Effect of oral prednisolone on the bronchoprotective effect of formoterol in patients with persistent asthma

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Asthma is usually treated with inhaled corticosteroids (ICS), in combination with bronchodilators [1]. Inhaled \( \beta_2 \)-agonists, both short- and long-acting, are the most potent bronchodilators in the treatment of asthma [2], whilst single doses of \( \beta_2 \)-agonists protect against a variety of bronchoconstrictive stimuli, including methacholine, adenosine 5'-monophosphate, exercise and allergen [3–6]. However, evidence is accumulating that regular use of \( \beta_2 \)-agonists results in a decrease in the initially large bronchoprotective effects by \( \beta_2 \)-agonists [7], whilst the bronchodilating effects are sustained [8]. This so-called tolerance to the bronchoprotective effect by \( \beta_2 \)-agonists appears to develop within one day of treatment with a long-acting \( \beta_2 \)-agonist (LAB) [9].

ICS are not effective in the attenuation of tolerance. Several studies have now shown that neither pre-existing regular treatment with ICS [10–12], or concomitant start of treatment with inhaled corticosteroids combined with LAB [13] seem to prevent the development of tolerance to the bronchoprotective effects by LAB in patients with asthma. To date, the effects of oral glucocorticoids on tolerance to the bronchoprotective effects by long-acting bronchodilators have not been examined. This is relevant during asthma exacerbations, when patients are more likely to increase the use of bronchodilators and it would be interesting to know whether or not oral steroid treatment can enhance the efficacy of bronchodilators. The aim of this study was to examine whether a short course of prednisolone (30 mg day\(^{-1} \) for 7 days) can reverse already existing tolerance which already exists, to the bronchoprotective effects by LAB in patients with moderate to severe persistent asthma who are using high doses of ICS in combination with LAB regularly.

**Methods**

**Patients**

Twenty-four nonsmoking patients with moderate to severe persistent asthma were selected and invited to participate in this study (table 1). In all patients, symptoms were controlled by regular treatment with
ICS together with LAB. Ten patients were using budesonide (range 800–1600 µg daily), one patient was using beclomethasone dipropionate (800 µg daily) and 13 patients were using fluticasone dipropionate (range 1000–2000 µg daily). In addition, all patients used short-acting β2-agonists as a rescue medication. Treatment with ICS and LAB had been stable during the past six months or longer. Forced expiratory volume in one second (FEV1) was >60% predicted [14] and reversible by >12% pred or within the normal range after bronchodilation (400 µg inhaled salbutamol) in all patients. Furthermore, all subjects were hyperresponsive to histamine, as shown by a provocative concentration causing a 20% fall in FEV1 (PC20) <4 mg·mL−1 [15]. None of the patients had suffered from a respiratory tract infection in the 2 weeks prior to the study, nor had they been treated with oral prednisolone in the 3 months before the study. The study was approved by the Hospital Medical Ethics Committee. Written informed consent was obtained from all patients.

Design

This was a double-blind, randomized, placebo-controlled, parallel group study (fig. 1). Inclusion criteria were assessed on 2 separate days, after which the patients enrolled to the run-in period and were instructed to use a formoterol Turbuhaler® (12 µg per metered dose, Oxis® Turbuhaler®, Astra, Sweden) twice daily, instead of their own long-acting bronchodilator for the duration of the study. After the two weeks run-in period, patients entered a 2 week baseline period. At days 5 and 7 of the baseline period, PC20 histamine was measured 30 min after single dose inhalation of placebo or formoterol (12 µg) in a double-blind fashion, administered in randomized order. After completion of the baseline period, patients were randomized to use either prednisolone 30 mg or identical placebo tablets for 7 consecutive days. At 24 h after administration of the first dose of oral treatment (placebo or prednisolone 30 mg), PC20 histamine was measured 30 min after (single-blind) inhalation of formoterol (12 µg). At days 5 and 7 of the oral treatment period, PC20 histamine was again obtained 30 min after double-blind, randomized, single dose inhalation of placebo or formoterol (12 µg), the order of which being the same as during the baseline period (fig. 1). The order of formoterol and placebo inhalation was randomized per oral treatment group.

