The anti-inflammatory profile of inhaled corticosteroids combined with salmeterol in asthmatic patients

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Inhaled corticosteroid (ICS) therapy such as fluticasone propionate (FP) is effective in moderate-to-severe asthma, but for patients on ICS who still experience symptoms, treatment guidelines recommend either increasing the dose of ICS or adding a long-acting β2-agonist such as salmeterol or formoterol. Several studies have now shown that adding salmeterol provides greater clinical benefit than increasing the dose of ICS, raising the question of whether salmeterol has an additive or complementary anti-inflammatory effect to that of ICS.

Recent studies on bronchial biopsies and bronchoalveolar lavage from asthmatic patients treated with either salmeterol, FP or placebo in addition to low-dose ICS have demonstrated that addition of salmeterol produces independent or additional reductions in several pro-inflammatory cells, cytokines and cell adhesion molecules compared with FP. Such complementary anti-inflammatory effects may explain the improved control of asthma symptoms and exacerbations observed when salmeterol is added to low-dose ICS therapy, and may help to modify the long-term sequelae of asthma. These findings also indicate, contrary to earlier speculation, that salmeterol does not have a pro-inflammatory effect or mask persistent airway inflammation.

This review presents the results of recent studies and suggests possible mechanisms for the additional anti-inflammatory effects of salmeterol.

Key words: salmeterol; fluticasone propionate; inhaled corticosteroids; asthma; airway inflammation; leucocytes.

Introduction

Asthma is considered to be an inflammatory disease of the airways, and exacerbations of asthma are usually associated with worsening of the underlying inflammation (1,2). In addition, chronic inflammation may eventually lead to thickening and fibrosis of the airway wall (airway remodeling), which can in turn cause reduced distensibility or fixed obstruction (3–7). Thus, current asthma treatment regimens are designed to modify the disease process by suppressing inflammation as well as providing relief from symptoms.

The anti-inflammatory effects of inhaled corticosteroids (ICS) such as fluticasone propionate (FP) are well established. Inhaled FP in a broad range of dosages has been shown to combine high topical potency with low systemic activity (8,9). From a clinical point of view, ICS may have a long-term disease-modifying effect and protect against fatal attacks by suppressing airway inflammation, as well as providing improvements in lung function and airway reactivity (10). International guidelines therefore recommend the introduction of regular, low-dose ICS in the early stages of asthma (11). However, some patients may be inadequately controlled by low-dose ICS alone, and the next step in treatment is either to increase the dose of ICS or add a long-acting β2-agonist (LABA) such as salmeterol or formoterol (1). At present, there is some debate over which of these options is preferable; however, several studies have shown that addition of a LABA to the existing dose of ICS improves symptom control and lung function to a greater extent than increasing the ICS dose (12–15).

The clinical benefits of combining salmeterol and ICS raise the question of whether salmeterol can, independently of its long-lasting bronchodilator and functional antagonist effects, add to or complement the anti-inflammatory activity of ICS in asthma. Two studies have now addressed...
this question, using bronchial biopsy and bronchoalveolar lavage (BAL) to obtain direct information on changes in airway inflammation during treatment. These studies are reviewed here.

**Bronchial biopsy/BAL studies**

Changes in various cellular markers and mediators of inflammation have been investigated in bronchial biopsies and BAL fluid obtained from over 100 asthmatic patients who participated in two randomized, double-blind, parallel-group studies (16–19). Both studies were conducted in patients with stable but symptomatic asthma who had been using the same dose of ICS [either beclomethasone dipropionate (BDP), budesonide or FP] for up to 12 months, i.e. patients for whom 'step 3' treatment (11) would be recommended. The studies were also designed so that any improvement or worsening of airway inflammation caused by salmeterol could be detected. Both studies comprised a 2-week run-in period, during which patients had to exhibit incomplete symptom control despite continuing their existing ICS, and a 12-week treatment period during which they were randomized to receive either ICS alone (at low or high dose) or salmeterol plus low-dose ICS, as outlined in Table 1. Bronchial biopsies and BAL were performed at randomization and after completing treatment, in line with current safety guidelines (20).

