Inhaled Beclomethasone Dipropionate Reverts Tolerance to the Protective Effect of Salmeterol on Allergen Challenge*

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**Study objective:** One week of regular treatment with salmeterol can induce tolerance to the protective effect of a β₂-agonist on early airway response to allergen (EAR). The objective was to assess whether inhaled corticosteroids revert tolerance to salmeterol.

**Study design:** The study had a randomized, double-blind, placebo-controlled design.

**Patients and methods:** Twelve subjects with mild allergic asthma and positive result of specific bronchial provocation test (sBPT) to allergen underwent three sBPTs, separated by 1 week. sBPT was done in all subjects after a single dose (T₁) and after 1 week of regular treatment with inhaled salmeterol (50 µg bid) (T₂) in order to induce tolerance. Subjects were then randomized to receive either the same dose of salmeterol + beclomethasone dipropionate (BDP, 500 µg bid) (group 1, n = 6) or placebo + BDP (group 2, n = 6) for 1 week before sBPT (T₃).

**Results:** After a single dose of salmeterol (T₁), all subjects were protected against EAR, whereas after 1 week of regular treatment, the protective effect of salmeterol was totally or partially lost (T₂). Maximum FEV₁ percent fall (MaxΔFEV₁%) after allergen inhalation was significantly higher at T₂ than at T₁. All subjects except one of group 1 were protected against EAR after salmeterol + BDP (T₂), and MaxΔFEV₁% at T₃ (median, 12%; range, 4 to 6%) was significantly lower than T₂ (median, 22%; range, 12 to 43%; p < 0.05 by Wilcoxon test). Subjects of group 2 did not show any significant protection against EAR after placebo + BDP treatment (T₃) MaxΔFEV₁% at T₂ (median, 31%; range, 9 to 40%) and T₃ (median, 31%; range, 3 to 42%; not significant).

**Conclusions:** In conclusion, the addition of inhaled BDP partially restored the bronchoprotective effect of salmeterol on allergen challenge that was lost after 1 week of regular treatment with salmeterol alone. This ability of BDP in reverting tolerance cannot be ascribed to a direct effect of corticosteroids per se on allergen challenge in this group of asthmatics.

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**Key words:** allergen challenge; asthma; β₂-agonist; inhaled corticosteroids; salmeterol; tolerance

**Abbreviations:** BDP = beclomethasone dipropionate; BU = biological unit; EAR = early airway response; MaxΔFEV₁% = maximum decrease in FEV₁ after allergen inhalation; PEF = peak expiratory flow; PI = protection index; sBPT = specific bronchial provocation test; T₀ = screening sBPT; T₁ = sBPT protected by a single dose of salmeterol (50 µg); T₂ = sBPT after 1 week of regular treatment with salmeterol (50 µg bid); T₃ = sBPT after 1 week of regular treatment with salmeterol (50 µg bid) and BDP (500 µg bid) in group 1 or placebo and BDP (500 µg bid) in group 2

Several studies have demonstrated that short-term regular treatment with salmeterol leads to tolerance to the bronchoprotective effect of salmeterol on bronchoconstricting stimuli, such as methacholine or exercise.¹²

In a previous study, it was shown that the protective effect of a single dose of salmeterol on allergen challenge was lost after regular treatment with salmeterol for 1 week.³ The patients in this study had mild asthma and used bronchodilators as needed, but not inhaled corticosteroids.

Corticosteroids have been proven to prevent or reverse tachyphylaxis induced in vitro by β₂-agonists,⁴⁻⁶ but few studies have been carried out in vivo. A previous study reported that a single dose of systemic steroids can restore the bronchodilator activity of β₂-agonists in seven asthmatic patients.⁷

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Because inhaled corticosteroids alone do not prevent tolerance to the protective effect of salmeterol on methacholine challenge, it is not clear whether therapeutic doses of inhaled corticosteroids may revert the tolerance to salmeterol that occurs in previously untreated patients.