Lung function measurements for each patient were performed at the same time of day ± 2 h. Patients were instructed to use the oral treatment before breakfast, lunch or dinner, whichever was closest to their time of lung function measurement. In that way, the oral treatment was taken at 24±2 h before lung function measurements. Patients withheld their formoterol for 24 h before each study visit, and short-acting bronchodilators for 8 h. They continued usage of their regular ICS throughout the study in the morning and evening, as usual, with no change in the dosage of ICS. Furthermore, patients were asked to refrain from caffeine-containing beverages for 4 h before each visit.

**Spirometry and challenge tests**

FEV1 was recorded from standardized maximal expiratory flow/volume curves using a calibrated Masterlab pneumotachograph (Jaeger, Würzburg, Germany). Spirometric reversibility was determined.
by measuring FEV1 before and 15 min after inhalation of 400 µg salbutamol, administered by a metered dose inhaler (MDI) connected to an aerosol chamber, and expressed as an increase in FEV1 in % pred.

Bronchial hyperresponsiveness to histamine was measured using the 2 min tidal breathing method [15]. Serial doubling concentrations of histamine-diphosphate (0.03 – 32 mg·mL⁻¹) were administered to the patient by a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, PA, USA) at 5 min intervals. The response to histamine was measured by FEV1 at 30 and 90 s after inhalation of each concentration. Challenge tests were discontinued if FEV1 fell by > 20% from baseline, or if the highest concentration of histamine had been administered. After completion of each challenge test, 400 µg of salbutamol was given to the patient.

Statistical analysis

PC20 values were calculated by linear interpolation of the last two points on the log concentration-response curve. If FEV1 had not fallen by > 20% from baseline after administration of the highest concentration of histamine, PC20 was censored to 64 mg·mL⁻¹ (which was necessary on 2 occasions). All PC20 values were (natural) log-transformed before statistical analysis. Data are presented as mean ± SD and PC20 values as geometric mean ± SD in doubling concentrations. The protective effect by formoterol at baseline and during the treatment period was calculated as the difference between postformoterol PC20 and postplacebo PC20, expressed in doubling concentrations. The change in protective effect of formoterol on PC20 histamine during the study was defined as the difference between the protective effect of formoterol during treatment compared to the baseline period, expressed in doubling concentrations of histamine. Between-group differences in parameters were tested by an unpaired t-test. Within-group changes were tested by repeated measured analysis of variance (MANOVA) and then by a paired t-test. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA). A probability value of < 5% was considered statistically significant.

With the sample size used in this study (12 subjects in each treatment arm), α = 0.05 (two-sided), β = 0.10 (one-sided; power = 80%) and the standard deviation in bronchoprotective effect in the study group at baseline (1.8), the study was powered to detect a difference in bronchoprotective effect of 2 doubling concentrations of histamine between the placebo and prednisolone treated patients.

Results

Twenty-two patients completed the study. Two patients in the prednisolone group were withdrawn; one because of a nonrespiratory illness during the study, and one because of a protocol violation. At inclusion, the patients in the prednisolone and placebo group did not differ in relevant magnitude with respect to age, FEV1, reversibility in FEV1, PC20 histamine and dosage of ICS (table 1).

Prechallenge FEV1 did not change by prednisolone or placebo treatment (p > 0.2, table 2). PC20 after single-dose formoterol and placebo inhalation, during oral placebo and prednisolone treatment are shown in table 3. The postplacebo PC20 and postformoterol PC20 were not different within and between the prednisolone and placebo treated groups at any of the visits (p > 0.3). However, postformoterol PC20 tended to be improved at 24 h after starting prednisolone treatment (p = 0.08 compared to postformoterol PC20 at baseline).

