Formoterol Attenuates Neutrophilic Airway Inflammation in Asthma*

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Study objectives: Airway neutrophil levels are increased in patients with severe asthma and during asthma exacerbations. Long-acting β₂-agonists (LABAs), such as formoterol, reduce the number of asthma exacerbations. While β₂-agonists may affect neutrophil function in vitro, it is uncertain whether they have effects on neutrophilic inflammation in asthmatic patients in vivo.

Design: In a double-blind randomized crossover study, we evaluated the effects of 4 weeks of treatment with formoterol (Turbuhaler), 24 μg bid, compared to placebo on sputum neutrophil numbers and interleukin (IL)-8 levels in asthmatic patients. Therapy with budesonide (administered via Turbuhaler), 400 μg bid for 4 weeks, was added at the end as a “gold standard” antiinflammatory effect comparison.

Patients: We studied 15 steroid-naı¨ve nonsmoking patients who ranged from 19 to 51 years of age and had mild persistent asthma.

Results: Formoterol therapy significantly reduced sputum IL-8 levels and neutrophil numbers compared to placebo. There was a significant correlation between the reduction in sputum IL-8 levels and the number of neutrophils, indicating that formoterol may attenuate neutrophilic airway inflammation by inhibiting IL-8 production.

Conclusions: Our data suggest that the LABA formoterol reduces neutrophilic airway inflammation in patients with mild asthma and that this might be beneficial in preventing asthma exacerbations. (CHEST 2005; 128:1936–1942)

Key words: asthma; formoterol; interleukin-8; long-acting β₂-agonist; neutrophil

Abbreviations: eNO = exhaled nitric oxide; IL = interleukin; LABA = long-acting β₂-agonist; NF = nuclear factor; NO = nitric oxide; PC₂₀ = provocative concentration of a substance causing a 20% fall in FEV₁; ppb = parts per billion

Asthmatic airways are infiltrated with a variety of activated inflammatory cells, including mast cells, eosinophils, and T lymphocytes. Eosinophils are characteristic of asthmatic inflammation and may play a role in the pathophysiology of asthma. However, more recent data confirm the considerable heterogeneity of the asthma phenotype, and many subjects may exhibit an airway disease that is characterized by sputum neutrophilia rather than the expected eosinophilic pattern.¹,² Neutrophils have been implicated in severe steroid-dependent bronchiolar asthma and during acute exacerbations and in cases of sudden-onset fatal disease.³–⁶ On the other hand, the role of airway neutrophils in the pathogenesis of mild persistent asthma has not been established, although there is much circumstantial evidence to support the existence of neutrophilic airway inflammation in patients with mild asthma.¹

Airway neutrophilia in patients with asthma is likely to be multifactorial, and is dependent on a complex interplay of lipid mediators and chemokines from both resident airway cells and inflammatory cells in addition to enhanced adhesion molecule expression. Interleukin (IL)-8, a CXC chemokine, is produced by the bronchial epithelium⁷–⁹ and macrophages,¹⁰–¹² and is a potent neutrophil activator and chemoattractant.¹³,¹⁴ Patients with acute severe asthma exhibit an increase in IL-8 levels and neutrophil numbers in the airways.¹⁵,¹⁶ However, little is known about the role of IL-8 and neutrophil airway inflammation in the pathogenesis of the milder forms of asthma.
Long-acting β₂-agonists (LABAs) show a range of in vitro and in vivo antineutrophil actions. Such effects might contribute to a reduction in the number of exacerbations seen in asthmatic patients with LABA treatment. Formoterol inhibits the release of oxidants from human neutrophils and inhibits the adhesion of neutrophils to postcapillary venules in the airways of rats, suggesting that it may inhibit neutrophil inflammation. One study showed that salmeterol significantly reduced the numbers of neutrophils and the amount of myeloperoxidase in bronchial biopsy specimens from asthmatic patients. Another study showed that the addition of inhaled salmeterol to therapy with an inhaled corticosteroid reduced the concentrations of IL-8 and myeloperoxidase in the BAL fluid of asthmatic patients, although it did not reduce the numbers of neutrophils. These data suggest that LABAs have an inhibitory effect on neutrophilic inflammation in asthma patients, although their mechanism of action is uncertain. We investigated the effect of formoterol on IL-8 concentrations and neutrophil numbers in the induced sputum of mild asthmatic patients who were not treated with inhaled corticosteroids.

