The effect of inhaled corticosteroids on bronchoalveolar lavage cells and IL-8 levels in stable COPD patients

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Summary Chronic obstructive pulmonary disease (COPD) is characterised by a chronic inflammatory process in the large and small airways, as well as in the lung parenchyma. Although the role of oral corticosteroids in the management of acute exacerbations of COPD is well documented, its role in stable COPD is not clear. We examined the anti-inflammatory effect of inhaled budesonide on the percentage of neutrophils and on interleukin-8 (IL-8) levels in bronchoalveolar lavage (BAL) and their correlation with spirometry and symptom scores. Twenty-six patients with stable COPD were randomised, in a double-blinded, placebo-controlled trial with either 800 \textmu g of inhaled budesonide or placebo for a 6-month period.

The budesonide-treated subjects had significant reductions in IL-8 levels in the BAL after therapy (mean ± SEM, 1.53 ± 0.72 at baseline vs. 0.70 ± 0.48 ng/ml at 6 months, \( P = 0.004 \)) and a reduction in the mean percentages of neutrophils (17.16 ± 2.67\% vs. 13.25 ± 2.28\% \( P = 0.002 \)). The improvement in sputum production was of borderline (\( P = 0.058 \)) significance but there was no improvement in lung function.

In stable patients with COPD, treatment with inhaled budesonide for a period of 6 months has a positive effect on markers of lung inflammation, as assessed by reduction in percentage neutrophils and IL-8 concentration in BAL.

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KEYWORDS
COPD; Interleukin-8; Bronchoalveolar lavage; Inhaled; Steroids; Budesonide

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Introduction

Chronic obstructive pulmonary disease (COPD) is one of the leading causes of death of increasing prevalence; it is expected to be the fourth leading cause of death worldwide within the next 20 years. Despite the enormous burden of the disease in health, economic and personal terms, there is lack of specific targeted treatments for this disease. Only smoking cessation and oxygen therapy have been shown to alter the progression of the disease.

COPD is characterised by a persistent airflow obstruction with reduction in the maximum expiratory flow. The pathogenesis of COPD is multifactorial, involving airway inflammation, protease–anti-protease imbalance, oxidative stress and recurrent infection. The inflammatory process, which contributes to the airway narrowing of the small airways, manifests as increased number of inflammatory cells such as neutrophils and CD8+ T lymphocytes; these cells have been implicated in the development and progression of COPD. Interleukin-8 (IL-8), one of the mediators that have a role in the pathogenesis of COPD, along with LTB4 and TNF-alpha, is a cytokine with potent neutrophil chemotactic and activation properties.

Both inhaled and oral corticosteroids have anti-inflammatory effects, causing improvements in symptoms and pulmonary function in the treatment of asthma. Although the role of oral corticosteroids in the management of exacerbations of COPD is well-documented, the role of inhaled steroids in stable COPD is not clear. A number of studies have been performed examining the effects of inhaled steroids variously upon measures of inflammation and of lung function, with conflicting results. Of those studies that investigated airway inflammation, we are only aware of two which evaluated lavage specimens, the others using sputum samples.

The aim of this study is to see if airway inflammation in COPD would be changed by a 6 months course of budesonide therapy, given at a dose of 800 mg daily, and to further assess whether these changes improved symptoms and lung function tests in these patients.

Materials and methods

Patients

Twenty-six patients with a medical history and clinical and radiological findings consistent with stable mild to moderate COPD, according to GOLD classification (8), were included in the study. Eight subjects, whose postbronchodilator FEV1 value was greater or equal than 80% of the predicted, were considered as mild COPD and 18 subjects whose FEV1 value was in the range between 50% and 80% of the predicted were considered as moderate (2a) COPD. All subjects had a smoking history of at least 20 pack-years and they had all ceased smoking at least 1 year prior to enrollment.

Inclusion criteria were: (1) FEV1/FVC < 70% and FEV1 > 50% the predicted value, (2) reversibility with inhaled-/β2-agonists (400 μg salbutamol) of less than 200 ml or less than 12% of predicted FEV1, (3) stable COPD defined as no acute exacerbation within the preceding 3 months, (4) no history of systemic disease or other pulmonary disease, (5) no therapy with inhaled or systemic corticosteroids within 3 months prior to entry into the study and (6) no history of asthma or atopy.

All of the patients were on therapy with inhaled salbutamol and ipratropium bromide. In nine patients, sustained-released theophylline was also being given.

