Inhaled Corticosteroids in Stable COPD Patients*
Do They Have Effects on Cells and Molecular Mediators of Airway Inflammation?

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Study objective: To investigate possible changes in cells and molecular mediators of airway inflammation following inhaled steroid treatment of stable COPD patients.

Design: Six-week open preliminary prospective study.

Setting: A university respiratory disease clinic.

Patients: Stable COPD patients with mild disease.

Intervention: Six-week treatment with inhaled beclomethasone (1.5 mg die).

Measurements: The levels of interleukin (IL)-8, myeloperoxidase, eosinophilic cationic protein and tryptase, and cell numbers in bronchial lavage specimens were determined, and the symptom score, the endoscopic bronchitis index, and functional parameters were recorded.

Results: After treatment there were significant reductions in the lavage levels of IL-8 ([mean ± SEM] 1,603.4 ± 331.2 vs 1,119.2 ± 265.3 pg/mL, respectively; p = 0.01) and myeloperoxidase (1,614.5 ± 682.3 vs 511.2 ± 144.2 μg/L, respectively; p = 0.05), in cell numbers (250.6 ± 27.7 vs 186.3 ± 11.5 cells × 10³/mL, respectively; p = 0.04), neutrophil proportion (59.7 ± 14.3% vs 31.5 ± 10.1%; p = 0.01), symptom score (4.5 ± 0.6 vs 1.4 ± 0.5; p = 0.01), and bronchitis index (8.5 ± 0.8 vs 5.5 ± 0.7; p = 0.007).

Conclusions: In stable patients with COPD, inhaled steroid treatment may induce changes on some cellular and molecular parameters of airway inflammation.

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Key words: airway inflammation; COPD; interleukin-8; myeloperoxidase

Abbreviation: IL = interleukin

It has been shown that airway inflammation plays an important role in the pathogenesis of COPD.1–12 Thus, treatment with anti-inflammatory medication has been advocated, and a number of both short-term and long-term controlled trials using inhaled corticosteroids and evaluating clinical and functional parameters have been published.13–27 However, the results of these studies often are conflicting,28 and international guidelines recommend that steroids should be used only in certain subpopulations of patients, although these subpopulations are not easily recognizable before a steroid trial.29,30

Surprisingly, only a few studies have investigated the biological effects of inhaled steroids on airway inflammation. One study, to the best of our knowledge, was performed by evaluating airway lavage specimens,17 and three studies were performed by evaluating sputum samples.31–33 The aim of this preliminary study was to investigate the effects on airway inflammation of short-term treatment with high-dose inhaled corticosteroids in stable patients with mild COPD by comparing cells and soluble mediators of inflammation in bronchial lavage specimens obtained before and after treatment.

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To obtain preliminary data on the possible changes in cells and mediators of airway inflammation in stable patients with COPD, we designed a short-term open clinical study. We studied eight patients (seven men and one woman; mean [± SEM] age, 61.1 ± 2.6 years) with chronic bronchitis and COPD. The inclusion criteria were the following:

1. FEV\textsubscript{1} < 75% of predicted values (actual observed values, 69.8 ± 2.1% of predicted) and no improvement in FEV\textsubscript{1} values of > 12% and ≥ 200 mL after inhalation of 200 mg salbutamol;
2. FEV\textsubscript{1}/FVC ratios after bronchodilator use that are < 88% of predicted in men and < 89% of predicted in women (actual observed values, 63.7 ± 1.4% of predicted); and
3. A history of cigarette smoking, with a minimum of 16.5 pack-years in patients who were current smokers at the time of evaluation. The patients had failed a smoking cessation program.

The exclusion criteria were the following:

1. Occupational or other type of exposure to substances known to cause lung disorders;
2. A history of systemic or other pulmonary disease or of congenital and/or acquired systemic immunodeficiency;
3. Bronchitic exacerbation within the preceding month;
4. Therapy with inhaled or systemic corticosteroids within 3 months;
5. Since it is known that among patients with COPD who respond to steroids many may present with features of asthma,29,30 particular emphasis was put on excluding from the study subjects who had atopy, or a personal or family history of allergic disease, or seasonal or recurrent wheezing, childhood asthma, or respiratory problems. In this context, all patients underwent skin tests for common allergen extracts, and all of them had negative skin tests.

