmeterol therapy for 3 days (72 h). However, this withdrawal time period is not sufficient to recover the baseline bronchoprotective efficacy of the first dose of salmeterol.

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Key words: allergen challenge; asthma; β₂-agonist; tolerance

Abbreviations: BU = biological units; EAR = early airway response; PI = protection index; pMDI = pressurized metered-dose inhaler; sBPT = specific bronchial provocative test; T₀ = screening allergen challenge; T₁ = sBPT after a single dose of salmeterol, 50 μg; T₂ = sBPT after 1 week of regular treatment with salmeterol, 50 μg bid; T₃ = sBPT after 4 days of regular treatment with salmeterol, 50 μg bid, and placebo for 3 days and single dose of salmeterol, 50 μg

Regular treatment with inhaled salmeterol can induce tolerance to its bronchoprotective effect on methacholine or specific bronchial provocative test (sBPT) with allergen, and this phenomenon occurs very early. One week of regular treatment is sufficient to give prominence to loss of the protective ability of salmeterol on allergen challenge.¹ Bhagat et al² have shown that salmeterol can induce tolerance to bronchoprotective ability against methacholine at 24 h, and this is evident even in subjects receiving regular treatment with inhaled corticosteroids.³

In vitro, some studies⁴,⁵ have demonstrated that short exposure (3 h) of peripheral lymphocytes and neutrophils to isoprotenerol induces a significant
hyporesponsive state of these cells to β-agonists, and this state was attenuated by the concomitant incubation with corticosteroids.5,6 Two weeks of treatment with oral terbutaline determined a remarkable and selective depression in β2-adrenoreceptor sensitivity of human alveolar macrophages, and this phenomenon was not influenced by concomitant use of inhaled or systemic corticosteroids.7 However, it is proven that desensitization mechanisms, such as uncoupling of the receptors from adenylate cyclase and internalization of uncoupled receptors, may be reversed within minutes or hours, respectively, from removal of the agonist.8 After days of agonist exposure, a net loss of cellular receptors occurs, and several days of removal of agonists are needed to reverse this process.9

In vivo, the effect of corticosteroids on tolerance to the protective effect of long-acting β2-agonists on various bronchial stimuli is not clear. Some studies10,11 failed to find any positive effect of inhaled corticosteroids on tolerance induced by regular use of β2-agonists, but others12,13 demonstrated the efficacy of inhaled beclomethasone or budesonide in reverting airway subresponsiveness or tolerance after regular treatment with inhaled formoterol or salmeterol.

To our knowledge, no studies have investigated whether the cessation of therapy with inhaled long-acting β2-agonists can partially or totally restore their bronchoprotective ability on methacholine or on sBPT with allergen. Therefore, no data have been reported on the withdrawal time required to obtain the baseline bronchoprotective ability. To address this issue, in this study, subjects with proven tolerance to the protective effect of salmeterol on sBPT with allergen induced by 1 week of regular treatment with salmeterol, withdrew regular inhaled salmeterol for 4 days and then changed to inhaled salmeterol with placebo (two puffs bid) for 3 days (72 h) and underwent sBPT with allergen after a single dose of salmeterol, 50 μg (T3). The sequences of treatment were single blind. To obtain a single-blind protocol, during the weeks between T1 and T2 and between T2 and T3, all subjects assumed inhaler salmeterol or placebo by two different pressurized metered-dose inhaler (pMDI) canisters, one for the first 4 days, and one for the subsequent 3 days. The technical personnel in charge of the pulmonary function tests were not aware of the patient’s treatment periods. Each sBPT with allergen was performed at the same time of the day (12 noon), 1 h after the last drug inhalation, by administering the same provocative dose of allergen as in T0 (Fig 1).

sBPT With Allergen

sBPT was performed with allergens standardized in biological units (BU) according to a method described previously.15 Allergen extract solution was delivered by a jet nebulizer (model 646; DeVilbiss; Somerset, PA) connected to a dosimeter driven by compressed air. Lyophilized allergen extract (NeoAbello` ; Milano, Italy) was dissolved in saline solution in order to obtain two solutions with different concentrations, 1 BU/mL and 10 BU/mL. The nebulizer was filled with 3 mL of diluent (phenol 0.4%.