### BRONCHIAL BIOPSY RESULTS

In the first study, baseline biopsies from 40 patients showed similar numbers of mast cells, macrophages and total and activated T-lymphocytes to those in 11 non-asthmatic controls (17). However, total and activated eosinophil counts (as detected by EG1+ and EG2+ cell markers, respectively) were elevated. In this study, all patients continued taking their ICS treatment at the same dosage as during the run-in period; in addition, they were randomized to receive twice daily treatment with either FP 100 µg ('high-dose ICS'), salmeterol 50 µg (SALM/ICS), or placebo ('low-dose ICS') (Table 1). After treatment, numbers of EG1+ eosinophils were significantly reduced from baseline in the SALM/ICS group but not in the other two groups (Fig. 1). There were no significant reductions in numbers of any other cell types. However, patients in both the SALM/ICS and high-dose ICS groups showed a trend towards a decrease in EG2+ eosinophils, compared with the low-dose ICS group (17). These contrasting results may reflect a difference in sensitivity of eosinophils to corticosteroid treatment, as the pre-study ICS treatment might already have minimized the numbers of corticosteroid-sensitive FG1+ cells. Thus, the remaining FG1+ eosinophils would be relatively resistant even to large doses of corticosteroids (21). The additional effects of salmeterol on eosinophils indicate that it may increase their corticosteroid-sensitivity or may act via an independent pathway. Indeed, salmeterol has previously been shown to potentiate

| Table 1. Entry criteria, treatment regimens and assessment schedules used in parallel-group bronchial biopsy/BAL studies |
|-------------------------------|-------------------------------|
| **First study (n=45)**        | **Second study (n=56)**       |
| [Ward et al. 1998 (16); Li et al. 1999 (17)] | [Sue-Chu et al. 1999 (18); Wilson et al. 2000 (19)] |
| **Enrollment**                |                                |
| Age 20–70 years, non-smoker; no acute respiratory tract infection, change in asthma medication or hospitalization for asthma within the previous 4 weeks; FEV1 ≥ 60% of predicted normal value | Non-smoker; FEV1 ≥ 50% of predicted; reversibility or BHR to methacholine (PC20 ≤ 8 mg) |
| **Run-in period (2 weeks)**   |                                |
| BDP or BUD 100–500 µg day⁻¹ | BDP or BUD 800–1200 µg day⁻¹, or FP 400–500 µg day⁻¹ |
| Symptom score > 2, rescue β₂-agonist treatment and/or > 15% diurnal variation in PEF on ≥ 7 days | Daytime symptoms or rescue β₂-agonist treatment on ≥ 6 days, or nocturnal symptoms or > 20% diurnal variation in PEF on ≥ 4 days |
| **Randomization**             |                                |
| Bronchial biopsy and BAL collection | PC20 to methacholine and bronchial biopsy |
| **Treatment period (12 weeks):** |                                |
| Low-dose ICS                  | Change to FP 200 µg bd         |
| Maintain existing ICS, add placebo | Change to FP 500 µg bd         |
| High-dose ICS                 | Maintain existing ICS, and FP 100 µg bd | Maintain existing ICS, and salmeterol 50 µg bd |
| Additional salmeterol         | Maintains salmeterol 50 µg bd plus FP 200 µg bd | Maintains salmeterol and bronchial biopsy |
| **Post treatment**            |                                |
| Bronchial biopsy and BAL collection | PC20 to methacholine and bronchial biopsy |

BAL, bronchoalveolar lavage; BDP, beclomethasone dipropionate; BHR, bronchial hyperreactivity; BUD, budesonide; FEV1, forced expiratory volume in 1 sec; FP, fluticasone propionate; ICS, inhaled corticosteroid; PC20, concentration required to provoke a 20% decrease in FEV1; PEF, peak expiratory flow.
concentrations of IL-8, a neutrophil chemoattractant, were the airway mucosa (30). In the ICS group (from 2.1 to 1.6%) (16). In addition, long-term airway remodelling by reducing the vascularity of the pro-apoptotic effects of corticosteroids on eosinophils (22).