A symptom score, comprising scores for cough, sputum, and dyspnea, was recorded for each patient, as previously described.2 Peripheral blood was drawn from each subject, and a CBC count, including a leukocyte differential count, and a determination of the levels of total IgE (Prist; Pharmacia; Uppsala, Sweden) were performed. To investigate airway inflammation, fiberoptic bronchoscopy (model 1T10 bronchoscope; Olympus; Tokyo, Japan) with inspection of bronchial mucosa and of all segmental bronchi was performed in all subjects. Bronchoscopies all were performed by the same investigator (A.P.). During bronchoscopy, bronchial lavage was performed by introducing 50 mL prewarmed, sterile saline solution through the bronchoscope that was wedged into a segmental bronchus, usually in the middle lobe or in the lingula, and then aspirating the fluid almost immediately.6 Bronchial lavage has been shown to be useful for investigating the changes associated with airway inflammation.2,3,5,17 The lavage fluid then was processed to separate cells from supernatants. The total and differential cell counts were performed, the latter by using the May-Grunwald-Giemsa and toluidine blue methods (Sigma; St. Louis, MO).6 Supernatants from bronchial lavages were assayed for the levels of interleukin (IL)-8 (enzyme-linked immunosorbent assay; Amersham International; Buckinghamshire, UK) after concentration of the samples using concentrators (Centriprep; Amicon; Beverly, MA), and for the levels of myeloperoxidase, eosinophilic cationic protein, and tryptase (Pharmacia).6

Thereafter, the treatment of patients with inhaled beclomethasone, 1,500 μg/d (500 μg tid with a metered-dose inhaler), was initiated. Patients were asked to continue smoking the same number of cigarettes per day as at the time of entry. The smoking patterns of the study subjects were followed by self-reported questionnaires filled out at the second visit after treatment had been stopped and also by follow-up calls at the third week of treatment. During the study, no patients needed a change in dosage of the inhaled β-agonist, anticholinergic, or theophylline. At six weeks of therapy, patients were reevaluated with a physical examination and pulmonary function tests, with the symptom score being recorded again, then a second bronchoscopy was performed, with procedures and sampling processes as discussed above. Lavages were performed in each subject in the same segment both before and after treatment.

This study protocol was approved by the local ethics committee. Each subject gave informed consent. Group data are expressed as mean ± SEM. Changes observed after treatment were tested for significance using the Wilcoxon signed rank test for paired samples. A p value < 0.05 was regarded as significant. Statistical analysis was performed by using computer software (SPSS, version 4.0; SPSS; Chicago, IL).

### RESULTS

The clinical and functional baseline characteristics of COPD patients are shown in Table 1.

<table>
<thead>
<tr>
<th>Patient No./Age, yr/Sex</th>
<th>Smoking History, pack-yr</th>
<th>IgE, IU/mL</th>
<th>Disease Duration, yr</th>
<th>Blood Eosinophils, No./μL</th>
<th>PaO\textsubscript{2}, mm Hg</th>
<th>PaCO\textsubscript{2}, mm Hg</th>
<th>FEV\textsubscript{1}, % predicted</th>
<th>FEF\textsubscript{25-75}, % predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/69/M</td>
<td>50</td>
<td>10.4</td>
<td>7</td>
<td>120</td>
<td>72.2</td>
<td>44.7</td>
<td>71</td>
<td>63</td>
</tr>
<tr>
<td>2/69/M</td>
<td>55</td>
<td>49.6</td>
<td>9</td>
<td>40</td>
<td>70</td>
<td>44.6</td>
<td>74</td>
<td>82</td>
</tr>
<tr>
<td>3/55/M</td>
<td>40</td>
<td>40.8</td>
<td>3</td>
<td>170</td>
<td>68</td>
<td>38.2</td>
<td>75</td>
<td>73</td>
</tr>
<tr>
<td>4/57/M</td>
<td>80</td>
<td>90.8</td>
<td>3</td>
<td>140</td>
<td>78.9</td>
<td>34.5</td>
<td>60</td>
<td>52</td>
</tr>
<tr>
<td>5/48/M</td>
<td>16.5</td>
<td>11.2</td>
<td>5</td>
<td>120</td>
<td>71.6</td>
<td>40.5</td>
<td>61</td>
<td>71</td>
</tr>
<tr>
<td>5/67/M</td>
<td>50</td>
<td>48.9</td>
<td>3</td>
<td>240</td>
<td>77</td>
<td>43.8</td>
<td>73</td>
<td>89</td>
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<tr>
<td>7/63/M</td>
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<td>5</td>
<td>80</td>
<td>65.3</td>
<td>46</td>
<td>70</td>
<td>57</td>
</tr>
<tr>
<td>8/61/F</td>
<td>24</td>
<td>1.3</td>
<td>20</td>
<td>60</td>
<td>72</td>
<td>45.3</td>
<td>75</td>
<td>71</td>
</tr>
<tr>
<td>Mean</td>
<td>61.1</td>
<td>40.3</td>
<td>6.8</td>
<td>121.2</td>
<td>71.8</td>
<td>42.2</td>
<td>69.8</td>
<td>69.7</td>
</tr>
<tr>
<td>SEM</td>
<td>2.6</td>
<td>8.5</td>
<td>10.8</td>
<td>2</td>
<td>22.7</td>
<td>5</td>
<td>1.4</td>
<td>2.1</td>
</tr>
</tbody>
</table>