The bronchoprotective effect by formoterol, i.e. difference between PC20 after formoterol and placebo inhalation expressed in doubling concentrations, was not different between the prednisolone and placebo groups at baseline (p > 0.8), and did not change within and between the groups during treatment (p > 0.5, fig. 2). Finally, the mean (95% confidence interval) change in bronchoprotective effect by formoterol was not significantly different between the two treatment groups (p > 0.4), being 0.14 doubling dose (DD) (-0.54 – 0.82) in the placebo group and -0.14 DD (-0.97 – 0.68) in the prednisolone group.

Discussion

The results of this study show that in patients with moderate to severe persistent asthma, treated with
Data are presented as geometric mean ± SEM in doubling concentrations. #: d5/7: double-blind, randomized pretreatment with placebo or formoterol inhalation was administered to the patient on days 5 and 7 of the baseline and oral treatment period. The randomized order was the same during the two periods; ~: p = 0.08 compared to postformoterol PC20 during baseline.

regular ICS and LAB, addition of oral prednisolone 30 mg·day⁻¹ for 7 days does not improve the bronchoprotective effects by formoterol. However, PC20 to histamine after formoterol inhalation tended to be temporarily increased at 24 h after the first dose of prednisolone, as compared to postformoterol PC20 at baseline. These findings suggest that one week of treatment with oral prednisolone does not restore the bronchoprotective effects of formoterol, nor does it influence the bronchodilating effects of formoterol in patients with moderate to severe persistent asthma using regular ICS in combination with long-acting bronchodilators.

This is the first study demonstrating that the bronchoprotective effect by the LAB formoterol is not affected by 7-days of treatment with oral prednisolone (30 mg) in patients with asthma who are already treated with high doses of inhaled corticosteroids and long-acting bronchodilators. However, prednisolone exhibited some acute effects, since at 24 h after starting oral treatment the postformoterol PC20 tended to be increased by almost two doubling concentrations of histamine from baseline postplacebo PC20, as compared to one doubling concentration in the placebo group. Remarkably, this change in postformoterol PC20 was not maintained during continued treatment with prednisolone and formoterol, since at the end of the treatment period neither the level nor the shift in postformoterol PC20 appeared to be different from the values at baseline or from the placebo group.

The present results were obtained using a standardized and validated method to measure bronchial hyperresponsiveness [15]. Furthermore, first, nonsmoking patients with asthma, controlled by high doses of inhaled steroids and LAB were selected. All patients had stable disease, and had no change in their medication during the past months. It is likely that the patients had already established tolerance to the bronchoprotective effect by their own long-acting bronchodilator, since they used LAB regularly [10, 12, 13]. However, given the present objective and study design, the degree of the pre-existing bronchoprotective effect could not be established, since this would have required discontinuation of regular LAB therapy. To ensure that all patients had an adequate duration of treatment by formoterol, they participated in a 2-week run-in period, before entering the baseline period of the study. This design succeeded in inducing tolerance since the bronchoprotective effect by single-dose formoterol at baseline was less than one doubling concentration of histamine, which is far less than that observed in patients not treated by regular LAB [6] and comparable to the residual bronchoprotective effect after a few weeks of such treatment [7, 12, 13]. Second, to study the short-term effects of oral prednisolone on bronchoprotection bronchial hyperresponsiveness to histamine was also measured after formoterol inhalation at 24 h after starting oral treatment. It would have been interesting to have paired measurements of bronchial hyperresponsiveness to histamine at this timepoint after inhalation of placebo and formoterol, as was done at baseline and during the treatment period, in order to examine the acute effects of prednisolone on the bronchoprotective effects by formoterol. However, PC20 measurements after randomized pretreatment could not be performed on the same day due to the duration of action of formoterol. Furthermore, the authors wanted to be sure that all subjects had used at least 2 doses of formoterol in the period before the PC20 measurement since this has been shown to induce tolerance [9]. Third, PC20 histamine was measured at 30 min after single-dose inhalation since formoterol has a relatively rapid onset of action (within 15 min) [16], with an optimal protective effect coinciding with the time of the histamine challenge. Finally, the dose of prednisolone, (30 mg per day for one week) chosen in this study was based on the current recommendations for the treatment of acute exacerbations of asthma [1]. Therefore, this enabled the examination of whether or not conventional treatment of an asthma exacerbation can improve the bronchoprotective (and bronchodilating) effect of bronchodilators, the most potent rescue medication in an exacerbation.