Informed content was obtained from the patients and this study was approved by the Ethics Committee of the Ege University, Izmir, Turkey.

Study design

The study was a randomised, double-blinded and placebo-controlled design. Subjects received treatment with either budesonide 400 μg (Astra-Zeneca) twice a day or placebo, delivered by dry powder inhaler. Patients were randomised by a computer-generated, blinded randomisation list. The treatment group was randomly assigned and was balanced at a ratio of 1:1. There was no seasonal difference with regard to viral infections, as the intervention started at the beginning of April and ended in November for both groups.

Spirometric tests, BAL analysis with bronchoscopy were ascertained on entry into the study and after 6 months of treatment. The patients maintained weekly diary cards in which they noted any changes in symptoms from their baseline conditions. Dyspnoea was scored as "0" for no symptoms, "1" for one–two episodes of breathlessness daily, "2" for three or more episodes and "3" for breathless most of the time. Cough was scored as "0" for none, "1" for one–two episodes daily, "2" for three or more episodes or "3" for persistent cough. Sputum production was ranked as "0" for none, "1" production only on rising, "2" for occasional sputum production and "3" for frequent...
episodes. All patients were assessed every 2 months for the symptoms, treatment compliance and side effects. When patients noticed two of the following symptoms—increase in dyspnoea, sputum purulence, increased sputum volume, a cold or fever, they telephoned a member of the clinician team and were seen by a doctor within 24 h to detect the exacerbations.

Spirometry

Pulmonary function tests were performed by the standard method using a dry rolling-seal spirometer. Three technically adequate maneuvers were required and the best values for postbronchodilation FVC and FEV₁ was accepted.

Bronchoscopy

Trans-nasal fiberoptic bronchoscopy was performed using an Olympus flexible fiberoptic bronchoscope, following the guidelines of the National Institutes of Health. Premedication included atropine (0.5 mg-IM) administered 30 min before the procedure, with local upper airways anaesthesia with 5 ml of 2% Lidocaine. All bronchoscopies were performed in the morning about 10:00 am to avoid diurnal variations. To perform lavage, the bronchoscope was wedged into the segmental bronchus of the middle lobe and 100 cm³ of sterile warmed saline solution was infused. Fluid was gently aspirated immediately after the infusion had been completed and was collected in a sterile container. The fluid was immediately centrifuged at 500 g for 10 min. Supernatants were removed and frozen in 1 ml sterile polystyrene tubes at −80 °C.

IL-8 count

IL-8 concentrations were determined by two-site sandwich IL-8 specific enzyme-linked immunosorbent assay (ELISA-Chemikline). The concentration of IL-8 in the samples was calculated by comparison to the curve obtained with different concentrations of standards included in each kit. Tests were done twice for validation.

BAL cell counts

The cell pellet was washed with phosphate-buffered saline solution. Cells were resuspended in Hank’s balanced salt solution and counted using a haemocytometer chamber. Cytocentrifuges were stained by the May–Grünwald Giemsa method. The differential cell counts of macrophages, lymphocytes, neutrophils and eosinophils were made under light microscopy at ×400 magnification, counting approximately 300 cells. The cells were counted by our pathologist who was also blinded. All cell data are expressed as percentages.

Statistical analysis

Parametric data are expressed as the mean±SEM. Baseline comparability of the treatment and placebo groups was assessed for age, sex, smoking history and spirometry. Parametric data were compared using Student’s t test. Nonparametric data comparisons were made using Mann–Whitney U test between the two groups. Comparisons between baseline and end of treatment data from treatment and placebo group were made using Wilcoxon’s signed rank test (2-tailed). Correlations between different parameters were tested with Spearman’s rank test. In each case, a P value of <0.05 was considered significant.

Results

Initially a total of 26 ambulatory patients with a medical history and clinical and radiological findings consistent with stable COPD were included in the study. Three patients (two in the placebo group, one in the budesonide-treated group) were withdrawn from the study, as they suffered exacerbation of their disease and required systemic steroid therapy. One patient was excluded for failure to take the medication consistently. The results presented are an analysis of 22 subjects (12 budesonide-treated subjects and 10 placebo-treated subjects) who completed the study. The 6 months course of treatment with inhaled budesonide was well tolerated, with no significant side effects except for minor oral candidiasis in four patients. All patients underwent the bronchoscopic procedures without any complication (Table 1).