### Materials and Methods

#### Subjects

We investigated 10 nonsmoking subjects (8 men and 2 women; mean ± SD age, 24 ± 8 years) with mild intermittent allergic asthma in the stable phase of the disease. Asthma diagnosis was done according to international guidelines.14 All subjects were occasionally receiving (less than once a week) inhaled rescue short-acting β2-agonists, with no regular therapy. All subjects had a positive early asthmatic response (EAR) to sBPT with allergen (Dermatophagoides pteronyssinus) (screening allergen challenge [T0]). The main clinical findings in 10 asthmatic subjects included in the study are reported in Table 1.

#### Study Protocol

All subjects underwent sBPT with allergen after a single dose of inhaled salmeterol, 50 μg (T1). Subsequently, they underwent sBPT with allergen after 1 week regular treatment with inhaled salmeterol, 50 μg bid (T2); after that, they continued treatment with inhaled salmeterol for 4 days and then changed to inhaled salmeterol with placebo (two puffs bid) for 3 days (72 h) and underwent sBPT with allergen after a single dose of salmeterol, 50 μg (T3). The main clinical findings in 10 asthmatic subjects included in the study are reported in Table 1.

### Table 1—Characteristics of the Examined Subjects*

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age, yr</th>
<th>Sex</th>
<th>Smoker</th>
<th>Duration of Asthma, yr</th>
<th>FEV1 % Predicted</th>
<th>Methacholine PD20, mg</th>
<th>Provocative Allergen Dose, BU</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>Male</td>
<td>No</td>
<td>10</td>
<td>95</td>
<td>0.120</td>
<td>0.20</td>
</tr>
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<td>2</td>
<td>26</td>
<td>Male</td>
<td>No</td>
<td>15</td>
<td>93</td>
<td>0.180</td>
<td>0.80</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>Male</td>
<td>No</td>
<td>13</td>
<td>99</td>
<td>0.134</td>
<td>0.05</td>
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<td>4</td>
<td>27</td>
<td>Male</td>
<td>No</td>
<td>20</td>
<td>85</td>
<td>1.192</td>
<td>0.20</td>
</tr>
<tr>
<td>5</td>
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<td>18</td>
<td>88</td>
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<td>0.025</td>
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<tr>
<td>6</td>
<td>28</td>
<td>Male</td>
<td>No</td>
<td>22</td>
<td>79</td>
<td>0.306</td>
<td>0.80</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>Female</td>
<td>No</td>
<td>12</td>
<td>97</td>
<td>0.082</td>
<td>0.025</td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>Male</td>
<td>No</td>
<td>10</td>
<td>100</td>
<td>0.150</td>
<td>0.80</td>
</tr>
<tr>
<td>9</td>
<td>27</td>
<td>Male</td>
<td>No</td>
<td>5</td>
<td>91</td>
<td>0.120</td>
<td>0.40</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>Female</td>
<td>No</td>
<td>4</td>
<td>106</td>
<td>0.948</td>
<td>0.40</td>
</tr>
</tbody>
</table>

*PD20 = provocative dose causing a 20% fall in FEV1.
in saline solution) or allergen solution. After baseline spirometry, the patient inhaled three puffs of diluent, followed, at 10-min intervals, by a different number of allergen solution inhalations. The cumulative doses of allergen were, at each step, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 BU. FEV$_1$ was measured 10 min after the end of each series of allergen inhalations by means of a water-sealed bell spirometer connected to a computer (Olivetti 240; Biomedin; Padova, Italy). The inhalations were continued until FEV$_1$ decreased by 20% from the postdiluent value, and the total dose of delivered allergen was recorded. FEV$_1$ was then measured after 20 min, 30 min, and 60 min. A positive response was defined as a > 20% fall in FEV$_1$ from the postdiluent value. The degree of EAR was expressed as the maximum FEV$_1$ percentage fall.

**Statistical Analysis**

FEV$_1$ is expressed as mean ± SD. Maximum FEV$_1$ percentage fall during sBPT with allergen and FEV$_1$ percent of predicted value are expressed as median (range). Paired t test and analysis of variance for repeated measures were used to compare groups of observations for normally distributed data, while the Wilcoxon test and Friedman test were used for nonnormally distributed data. A p value < 0.05 was considered as significant.