*M = male; F = female; FEF\textsubscript{25-75} = mid-expiratory phase of forced expiratory flow.
Generally, the 6-week course of treatment with inhaled beclomethasone dipropionate was well-tolerated, and no significant side effects, except for a casual report of hoarseness, were recorded. All patients completed the study. All patients underwent bronchoscopic procedures without any significant complication.

The amounts of bronchial lavage fluid recovered during the bronchoscopy procedures that were performed before and after treatment were similar (before, 34 ± 4 mL; after, 32 ± 5 mL; p > 0.5). After treatment, there was a reduction in the bronchial lavage levels of IL-8 (before, 1,603.4 ± 331.2 pg/mL; after, 1,192 ± 265.3 pg/mL; p = 0.01) and myeloperoxidase (before, 1,614.5 ± 682.3 µg/L; after, 511.2 ± 144.2 µg/L; p = 0.05) (Fig 1). No significant changes were observed in the levels of eosinophilic cationic protein and tryptase. The total number of bronchial lavage cells declined significantly after treatment compared with baseline values (250.6 ± 27.7 vs 186.3 ± 11.5 cells × 10³/mL; p = 0.04) (Fig 1). This reduction was associated with important changes in the proportions of cells present in the bronchial lavage specimens. The percentage of neutrophils was significantly reduced in lavage specimens after treatment (before, 59.7 ± 14.3%; after, 31.5 ± 10.1%; p = 0.01) (Fig 1), while, conversely, there was an increase in the percentage of macrophages (before, 38.1 ± 14.4%; after, 60.9 ± 9.6%; p = 0.03) and lymphocytes (before, 1.4 ± 0.5%; after, 6.2 ± 1.6%; p = 0.008). No significant changes were observed in the proportions of eosinophils or mast cells.

After the 6-week course of inhaled beclomethasone, we observed a significant change in the cough score (before, 1.5 ± 0.2; after, 0.8 ± 0.3; p = 0.01) and in the dyspnea score (before, 1.0 ± 0.2; after, 0.75 ± 0.3; p = 0.01). No differences were seen for the sputum score. Accordingly, there was a significant reduction in the total symptom score (before, 4.5 ± 0.6; after, 1.4 ± 0.5; p = 0.01). During bronchoscopy, we observed after treatment a significant reduction in the erythema score (before, 2.0 ± 0.18; after, 1.5 ± 0; p = 0.01) and in the secretion score (before, 2.0 ± 0.32; after, 1.0 ± 0.18; p = 0.03). No significant differences were seen for the edema and fragility scores. Accordingly, there was a significant reduction in the total bronchitis index (before, 8.5 ± 0.8; after, 5.5 ± 0.7; p = 0.007).

A comparison of the functional data obtained before and after the 6-week treatment with inhaled beclomethasone showed no significant differences for the values of FEV₁ or of the midexpiratory phase of forced expiratory flow (not shown).