After the treatment period, the neutrophil percentage was significantly decreased in the budesonide-treated subjects. The mean neutrophil percentage before and after the budesonide treatment were 17.16±2.67% vs. 13.25±2.28%, respectively (P = 0.002) and the mean neutrophil percentage before and after the placebo treatment were 15.2±2.48% vs. 14.5±1.96%, respectively (P = 0.495). The mean BAL concentrations of IL-8 before and after the treatment period, in the budesonide group were 1.53±0.72 ng/ml, P = 0.004, respectively and in placebo group were 0.49±0.22 ng/ml,
P = 0.767, respectively (Table 2). When we considered the difference of IL-8 level and difference of neutrophil percentage between the groups, we also found that it was statistically significant. The mean IL-8 difference for the placebo and budesonide group, was respectively (mean ± SEM), 0.02 ± 0.17, and 0.83 ± 0.29 (P: 0.037). The mean neutrophil difference for the groups respectively was (mean ± SEM) 0.7 ± 0.84, and 3.9 ± 0.55 (P: 0.004).

One budesonide-treated patient had exceptionally high levels of IL-8 (Fig. 1); so there was a difference in baseline between the two groups of patients but the difference was not statistically significant. We found a decrease in IL-8 level in 11 patients in budesonide treated group, and we found a decrease in IL-8 level in four patients and an increase in IL-8 level in five patients in placebo group.

The budesonide-treated subjects demonstrated no statistically significant changes in the FVC and FEV1, nor did the placebo group (Table 3).

The amounts of BAL fluid recovered during the bronchoscopy procedures that were performed before and after treatment were similar and about 45% of the given saline solution was recovered. We also did not observe any difference between the groups.

There was statistically significant correlation between IL-8 and neutrophil count at baseline (for all 22 patients, r = 0.44, P = 0.041), the fall in IL-8 in the budesonide-treated arm correlated well.

**Table 1** Demonstrates the randomization of the patients.

<table>
<thead>
<tr>
<th></th>
<th>Budesonide-treated</th>
<th>Placebo-treated</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (male/female)</td>
<td>12 (11/1)</td>
<td>10 (7/3)</td>
<td>0.20*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.91 ± 1.68</td>
<td>65.9 ± 2.24</td>
<td>0.73</td>
</tr>
<tr>
<td>Smoking history pack-years</td>
<td>45.58 ± 6.16</td>
<td>44.4 ± 6.37</td>
<td>0.9</td>
</tr>
<tr>
<td>Spirometry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC (ml)</td>
<td>2500 ± 151.7</td>
<td>1890 ± 199.69</td>
<td>0.02†</td>
</tr>
<tr>
<td>FEV1 (ml)</td>
<td>1608 ± 150.48</td>
<td>1180 ± 147.42</td>
<td>0.06</td>
</tr>
<tr>
<td>FEV1 (%predicted)</td>
<td>61.1 ± 2.7</td>
<td>57.3 ± 3.1</td>
<td>0.56</td>
</tr>
<tr>
<td>FEV1/FVC %</td>
<td>64.4 ± 1.9</td>
<td>62 ± 2.0</td>
<td>0.43</td>
</tr>
<tr>
<td>Bronchoalveolar lavage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8 (ng/ml)</td>
<td>1.53 ± 0.72</td>
<td>0.488 ± 0.223</td>
<td>0.23</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>17.16 ± 2.67</td>
<td>15.20 ± 2.48</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.

* Mann–Whitney U test.
† Significant P value for FVC.

**Table 2** Bronchoalveolar lavage cellularity and IL-8 level.

<table>
<thead>
<tr>
<th></th>
<th>Budesonide-treated</th>
<th>Placebo-treated</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophages (%)</td>
<td>76.66 ± 3.0059</td>
<td>82.42 ± 2.28</td>
<td>0.003</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>17.167 ± 2.67</td>
<td>13.25 ± 2.28</td>
<td>0.002</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>7.0 ± 1.49</td>
<td>5.17 ± 0.93</td>
<td>0.031</td>
</tr>
<tr>
<td>IL-8 (ng/ml)</td>
<td>1.533 ± 0.72</td>
<td>0.703 ± 0.486</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.
with the fall in neutrophil count \( r = 0.75, P = 0.013 \) (Fig. 2). The fall in lymphocyte count in the budesonide-treated arm did not correlate with the fall in IL-8.

Analysis of symptoms showed that there was no significant change in cough or dyspnoea score between baseline and 6 months in either budesonide-treated or placebo groups. The budesonide-treated group showed an improvement in sputum production which just fell short of statistical significance (improved in six patients, worse in one, unchanged in five, \( P = 0.058 \)): the placebo arm showed no significant change in sputum production.