Protection index (PI) was computed as the percentage ratio between maximum FEV$_1$ percentage fall during sBPT with allergen after pharmacologic treatment (T$_1$, T$_2$, or T$_3$), and maximum FEV$_1$ percentage fall during T$_0$. A PI ≥ 50% was considered to represent a significant protection.

**RESULTS**

The baseline value of FEV$_1$ before sBPT with allergen was not significantly different among four sBPTs with allergen both as absolute value and as percent of predicted value (Table 2). EAR (ΔFEV$_1$ ≥ 20% with respect to postdiluent value) was completely abolished by a single dose of salmeterol, but it was still present after 1 week of regular treatment with salmeterol in all subjects. In T$_1$, the maximum FEV$_1$ percentage fall during sBPT with allergen was significantly lower with respect to all other sBPTs with allergen (ΔFEV$_1$ percentage median [range]: 3 [0 to 13] vs 39 [29 to 45] vs 29.5 [18 to 49] vs 23 [4 to 41] for T$_1$, T$_0$, T$_2$, T$_3$, respectively; p < 0.05).

The maximum FEV$_1$ percentage fall during sBPT with allergen was significantly lower after withdrawal of regular inhaled salmeterol (T$_3$) than after regular treatment with salmeterol (T$_2$) (ΔFEV$_1$ percentage median [range]: 23 [4 to 41] vs 29.5 [18 to 49] for T$_3$ and T$_2$, respectively; p < 0.05). PI of salmeterol on sBPT with allergen after salmeterol withdrawal for 3 days (T$_3$) was significantly higher than in T$_2$ (Fig 2). However, PI was significantly lower in T$_3$ than after a single dose of salmeterol (T$_1$) (Fig 2), and only 4 of 10 patients showed in T$_3$ a PI ≥ 50% (Table 2).

**DISCUSSION**

The salmeterol bronchoprotective effect on allergen-induced EAR, which was completely lost after 1 week of regular treatment with salmeterol, may be partially restored by the withdrawal of salmeterol therapy for 3 days (72 h). However, this withdrawal

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**Table 2—Maximum FEV$_1$ (Percentage Fall and PI During T$_0$, T$_1$, T$_2$, and T$_3$)**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Screening (T$_0$)</th>
<th>Single Dose (T$_1$)</th>
<th>Regular Treatment (T$_2$)</th>
<th>Salmeterol Withdrawal (3 d) (T$_3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>FEV$_1$, L</td>
<td>3.83 ± 0.8</td>
<td>3.84 ± 0.8</td>
<td>3.91 ± 0.8</td>
<td>3.82 ± 0.8</td>
</tr>
<tr>
<td>FEV$_1$, % of predicted</td>
<td>90 (78–113)</td>
<td>90 (78–119)</td>
<td>94 (79–115)</td>
<td>89 (71–110)</td>
</tr>
<tr>
<td>FEV$_1$, (percentage fall at T$_0$ provocative dose)</td>
<td>39 (29–45)</td>
<td>3 (0–13)</td>
<td>29.5 (18–49)</td>
<td>23 (4–41)†‡</td>
</tr>
<tr>
<td>PI</td>
<td>94 (65–100)</td>
<td>20 (11–49)</td>
<td>44 (2–86)†‡</td>
<td></td>
</tr>
<tr>
<td>No. of subjects with PI ≥ 50%</td>
<td>–</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD or median (range) unless otherwise indicated.

†p < 0.05 between groups by Friedman test.

‡p < 0.05 between T$_2$ and T$_3$ by Wilcoxon test.
time period is not sufficient to recover the baseline bronchoprotective efficacy of the first dose of salmeterol.

In vitro desensitization occurs in response to the binding of receptor with the agonist molecule. The mechanisms by which the desensitization can occur can be different and require different times, within minutes to hours, of receptor stimulation.8 However, in humans, 2 days of terbutaline administration led to a decrease in lymphocyte β2-adrenoreceptor density of 40 to 50%, and a withdrawal of terbutaline therapy for 4 days is required to reach the pretreatment level.9 Prednisone treatment can accelerate the recovery of β2-adrenoreceptor density until 8 to 10 h.9 Other studies19,20 have proven that the time required for complete recovery of lymphocyte β2-adrenoreceptors after terbutaline administration may be as long as 1 week.