Materials and Methods

Subjects

Eligible patients were stable and had experienced mild persistent asthma for at least 3 months prior to the study (seven men and eight women; age range, 19 to 51 years; mean [± SEM] age, 29.5 ± 3.58 years) [Table 1], none had received a course of therapy with oral or inhaled corticosteroids during this period. Asthma was diagnosed by the American Thoracic Society criteria. Subjects had a baseline FEV₁ of > 70% predicted and demonstrated a reversibility of FEV₁ after therapy with nebulized albuterol (2.5 mg) of ≥ 15% and 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, or the use of LABAs. Two patients were withdrawn from the study during the randomized treatment period because of their absence from regular visits. Written informed consent was obtained from each patient, and the study was approved by the Ethics Committee of the Royal Brompton and Harefield National Health Service Trust.

Study Design

This was a double-blind, randomized, placebo-controlled, and crossover study using 24 μg of formoterol via inhaler (Turbuhaler; GlaxoSmithKline; Research Triangle Park, NC) and identical placebo inhalers for 4 weeks each, with a 2-week washout phase between rounds of therapy. After the end of the crossover period, patients were administered single-blind budesonide via inhaler (two doses of 400 μg twice daily) for 4 weeks. Patients entered an initial 2-week run-in period in which inhaled ipratropium bromide, which was administered via powder metered-dose inhaler, was used as rescue medication to avoid the possible anti-inflammatory effect of short-acting β₂-agonist for the study duration. The primary end point was the change in sputum IL-8 levels after active treatment compared with placebo, with changes in sputum neutrophil numbers being the secondary end point. Other parameters studied were changes in FEV₁ and in levels of exhaled nitric oxide (eNO) and sputum α₁-macroglobulin. Measurements for sputum IL-8, sputum neutrophil numbers, and eNO levels were made before and after each randomized treatment. 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 Measurements

FEV₁ and FVC were measured using a dry wedge spirometer (Vitalograph; Buckingham, UK). Values are expressed as the percent predicted. 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. The measurement was performed at a screening 2 weeks before the first study day, as shown in Table 1, and during each visit before the beginning of sputum induction. Bronchial provocation test results were determined at the screening visit. The level of bronchial reactivity was assessed by methacholine challenge, which was performed according to a standardized technique. The methacholine PC₂₀ was determined by the linear interpolation of the concentration-FEV₁ response curve.

eNO

eNO was measured simultaneously by a chemiluminescence analyzer (model LR2000; Logan Research; Rochester, UK). The analyzer is sensitive to nitric oxide (NO) concentrations from 1 to 500 parts per billion (ppb) by volume, with a resolution of 0.3 ppb. The analyzer was calibrated using certified NO mixtures (ie, 90 and 436 ppb) in nitrogen (BOC Special Gases; Guildford, UK). Measurement was made by slow exhalation (5 to 6 L/min) from total lung capacity for 15 to 20 s against a low resistance (5 cm H₂O) to exclude nasal contamination.
Sputum Induction and Processing

Sputum was induced by the inhalation for 15 min of a 3.5% saline solution via an ultrasonic nebulizer (model 2000; DeVilbiss Co; Heston, UK), as previously described.21 Briefly, the whole sputum sample was processed with dithiothreitol (Sigma Chemicals; Poole, UK). The homogenized sputum was centrifuged at 300g for 10 min. The supernatant was separated and frozen at −70°C until further analysis. Total cell counts were made on a hemocytometer slide, using Kimura stain, and slides were prepared with a cytopsin device (Shandon; Runcorn, UK) and were stained with May-Grumwald-Giemsa stain. Differential cell counts were made by a blinded observer. Three hundred nonsquamous cells were counted on two slides for each sample. Differential cell counts are expressed as the percentages of nonsquamous cells.

IL-8 Assay

The concentration of IL-8 in sputum supernatant was determined using commercially available enzyme-linked immunosorbent assay kit (R&D Systems; Minneapolis, MN) according to the manufacturer’s instructions.

Sputum α2-Macroglobulin Assay

Because formoterol is reported to reduce airway microvascularity leakage in asthmatic patients,22 we also measured the levels of α2-macroglobulin, a marker of vascular leakage. Sputum levels of α2-macroglobulin were measured using a radioimmunoassay that was sensitive to 10 ng/mL. Rabbit antihuman α2-macroglobulin (Dakopatts; Copenhagen, Denmark) was used as antiserum and human serum (Behringwerke; Marburg, Germany) as standard. Human α2-macroglobulin (Cappel-Organon; Turnhout, Belgium) was iodinated using the lactoperoxidase method. Tracer and standard (or samples) were mixed with antiserum before adding goat antirabbit antiserum (AstraZeneca; Lund, Sweden). The bound fraction was measured using a gamma counter (Pharmacia; Uppsala, Sweden). The intra-assay and interassay coefficients of variation were between 3.8 to 6.0% and 3.1 to 7.2%, respectively.