In the second study, patients were switched from their existing ICS (BDP or budesonide 800–1200 μg) to twice-daily treatment with either FP 200 μg (FP 200 group), 500 μg (FP 500 group) or salmeterol 50 μg plus FP 200 μg (SALM/FP) (Table 1) (18). The baseline biopsies in this study showed that run-in ICS treatment had already reduced activated eosinophil numbers in all patients, and that the possibility of study treatments decreasing them further was negligible. Numbers of submucosal mast cells in post-treatment biopsies were significantly lower than baseline in patients receiving SALM/FP. Although a significant increase in total (CD3⁺) and helper/inducer (CD4⁺) T-lymphocytes was observed in the FP 200 group, numbers of these cells were unchanged in the other two groups; in addition, treatment with SALM/FP resulted in a reduction in the expression of HLA-DR and vascular cell adhesion molecule (VCAM)-1 (19).

**BAL RESULTS**

BAL fluid analysis reflects events occurring on the airway surface, and can be used to detect soluble mediators of inflammation. Since BAL allows small airways to be sampled as well as large ones, the information gathered may complement that provided by biopsies (20,23).

Compared with non-asthmatic controls, baseline BAL samples from 45 asthma patients in the Li et al study showed an "asthmatic signal", including significantly higher counts of lymphocytes and eosinophils (17). The neutrophil count was not elevated at baseline. However, consistent with previous reports (24,25), the number of neutrophils in BAL from patients receiving high-dose ICS increased significantly from baseline (from 1-4 to 3-5%; P = 0-001), whereas there was a trend towards a decrease in the SALM/ICS group (from 2-1 to 1-6%) (16). In addition, concentrations of IL-8, a neutrophil chemoattractant, were unchanged in the high-dose ICS group but fell significantly in the SALM/ICS group (16). In contrast, high-dose ICS significantly reduced the expression of the HLA-DR marker (P = 0-05) of CD4⁺ T-lymphocyte activation, with a trend towards a decrease in expression of the CD25 marker. These changes were not seen in the SALM/ICS group (17).

Taken together, the results of these bronchial biopsy and BAL studies indicate that the combination of a LABA and low-dose ICS therapy reduces the number of several types of pro-inflammatory cell in the airways, and reduces the production of various mediators of inflammation and cell adhesion molecules. These effects are more pronounced with the combination therapy than with ICS alone, suggesting a complementary anti-inflammatory effect. This concept is borne out by two recent studies. In the first (the FACET study), 1 year's treatment with formoterol 17 μg plus budesonide 400 μg produced a greater reduction of the number of exacerbations than budesonide alone in patients with asthma symptoms despite ICS therapy (14); in the second, patients who received salmeterol plus low-dose FP for 24 weeks showed a trend towards fewer exacerbations than those on high-dose FP (15). Furthermore, in a recent 1-year study in patients with moderate asthma, no significant changes in inflammatory cells and markers in induced sputum were found after twice daily treatment with either formoterol 12 μg/budesonide 100 μg or budesonide 400 μg alone (26), demonstrating that addition of a LABA to ICS therapy does not mask an increase in airway inflammation.

**REMODELLING OF AIRWAY VASCULATURE**

Remodelling of the airway wall over time is considered to be an important cause of reduced wall distensibility in mild asthma and fixed obstruction in more severe disease (3–7).

It has been suggested that mucosal oedema consequent to vascular proliferation may narrow the airway, possibly by increasing smooth muscle tone (7,27), and a bronchial biopsy study has demonstrated that the mucosal lamina propria contains substantially more blood vessels in asthmatic patients than in non-asthmatic controls (28). An increase in mucosal blood vessels may therefore reflect airway wall remodelling. ICS have recently been shown to reduce both the number of vessels and the area of the lamina propria that they occupy in asthmatic patients, in a dose-dependent manner (29).