Probably, the mechanisms underlying tolerance to the protective effect of salmeterol on sBPT with allergen in vivo are more complicated. β2-Adrenoreceptors on various inflammatory cells might be involved in this phenomenon. This fact might require a longer time to restore normal activity of β2-receptors than in vitro. Probably a time-course protocol will be useful to evaluate the effective required time to restore the baseline bronchoprotective ability of β2-agonists. Unfortunately, to our knowledge, no other studies evaluated these peculiar aspects.

Clinically, however, it is well known that regular use of short-acting β2-agonists may cause a significant deterioration in airways hyperreactivity21 and may determine a deterioration in asthma control.22 More recently, Hancox et al23 demonstrated that continuous treatment with inhaled terbutaline may lead to a reduced response to emergency β2-agonists during asthma exacerbations. These findings support the evidence that short-acting β2-agonists should be used only when required. This negative effect may be present even with long-acting β2-agonists. Lipworth and Aziz24 demonstrated that regular use of salmeterol or formoterol may reduce the efficacy of albuterol in preventing bronchoconstriction caused by methacholine. Furthermore, neither salbutamol nor salmeterol can reduce the maximal airway narrowing during methacholine-induced bronchoconstriction.25 These data may indicate that patients receiving regular treatment with inhaled long-acting β2-agonists might be more vulnerable to bronchoconstrictor stimuli as a consequence of β2-adrenoreceptor subsensitivity.

Our data complete and extend these findings. Three days of salmeterol therapy withdrawal cannot completely abolish its tolerance to the bronchoprotective effect on allergen challenge. These suitable results, albeit derived from a small selected sample of asthmatic subjects, might help to optimize the ideal duration of treatment with salmeterol and its relationship with rescue treatment use. Our data may suggest that a prolonged interval in long-acting β2-agonist therapy might be required to obtain the baseline clinical drug efficacy and to permit a positive effect of rescue therapy during asthma attacks. Furthermore, our data may suggest that tolerance to the protective effect of salmeterol on sBPT with allergen could be never completely restored by withdrawal of regular treatment with salmeterol.

However, to better explain whether and when the protective abilities of salmeterol are back to baseline, study protocols with withdrawal of salmeterol therapy >3 days are required. This may be relevant to identify which measure may be taken to blunt the tolerance. Temporary withdrawal of salmeterol treatment associated with concurrent treatment with inhaled corticosteroids, known to reduce tolerance,12 could minimize the occurrence of tolerance.

Another possible important implication of our findings is the clear indication for the design of the future study protocol about tolerance to the bronchoprotective effect of β2-agonists. Many studies26–29 considered asthmatic subjects previously treated before the study protocols, and a various washout period (1 to 6 months) from inhaled β2-agonists should be used only when required. Inhaled β2-agonists and other inhaled therapy was considered sufficient to restore the baseline bronchoprotective ability of these drugs compared to regular use. However, in these studies, rescue therapy was allowed until the study protocol without any washout period. The time required for a suitable washout period is not well determined. Three days only, for instance, does not allow enough time to restore the original ability of the drug in protecting against
various stimuli. These findings suggest that, to determine the degree of tolerance observed avoiding any effects of previous treatment, it is mandatory to study subjects not regularly treated previously.

We studied asthmatic patients with mild intermittent asthma and without regular treatment with inhaled corticosteroids. Salmeterol is clearly not indicated for these subjects. However, we studied the same type of patients as were in our previous study, in order to evaluate the time course of the recovering of the bronchoprotective effect. We have already demonstrated that in patients with the same degree of asthma severity, inhaled corticosteroids can attenuate the development of salmeterol tolerance. We studied a small number of subjects, eight men and two women. However, this number of subjects is compatible with the study population from previous articles about tolerance, therefore, tolerance to the protective effect of salmeterol on allergen challenge seems not to be affected by the sex or the age of the patients studied.

In conclusion, we have shown that the withdrawal of inhaled salmeterol therapy for 3 days can only slightly improve its bronchoprotective ability on sBPT with allergen, which was completely lost after 1 week of regular treatment with salmeterol. Further studies are needed to evaluate whether the baseline bronchoprotective ability of the first dose of the drug can be restored by a longer time period of salmeterol therapy withdrawal.

REFERENCES