To address this issue, subjects with tolerance to the bronchoprotective effect of salmeterol on allergen challenge induced by 1 week of treatment with salmeterol were treated for a further week with salmeterol and beclomethasone dipropionate (BDP), after which they again underwent allergen challenge. As control, a group of subjects with tolerance to inhaled salmeterol underwent allergen challenge after 1-week treatment with BDP + placebo.

**Materials and Methods**

**Subjects**

We investigated a group of nonsmoking asthmatic subjects, in a stable phase of the disease. Asthma diagnosis was done according to international guidelines. All subjects had a positive skin prick test to one or more common airborne allergens, positive methacholine challenge test, and developed an early airway response (EAR) after specific bronchial provocation test (sBPT) with allergen (*Dermatophagoides pteronyssinus*). All subjects were occasionally (≤1 time a week) taking inhaled short-acting *β*2-agonists, with no regular therapy. At the time of present investigation, all subjects were classified as having mild intermittent asthma.

Of the 18 subjects screened for the study, we selected 12 subjects (10 were male and 2 were female), with a mean age of 20 ± 4 years, who had demonstrated the occurrence of tolerance to salmeterol on allergen challenge after 1 week of treatment with salmeterol. The main clinical findings of 12 asthmatic subjects who develop tolerance to the bronchoprotection ability of salmeterol, and of 6 asthmatics subjects who did not develop tolerance, are reported in Table 1.

**Study Protocol**

The study had a randomized, double-blind, placebo-controlled design, in order to assess whether the addition of inhaled BDP (500 μg bid) restores the protective effect of salmeterol on allergen challenge in patients who had previously developed tolerance to salmeterol.

During the first part of the protocol, all subjects with a previous positive response to allergen challenge (T0) again underwent allergen challenge after a single dose (50 μg) of inhaled salmeterol (T1), followed by a second allergen challenge after regular treatment with inhaled salmeterol (50 μg bid) for 1 week (T2) (Fig 1).

In the second part of the protocol, subjects were randomized in two groups: (1) group 1 (n = 6 subjects) receiving salmeterol (50 μg bid) and BDP (500 μg bid); and (2) group 2 (n = 6 subjects) receiving BDP (500 μg bid) and placebo (two puffs bid). After 1 week of randomized treatment, all subjects underwent a third bronchial challenge (T3) after BDP + salmeterol in group 1 or BDP + placebo in group 2.

Each allergen challenge was performed at the same time of the day (12 AM), 1 h after the last drug inhalation, by administering the same provocative dose of allergen as in the screening challenge. Between T1, T2, and T3, all subjects recorded diurnal and nocturnal symptom score (ranging from 0 to 4 for each day or night) and morning and evening peak expiratory flow (PEF) on a diary card.

**sBPT With Allergen**

sBPT was performed with allergens standardized in biological units (BU) according to a method described previously. Aller-
gen 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 to obtain two solutions with different concentrations, 1 and 10 BU/mL. The nebulizer was filled with 3 mL of diluent (phenol 0.4% in saline solution) or allergen solution. After baseline spirometry, the patient inhaled three puffs of diluent, followed at 10-min intervals by a different concentration of allergen solution inhalations. The cumulative doses of allergen were, at each step, as follows: 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 BU. FEV1 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 FEV1 decreased by 20% from the postdiluent value, and the total dose of delivered allergen was recorded. FEV1 was then measured after 20, 30, and 60 min. A positive response was defined as a >20% fall in FEV1 from the postdiluent value.

The degree of EAR was expressed as the maximum decrease in FEV1 after allergen inhalation (MaxΔFEV1%) and as the area under the curve, computed by trapezoid integration in the first hour after the last dose of inhaled allergen.

**PEF Flow Monitoring**

All subjects were asked to monitor PEF by means of a mini-Wright peak flowmeter (Clement Clarke; Harlow Essex, UK) two times daily for 7 consecutive days between two sBPTs. On each occasion, the subjects performed three blows and recorded the highest value on a diary card. Subjects were also instructed to measure PEF each time they had dyspnea or wheeze, to use only rescue β2-agonist, and to repeat measurements 10 min after β2-agonist inhalation. PEF values were entered into a computer (M280 Olivetti; Biomedin) programmed to obtain, for each record, several indexes of daily variability. For analysis we used the mean maximal amplitude (maximum daily value minus minimum daily value, in percentage of daily mean value) on all days of monitoring.