In a subsequent biopsy study in 34 asthma patients receiving low-dose ICS treatment who were given supplementary twice daily treatment with either salmeterol 50 μg or FP 100 μg (30), the number of vessels in the lamina propria was significantly reduced by treatment with ICS plus salmeterol and not with ICS plus FP; indeed, addition of salmeterol to ICS resulted in a normalization of vessel number to the value observed in 25 non-asthmatic controls (Fig. 2), suggesting that salmeterol may be able to influence long-term airway remodelling by reducing the vascularity of the airway mucosa (30).
way smooth muscle and reduced the concentration of the regulatory protein cyclin D1, actions which were not inhibited thrombin-stimulated hyperplasia of human airway smooth muscle in vitro (37,38). In addition, epithelial, vascular and inflammatory cells produce a range of cell surface adhesion molecules which assist leucocyte infiltration into the airway wall (39). Addition of salmeterol 50 µg twice daily to low-dose ICS therapy for 12 weeks reduced the levels of IL-8, IL-4 and VCAM-1 in BAL samples from asthma patients (16,19), suggesting that prevention of cytokine or adhesion molecule expression may have been responsible for the reductions in leucocyte numbers recorded in that study.

With respect to the potential effects of salmeterol on long-term airway remodelling, the enhanced vascularity of the airway mucosa may stem from elevated levels of angiogenic growth factors, which include IL-8 (40-42). In contrast with FP, salmeterol reduced both the vascularity of the lamina propria (30) and IL-8 levels in BAL samples from asthmatic patients (16,19), suggesting that prevention of cytokine or adhesion molecule expression may have been responsible for the reductions in leucocyte numbers recorded in that study.

Possible mechanisms

Inflammation involves the influx into the bronchial wall of a variety of pro-inflammatory cells, which when activated induce the expression and release of a complex array of inflammatory mediators. Among the many chemotactic cytokines for pro-inflammatory cells, IL-8, which is produced by macrophages, mast cells and epithelial cells on the airway surface (31-33), recruits predominantly neutrophils. Since IL-8 can also induce bronchial hyperresponsiveness in animals (34), it may play an important role in the pathogenesis of asthma. While the effects of ICS on IL-8 expression remain unclear, some studies have shown increased levels during treatment with corticosteroids (35,36). Similarly, IL-4 is a major inflammatory cytokine found in the airway; it has been shown to induce goblet cell production and secretion of mucus and mucous glycoconjugates in an animal model and in human bronchial epithelial cells in vitro (37,38). In addition, epithelial, vascular and inflammatory cells produce a range of cell surface adhesion molecules which assist leucocyte infiltration into the airway wall (39). Addition of salmeterol 50 µg twice daily to low-dose ICS therapy for 12 weeks reduced the levels of IL-8, IL-4 and VCAM-1 in BAL samples from asthma patients (16,19), suggesting that prevention of cytokine or adhesion molecule expression may have been responsible for the reductions in leucocyte numbers recorded in that study.

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The actual mechanisms underlying all the effects exerted by salmeterol remain unclear. However, most types of cell can express β2-receptors, and prolonged stimulation of these receptors might influence some of the pathways leading to airway inflammation in asthma. Animal studies have shown that β2-receptor stimulation by salmeterol can prevent platelet activating factor-induced adhesion of leucocytes to mucosal blood vessels (47) and eosinophil accumulation in the airway lumen (48). Furthermore, glucocorticoids have been shown to induce the expression of β2-receptor mRNA, prevent β2-agonist-induced down-regulation of β2-receptors, and increase the number and function of β2-receptors in human respiratory tissue (49-51). These may help to explain the additional anti-inflammatory effects seen when salmeterol is combined with ICS.

Conclusions

Recent bronchial biopsy and BAL studies have demonstrated that treatment with a combination of salmeterol and ICS produces independent or additional reductions in several pro-inflammatory cells, cytokines and cell adhesion molecules compared with ICS alone. There may also be an independent inhibitory effect of salmeterol on angiogenesis in asthma patients treated with ICS. Some of these effects may explain the improved control of asthma symptoms observed when salmeterol is added to low-dose ICS therapy, and may help to modify the long-term sequelae of asthma.

These findings importantly indicate that salmeterol does not have a pro-inflammatory effect or mask persistent airway inflammation in the presence of ICS. There is encouraging evidence of a complementary interaction between salmeterol and FP which results in enhanced anti-inflammatory activity. More studies are needed to determine why this occurs, and what the clinical implications of the interaction may be.

References

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