Table 3. – Provocative concentration of PC20 histamine causing a 20% fall in forced expiratory volume in one second after placebo and formoterol pretreatment during the baseline and treatment period in the placebo and prednisolone group

<table>
<thead>
<tr>
<th></th>
<th>Placebo inhalation</th>
<th>Formoterol inhalation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline d5/7ᵃ</td>
<td>Treatment d5/7ᵃ</td>
</tr>
<tr>
<td>Oral placebo</td>
<td>0.8 ± 0.7</td>
<td>0.8 ± 0.6</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>0.8 ± 0.8</td>
<td>1.0 ± 0.7</td>
</tr>
</tbody>
</table>

Data are presented as geometric mean ± SEM in doubling concentrations.ᵃ: d5/7: double-blind, randomized pretreatment with placebo or formoterol inhalation was administered to the patient on days 5 and 7 of the baseline and oral treatment period. The randomized order was the same during the two periods; ~: p = 0.08 compared to postformoterol PC20 during baseline.
What are the mechanisms underlying the results? With prolonged usage of LAB, and prolonged β₂-receptor occupancy, downregulation of the β₂-receptor is likely to occur [17], through uncoupling of the receptor from the membranous Gₛ protein, internalization of the receptor, a decline in steady state β₂-receptor messenger ribonucleic acid (mRNA) and/or reduced transcription [18–20]. Such downregulation or uncoupling of β₂-receptor numbers can be reflected by a reduced protective effect of LAB against bronchoconstrictor stimuli such as methacholine [7] and exercise [4]. Following treatment with high dose intravenous glucocorticoids, an upregulation of β₂-receptor numbers on circulating lymphocytes, due to increased transcription [21], can be observed in patients with asthma within hours of the start of treatment [22]. This is in keeping with the present results which show a trend towards an increase in PC₂₀ after formoterol inhalation at 24 h after the start of prednisolone treatment, compatible with β₂-receptor upregulation. With continued use of oral glucocorticoids and LAB, β₂-receptor numbers may again be downregulated, resulting in renewed development of tolerance to the bronchoprotective effect by long-acting bronchodilators, as shown by the lack of difference between the bronchoprotective effect by formoterol before and after a week treatment with oral prednisolone.

What are the clinical implications of the study? Long-acting β₂-agonists are indicated in the treatment of patients with moderate and severe persistent asthma for relief of symptoms [1]. With continued use of long-acting β₂-agonists, these patients develop tolerance to the bronchoprotective effect of these drugs [7], which is not prevented by concomitant treatment with inhaled corticosteroids [10–13], oral theophylline [23], or oral glucocorticoids. Furthermore, exposure to allergens may result in β₂-receptor dysfunction [24], possibly on top of the existing downregulated β₂-receptor numbers [17]. Thus, in case of an allergen-induced asthma exacerbation, β₂-agonists may not be effective due to receptor downregulation and dysfunction, while at the same time patients are more likely to increase their use of bronchodilators. Although a short course of oral glucocorticoids is very effective in improving symptoms and reducing airway inflammation during an acute exacerbation [25], it does not reverse tolerance to the protective effects of β₂-agonists. This phenomenon needs further ex vivo examination of β₂-receptor numbers on circulating lymphocytes, or, preferably, on human bronchial smooth muscle.

References

18. Nishikawa M, Mak JCW, Shirasaki H, Barnes PJ.