**Statistical Analysis**

FEV1 is expressed as mean ± SD. Maximum FEV1% fall after allergen challenge and the area under the curve 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 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 percent ratio between maximum FEV1 percent fall during bronchial challenge after pharmacologic treatment (T0, T2, or T3) and maximum FEV1 percent fall during screening sBPT (T0). A PI of ≥50% was considered as significant.

**RESULTS**

Baseline values of FEV1 were not significantly different among allergen challenges, both as absolute values and as percent of the predicted value (FEV1, L [FEV1%]: 3.9 ± 0.8 [99 ± 14%]; 4.0 ± 0.8 [101 ± 15%]; 4.1 ± 0.7 [103 ± 13%]; 4.1 ± 0.7 [104 ± 12%] in T0, T1, T2, and T3, respectively). A single dose of salmeterol inhibited allergen-induced EAR in all subjects, whereas after 1 week of salmeterol treatment, a significant reduction in the protective effect of salmeterol on EAR was observed, both when MaxΔFEV1% and the area under the curve during allergen challenge were considered (Table 2). The loss of the protective effect of 1 week of salmeterol treatment was of similar degree both in group 1 and group 2.

Subjects treated with inhaled BDP in addition to salmeterol for 1 week (group 1) showed a partial recovery of the protective effect of salmeterol on allergen challenge (Fig 2). Subjects treated with BDP alone for 1 week (group 2) showed no protective effect of BDP on early response to allergen inhalation (Fig 3).

Table 2 shows the maximum decrease in FEV1 and the area under the curve during sBPT at T1, T2, and T3. There was a significant difference among tests in each group (p < 0.05, Friedman test). In group 1, MaxΔFEV1% during sBPT at T3 was significantly lower than at T2, and it was no different from T1. Similar results were obtained by considering the area under the curve. In group 2, MaxΔFEV1% and the area under the curve at T3 were similar to the values measured at T2, and significantly different from T1.

When individually considered, in group 1, only

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**Figure 1. Protocol schedule.** T0 = screening sBPT, T1 = sBPT after a single dose of salmeterol (SALM, 50 µg), T2 = sBPT after 1 week of regular treatment with salmeterol (50 µg bid), T3 = sBPT after 1 week of regular treatment with salmeterol (50 µg bid) and BDP (500 µg bid) in group 1, or placebo (PLAC) and BDP (500 µg bid) in group 2.
two subjects were protected against EAR at T2 (PI ≥ 50%), while five subjects were protected against EAR in T3. However, in group 2, only one subject was protected at T2 (PI ≥ 50%) as well as at T3.

The stability of the disease during the study protocol was confirmed by the low symptom score and PEF variability. When all subjects were considered, no significant difference was observed between the 2 weeks of the study protocol (week between T1 and T2; week between T2 and T3) in diurnal symptom score (mean ± SD: 0.6 ± 0.8 vs 0.6 ± 1.2, respectively), nocturnal symptom score (mean ± SD: 0.1 ± 0.3 vs 0.1 ± 0.2), PEF variability (maximal amplitude daily mean: 4.7 ± 2 vs 5.3 ± 3), and use of rescue salbutamol (number of actuations in 1 week: 0.5 ± 0.7 vs 0.5 ± 0.9). Similar results were obtained when group 1 and group 2 subjects were separately considered.

**Discussion**

After 1 week of treatment, salmeterol loses its protective effect on allergen challenge, but this protective effect is partially restored by BDP. By itself, BDP does not afford protection against decrease in pulmonary function caused by allergen challenge, because BDP alone does not determine any significant change on EAR in comparison to pretreatment baseline sBPT.