**Discussion**

Our results suggest that treatment with inhaled budesonide for a period of 6 months has a positive effect on lung inflammation, as assessed by percentage of neutrophils and IL-8 concentration in bronchoalveolar lavage (BAL), but this therapy failed to improve lung function or daily symptoms in patients with stable COPD. Like previous studies, we decided to document the effect of inhaled corticosteroids on symptomatic and functional parameters for 6 months, to allow time for the

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**Table 3** Spirometric changes.

<table>
<thead>
<tr>
<th></th>
<th>Budesonide-treated</th>
<th>Placebo-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
</tr>
<tr>
<td>FEV(_1) (ml)</td>
<td>1608.33 ± 150.48</td>
<td>1662.5 ± 185.77</td>
</tr>
<tr>
<td>P</td>
<td>0.84</td>
<td>P = 0.047</td>
</tr>
<tr>
<td>FVC (ml)</td>
<td>2500 ± 151.75</td>
<td>2525 ± 191.93</td>
</tr>
<tr>
<td>P</td>
<td>0.84</td>
<td>P = 0.69</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.
reduced inflammation to modulate these parameters.

Our study has certain limitations. Randomisation was not perfect; in particular, there was a difference in mean IL-8 at baseline in the two arms, although this difference was not statistically significant. The higher mean IL-8 in the budesonide-treated group is largely due to an exceptionally high value in one patient (Fig. 1). The statistical significance of the change in IL-8 is retained if this one patient is excluded from the analysis. There was also a statistically significant difference in baseline FVC between the two groups. We were not able to ascertain total cell counts in the lavage specimens. The strengths of our study were that it examined BAL, while most previous studies have examined sputum. BAL should give a better assessment of small airways and alveolar inflammation. The only two previous studies of BAL have been small (Balbi examined only eight patients and Thompson 20 in the treated arm,7,9). Ours was a double-blind, placebo-controlled study. In our study, both subjective symptoms and objective lung function were determined.

Our study supported the previous findings of Balbi et al.7 that steroids can reduce concentrations of IL-8 in BAL. Llewellyn-Jones11 also showed a reduction in chemotactic activity of sputum after treatment with fluticasone. In contrast, both Culpitt et al.12 and Keatings et al.13 did not show any improvement across a range of inflammatory markers, including IL-8, in sputum. We demonstrated a significant reduction in neutrophil count in the budesonide-treated arm. This confirms previous results on BAL7 and sputum.9,10 The correlation between the change in IL-8 and change in neutrophil count, between baseline and 6 months in the budesonide-treated group, is consistent with the role of IL-8 in recruiting neutrophils and suggests that the reduced neutrophil count induced by steroid therapy is mediated by a reduction in IL-8, although it does not prove causality.

Of the subjective symptoms assessed, cough, dyspnoea and sputum production, we only found a possible improvement in sputum production, falling just short of statistical significance. Katsura and Kida14 showed improvements in both cough and sputum production in elderly patients. The 'Inhaled Steroids in Obstructive Lung Disease (ISOLDE) Study’ showed a moderation of the annual rate of deterioration of subjective symptoms as assessed by the St. George’s Respiratory Questionnaire but details of the effects on individual symptoms have not been published.15 We failed to demonstrate any improvement in FVC or FEV1 after budesonide treatment. Results of small studies have been mixed: both Bourbeau et al.16 and Confalonieri et al.9 have also failed to show an improvement in FEV1, while Thompson et al.6 and Wiener et al.17 both showed improvements. Two studies, larger than ours, showed small but significant improvements in FEV1 over 6 months.18,19 However, three studies, each sustained for a period of 3 years (ISOLDE, European Respiratory Society Study on COPD (EUROSCOP), and Copenhagen City Lung Study), failed to show a significant amelioration in the temporal decline in FEV1 over this longer period. Lack of an improvement in our, and other studies, may be due to sub-optimal dosing; it has been suggested on the basis of a meta-analysis that budesonide is required at a dose of 1.6 mg for an optimum effect.22

Conclusion

Our study supports the published data showing decrease in the inflammatory markers in BAL in stable COPD patients using long-term inhaled steroids, an improvement in sputum production of borderline significance but fails to show any improvement in cough, dyspnea or pulmonary function. The decision, therefore, to use inhaled corticosteroids in these patients is therefore still a matter of clinical debate with assessment of both objective and subjective parameters.

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References