**Discussion**

By comparing airway cell and soluble mediator data that were obtained from stable patients with mild COPD who are current smokers before and after short-term treatment with inhaled, high-dose beclomethasone, the results of this study show that some parameters of airway inflammation, namely, the number and proportions of inflammatory cells and the levels of two important mediators of inflammatory processes, together with some clinical and endoscopic data, may change after this therapy, while no changes occurred in the results of lung function tests.

COPD comprises various diseases in which not only the pathologic, clinical, and functional features, but also the biological phenomena that cause and maintain airway inflammation, may differ between baseline (ie, nonexacerbated) and acute exacerbations or according to the degree of airway obstruction history of cigarette smoking.
In this context, our previous characterization of airway inflammation in patients who are current smokers with mild airflow obstruction prompted us to test the hypothesis that in this subpopulation of patients airway inflammation might be sensitive to inhaled steroids. Although smoking cessation is the only proven successful therapeutic intervention that is able to reduce the decline of lung function, even the best cessation program have high failure rates, and smoking cessation by itself may not be able to suppress airway inflammation. \cite{12,29,30,36} In these patients, IL-8 is one of the chemotactic factors that is produced in the airways in response to the stimulus of cigarette smoke. This chemokine is able to recruit inflammatory cells, mainly granulocytes, into the airways and to activate them to release inflammatory mediators, such as myeloperoxidase and eosinophilic cationic protein.\cite{2,5,6}

This picture of an active inflammatory process that is ongoing in the airways is one reason to study the biological response to inhaled steroids in these COPD patients. COPD usually is characterized by a slowly progressive and largely irreversible airflow limitation over decades. In this scenario, it is conceivable that if steroids work in patients with COPD, their anti-inflammatory activity would be required in patients with a relatively “young” airway inflammation who have already developed significant, but still mild/moderate, airflow limitation together with clinical symptoms of disease. In contrast, patients with more advanced disease and severe airflow limitation may have features of airway inflammation such as the type and number of inflammatory cells in bronchial mucosa and more pronounced remodeling in airway walls\cite{29,30,36} that could be less prone to respond to treatment with anti-inflammatory medication.

The results obtained in this study after steroid treatment are consistent with the known anti-inflammatory effects of steroids, namely, that steroids are able to modulate the expression of many cytokine genes, including IL-8, and to inhibit granulocyte chemotaxis and degranulation,\cite{39–41} and are in agreement with the only (to the best of our knowledge) similar study previously conducted.\cite{17} After short-term therapy with an inhaled steroid, Thompson et al\cite{17} observed a reduction in the number of cells and in the levels of albumin, lactoferrin, and lysozyme in lavage specimens. Other investigators evaluating patients' sputum after steroid treatment found a reduction in the chemotactic activity\cite{31} and no changes in the amounts of myeloperoxidase and eosinophilic cationic protein,\cite{31,32} while others observed lower numbers of neutrophils.\cite{33}

Thus, the results of our study and of previous studies suggest that the treatment of stable patients with COPD with inhaled steroids may induce a biological response, which may be associated with changes in some clinical and endoscopic data. However, steroid treatment did not change the result of any lung function test in our patients, as was the case in other short-term trials using inhaled steroids, particularly in those including subjects who were smokers.\cite{14,16,18} Since this was an open pilot study including a small number of subjects and not using a placebo control group, caution is needed in interpreting the results, as it is difficult to draw conclusions on the significance of the changes observed. In this context, although it is unlikely, it is possible that some of the subjective symptomatic improvements and improvements in endoscopic scores also may have been observed with a placebo intervention.

Our study was not meant to be a controlled clinical trial. In this context, the scarcity of published data demonstrating the effects of steroids on airway inflammation in patients with COPD (the only article with lavage data\cite{17} was published in 1992) is in contrast with the widely diffused treatment of COPD patients with steroids. On the other hand, this lack of sufficient background data is a major drawback in designing a controlled clinical trial. Thus, our work was designed to provide preliminary data on possible changes that inhaled steroid treatment may induce on some parameters of airway inflammation in patients with COPD.

In conclusion, the results of this study and the discrepancy between the biological and functional response to steroids would perhaps suggest the need for further, larger, and controlled studies that are designed to evaluate whether subgroups of patients can be identified in whom inhaled steroids may induce changes in airway inflammation as well as in some clinical and/or functional outcome parameters.

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