Statistical Analysis

The results are expressed as the mean ± SEM. Changes in IL-8 levels and neutrophil numbers after treatment were compared using a paired t test. The r value (ie, Pearson correlation coefficient) was determined for the correlation of IL-8 levels and neutrophil numbers within the groups. 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).

RESULTS

The Effect of Treatment on eNO

The degree of airway inflammation, as determined by eNO levels, was not different before the start of each randomized treatment and was not affected by therapy with formoterol (Fig 1). In contrast, budesonide treatment significantly suppressed eNO levels over a period of 4 weeks when compared with the placebo group (p = 0.03) and the pre-budesonide treatment group (p < 0.05) [Fig 1]. In addition, budesonide treatment had a significant effect on eNO levels in comparison with formoterol treatment (p = 0.0026).

Effect of Formoterol Treatment on IL-8 Production

Sputum induction was successful in all of 13 patients studied. There were no significant differences in the baseline concentration of IL-8 before either formoterol or placebo treatment. Formoterol treatment for 4 weeks caused a significant reduction in sputum IL-8 levels when compared to placebo (−467 ± 171 vs 716 ± 162 pg/mL, respectively; p = 0.0004). Budesonide also gave a significant reduction in sputum IL-8 release compared with placebo (−434.4 ± 186 vs 716 ± 162 pg/mL, respectively; p < 0.01). An analysis of between-treatment group changes indicated that the reduction in IL-8 levels following the introduction of formoterol therapy was similar to that seen following budesonide therapy (−467 ± 171 vs −434.4 ± 186 pg/mL, respectively) [Fig 2]. Treatment with both formoterol and budesonide was significantly more effective in suppressing sputum IL-8 levels than placebo treatment.

Effect of Inhaled Formoterol on Sputum Cells

Formoterol administration caused a significant reduction in the absolute number of airway neutrophils compared with placebo (−823 ± 289 vs 591 ± 174, respectively; p = 0.0061) [Fig 3]. In contrast, budesonide had a slight but insignificant effect.
on absolute neutrophil numbers measured either by between-group analysis (−272 ± 118 vs 591 ± 174, respectively; difference not significant) or by post-treatment comparison (1,321 ± 357 vs −1,085 ± 240, respectively; difference not significant). However, total cell counts were reduced after budesonide treatment when compared to after placebo treatment (p < 0.05), confirming the anti-inflammatory action of budesonide (Table 2). By contrast, there was no effect of formoterol on total cell counts (Table 2).

There was a significant correlation between the percentage of neutrophils and sputum IL-8 levels in the formoterol group (r² = 0.35; p ≤ 0.05) [Fig 4, top, a]. In addition, we could demonstrate an inverse correlation between FEV₁ and sputum neutrophil numbers in those patients who were treated with formoterol, placebo, and budesonide (Fig 4, bottom, b).

**Effect of Formoterol on Sputum α₂-Macroglobulin Levels**

Only eight subjects exhibited detectable levels of α₂-macroglobulin (Fig 5). All values were very low, and there was no significant difference in mean α₂-macroglobulin concentrations after formoterol treatment when compared with placebo treatment (0.6 ± 0.1 vs 1.1 ± 0.2, respectively; p = 0.1) or with budesonide treatment (0.6 ± 0.1 vs 0.7 ± 0.2, respectively; p = 0.7).

**Discussion**

The study described here provides evidence that inhaled formoterol inhibits IL-8 production and attenuates neutrophilic inflammation in the airways of patients with mild asthma. The very low levels of α₂-macroglobulin suggest that little microvascular leakage is occurring in all subjects and that changes in leakage therefore cannot account for the effect of formoterol on reduced neutrophil numbers. We speculate that the formoterol-induced inhibition of IL-8 production might be due to the down-regulation of IL-8 gene transcription in airway epithelial cells and alveolar macrophages.