The development of tolerance to the effects of β2-agonists is well known.14 Tolerance to the bronchodilator effect has been reported for formoterol15 but not for salmeterol.16,17 Tolerance to the protective effect of salmeterol on bronchoconstriction induced by methacholine,1 exercise,2 and allergen challenge3 has been demonstrated. To our knowledge, there are no data available about the prevalence of this phenomenon in the general asthma population; in this study, tolerance to salmeterol occurred in 12 of 18 patients studied consecutively. In general, the tolerance to the bronchodilator effect of long-acting β2-agonist is less evident than the tolerance to the bronchoprotective effect.1,16,17 All studies considered only subjects with mild asymptomatic asthma who did not require regular anti-inflammatory treatment, including corticosteroids.

![Figure 2. Time course of fall in FEV1 from baseline (ΔFEV1 percent) in subjects of group 1 during allergen challenge test, performed as screening (T0), after pretreatment with a single dose of salmeterol (T1), after 1 week of regular treatment with salmeterol (T2), and after 1 week of regular treatment with salmeterol and BDP (T3). T2 was significantly different from T3 at any time points.](image-url)
can be argued that the concomitant treatment with inhaled corticosteroids, which are the cornerstone in asthma treatment, could prevent this negative phenomenon by some interaction with the expression and/or the function of $\beta_2$-adrenoreceptors in human airways.

In vitro studies reported that corticosteroids reduce or revert $\beta$-adrenoreceptor desensitization in blood lymphocytes. In contrast with our results, previous human studies reported that inhaled corticosteroids did not prevent the development of tolerance to the protective effect of inhaled $\beta_2$-agonists. Important differences can be found between other studies and ours in the selection of patients, in the drug used, and in the study design. Some authors studied subjects with mild asymptomatic asthma not regularly treated with inhaled corticosteroids, while other authors included subjects with moderate-to-severe chronic asthma regularly treated with inhaled corticosteroids. The $\beta_2$-agonist drug used for bronchoprotection was salmeterol in some studies and salbutamol in others. The bronchoconstricting stimulus was methacholine in all studies and was associated with allergen challenge in one study. Furthermore, the study protocols often have different durations of treatment and sequences of treatments. Finally, some authors use a parallel group design, others use crossover design. However, a positive effect of systemic corticosteroids on reverting the bronchodilator subsensitivity induced by formoterol and on restoring $\beta_2$-agonist bronchodilation in severe asthma has been demonstrated. Thus, the difference between our results and those of the previous studies may be ascribed to the different study protocol and to the different stimulus (allergen vs methacholine). We speculate that the tolerance induced by salmeterol on $\beta_2$-adrenoreceptors on mast cells, which are involved in the airway response to allergen challenge, might be more easily reverted by corticosteroids than the tolerance on $\beta_2$-adrenoreceptors on smooth muscle cells, which are involved in the response to methacholine challenge.

The protective effect of salmeterol + BDP association on allergen challenge might be ascribed to a direct effect of corticosteroids per se on airway responsiveness to allergen inhalation. Short-term treatment with inhaled corticosteroids prevents early airway response in a dose-dependent manner. In general, 1-week treatment with a low-to-moderate dose did not significantly influence the severity of airway response to allergen challenge. Our control group showed no significant change in the severity of EAR to allergen after 1 week of treatment with BDP alone. Therefore, we suggest that the lack of a significant airway response to allergen inhalation after 1 week of treatment with salmeterol + BDP in patients who had previously developed tolerance to the protective effect of salmeterol is due to a reversion of the tolerance by inhaled BDP. The mechanism of this effect of corticosteroids in reverting the tolerance is not known, but might be related to the ability of corticosteroids in increasing the density and/or the function of $\beta_2$-adrenoreceptors on inflammatory cells.
Our study included subjects with mild intermittent asthma who did not require regular antiasthma treatment. Further studies are needed to assess whether tolerance to the bronchoprotective effect of salmeterol occurs also in long-term treated patients with moderate asthma. Indeed, in patients regularly treated with salmeterol and inhaled corticosteroids, the tolerance to the protective effect of salmeterol may have a clinical relevance in the management of the disease.

In conclusion, we showed that the association of BDP to salmeterol reverts the tolerance to the protective effect of salmeterol on allergen challenge in a group of asthmatic subjects. This observation may partially explain the positive interaction between inhaled β2-agonists and corticosteroids in the long-term treatment of asthma.1–3

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