Bronchial epithelial cells in patients with asthma show increased expression of IL-8, 25 and so IL-8 is thus implicated in inflammatory cell chemotaxis in asthma. IL-8 is regulated primarily at the level of gene transcription. Several studies26,27 have shown that the sequence spanning nucleotides −1 to −133 within the 5' flanking region of the IL-8 gene are essential and sufficient for transcriptional regulation of the gene. This promoter region has DNA-binding sites for proinflammatory transcription factors, including nuclear factor (NF)-κB, activator protein-1, and NF-IL-6. All three transcription factors can act in concert to synergistically activate IL-8 promoter, especially the preferred cooperative interaction between activator protein-1 and NF-κB.28,29 Indeed, formoterol treatment reduces the epithelial expression of activated NF-κB.30 The formoterol-mediated inhibition of NF-κB may be the mechanism underlying the reduced IL-8 production observed in the present study.

The association between the levels of IL-8 and the number of neutrophils in the asthmatic airway remains controversial. Previous reports have suggested that there was no significant relationship between IL-8 levels and neutrophil numbers in patients with...
severe diseases and subjects with persistent asthma who were treated with inhaled corticosteroids. In contrast, the present study performed in patients with mild asthma demonstrated a significant association between the number of neutrophils and the concentrations of IL-8 in steroid-naïve asthmatic patients following formoterol treatment (Fig 4). This might reflect the fact that other neutrophil chemotactic factors may additionally be involved in patients with severe asthma.

In stable patients with asthma, the levels of sputum eosinophils, but not neutrophils, are elevated in induced sputum. In our patients with mild asthma, there was even a trend toward an increased number of airway neutrophils after placebo treatment. During spontaneous asthma exacerbations or following upper respiratory tract viral infections, a prominent neutrophilic inflammation has been observed in association with high levels of IL-8. The present study showed that the reduction in airway neutrophils was associated with the improvement of lung function in terms of FEV₁ (Fig 4, bottom). Formoterol may also prove to be a useful additional therapy, particularly in asthmatic patients with a neutrophilic pattern of inflammation who do not

![Figure 4](http://publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/22032/)

**Figure 4.** Top, a: correlation between the percentage of sputum neutrophils and IL-8 levels in asthmatic patients treated with formoterol. Bottom, b: relationship between FEV₁ percent predicted and sputum neutrophil numbers.

![Figure 5](http://publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/22032/)

**Figure 5.** α₂-Macroglobulin levels in induced sputum before and after 4 weeks of treatment with formoterol (24 µg bid), placebo, and budesonide (400 µg bid). Horizontal bars represent the means of eight individual values; levels in samples from the remaining five subjects were below the level of detection. See Figure 1 for abbreviations not used in the text.
respond as well to inhaled corticosteroids as patients with predominantly eosinophilic inflammation. 1

Although the anti-inflammatory activities of LABAs have been demonstrated in in vitro study and an animal study, the similar effects seen in these studies failed to be shown in human asthmatic patients. Earlier studies suggested that regular salmeterol treatment had no anti-inflammatory effects on airway inflammation in asthmatic patients. In contrast, the present study showed that formoterol therapy alone could attenuate neutrophilic airway inflammation in the induced sputum of asthmatic patients. The conflicting data could be explained by the fact that prior studies that could not demonstrate the anti-inflammatory effects of salmeterol examined actions on markers such as the number of CD4+ and CD8+ lymphocytes either in BAL fluid or bronchial biopsy specimens rather than on sputum or airway neutrophil numbers.

We also demonstrated that budesonide treatment attenuated neutrophil numbers and IL-8 levels to a lesser extent than formoterol. This raises the possibility that the mechanisms underlying anti-neutrophil recruitment into the airways by formoterol are distinct from those mediated by budesonide. The failure to show significantly reduced sputum neutrophilia with budesonide may result from the ability of corticosteroids to prolong neutrophil survival, and would also account for the failure to see any correlation between IL-8 levels and neutrophil numbers after budesonide treatment. Overall, however, budesonide therapy showed a significant reduction in total cell counts, reflecting an effect on levels of sputum eosinophils and macrophages (Fig 6). Furthermore, budesonide treatment caused a reduction in NO production, whereas this effect was not demonstrated after treatment with formoterol.

In summary, we have shown that formoterol reduces sputum IL-8 levels and neutrophilia independently of changes in microvascular leakage in steroid-naïve patients with mild asthma. However, due to the small size of this study further studies are required to confirm these data in a larger population of subjects with mild persistent asthma. In addition, studies examining the effects of formoterol therapy on tissue IL-8 levels and neutrophilia are awaited. The data further suggest that formoterol therapy may be a useful adjunct to complement inhaled corticosteroid therapy in patients with mild asthma, and particularly in those subjects who have neutrophilic exacerbations.

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