Effects Of Inhaled and Oral Glucocorticoids on Inflammatory Indices in Asthma and COPD

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The role of glucocorticoids in the treatment of chronic obstructive pulmonary disease (COPD) is controversial. We have previously described high numbers of neutrophils and high concentrations of the inflammatory cytokines interkeukin-8 (IL-8) and tumor necrosis factor-α (TNF-α), and of the cell activation markers eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), myeloperoxidase (MPO), and human neutrophil lipocalin (HNL) in COPD patients as compared with controls, and have postulated that the cytokines TNF-α and IL-8 play a role in propagating the inflammatory response in COPD. We have now studied the effects of inhaled and oral glucocorticoids on these inflammatory indices in induced sputum. Initially, we studied the effect of a 2-wk course of inhaled budesonide (800 mg twice daily for 2 wk) in 13 patients with severe COPD (mean FDV_1: 35% predicted). There was no clinical benefit in either lung function or symptom scores, and no significant change in the inflammatory indices as measured by total and differential cell counts and concentrations of TNF-α eosinophil activation markers ECP and EPO, and neutrophil activation markers MPO and HNL. Because the lack of anti-inflammatory effect might have been due to poor drug delivery as a result of severe airflow limitation, we undertook a study examining the antiinflammatory effect of oral prednisolone (30 mg daily for 2 wk) in patients with COPD and undertook the same measurements in 10 patients with atopic asthma. Sputum eosinophil numbers, ECP, and EPO were significantly reduced in the asthmatic patients but were not modified in COPD. This confirms the clinical impression that inhaled steroids have little antiinflammatory effect, at least in the short term in this group of patients, and suggests that the inflammatory process in COPD is resistant to the antiinflammatory effect of glucocorticoids. Keatings VM, Jatakanon A, Worsdell YM, Barnes PJ. Effects of inhaled and oral glucocorticoids on inflammatory indices in asthma and COPD. AM J RESPIR CRIT CARE MED 1997;155:542–548.

Chronic obstructive pulmonary disease (COPD) is clinically characterized by progressive airflow limitation and intermittent exacerbations, usually precipitated by infection. The main etiologic factor is cigarette smoking, and the cessation of smoking is the only intervention that causes an improvement in the natural history of the condition (1, 2). Long-term domiciliary oxygen therapy prolongs life in subjects with cor pulmonale secondary to COPD, and its beneficial effect on survival is presumably due to effects on the pulmonary circulation (3).

Both asthma and COPD are characterized morphologically by the presence of airway inflammation (4–7). In asthma, there are increased numbers of mast cells and eosinophils, and degranulation of these cells has been shown to occur following allergen challenge. There is increased expression of cytokines interleukin (IL)-2, IL-3, IL-4, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) in asthmatic patients compared with nonasthmatic controls (8). These cytokines contribute to the recruitment and activation of eosinophils into the airways. Cytokine gene expression is known to be reduced both *in vitro* (9) and *in vivo* (10) in response to corticosteroids, and this has been postulated as the mechanism of action of corticosteroids in this condition (11). In COPD, studies have shown a predominantly neutrophilic infiltrate (12, 13), and it is thought that the consequent protease burden, in addition to reduced antiprotease capacity, is contributory to the development of irreversible airflow obstruction. In addition to the predominantly neutrophilic infiltrate in the airway lumen, a lymphocytic infiltrate has been demonstrated in the airway submucosa and alveolar parenchyma (6, 7, 14). The mechanisms for this inflammatory response are not fully known. Recently, increased expression of the adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and E-selectin in the endothelium has been demonstrated in bronchial biopsy of patients with COPD (15), and there are increased concentrations of these adhesion molecules in the serum of patients with COPD compared with normal control subjects (16). We have previously described high concentrations of the cytokines IL-8 and tumor necrosis factor-α (TNF-α) in induced sputum from patients with COPD (17), and these cytokines may be involved in the recruitment of neutrophils and upregulation of adhesion molecules on the endothelium (18, 19). If steroids do play a role in the treatment of COPD, it is reasonable to suggest that they act via downregulation of the cytokines and adhesion molecules, with a consequent reduction in cell migration and activation. We have also found high concentrations of the eosinophil activation markers eosinophil cationic protein (ECP) and eosin-

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ophil peroxidase (EPO), and of the neutrophil granule proteins myeloperoxidase (MPO) and human neutrophil lipocalin (HNL) in induced sputum compared with the other subject groups, suggesting that a vigorous inflammatory process is ongoing in the airways of subjects with COPD (20).

Glucocorticoids, both inhaled and oral, are established as effective treatment in asthma, causing improvement in clinical symptoms and spirometry within a short period of time (21, 22). In COPD, however, no such short-term improvement is seen, and the unresponsiveness of FEV_1 is often used as a defining criterion for the exclusion of a diagnosis of asthma (22–24). But the role of glucocorticoids in the management of COPD is controversial (22, 23). Some studies have suggested that long-term oral prednisolone can reduce the deterioration of FEV_1, and clinical trials of the effect of long-term inhaled steroids are currently underway (24). The findings in clinical studies of corticosteroids in COPD are contradictory. Studies using inhaled steroids have shown a marginal increase in FEV_1 over a 3-wk period in patients receiving high-dose inhaled steroids (25), and other studies have suggested a reduction in long-term decline in FEV_1 in patients taking oral prednisolone at a dose of more than 7.5 mg/d (26, 27). These studies examined patients with a high level of reversibility following a use of bronchodilator, suggesting that many asthmatic subjects must have been included. The clinical effects of a 2-wk course of prednisolone compared with placebo showed that neither skin prick tests, radioallergosorbent test (RAST), sputum or blood eosinophilia, age, nor bronchodilator response predicted a response to the steroid. One study has shown a selective increase in the protease inhibitor alpha-1 chymotrypsin following 7 d of treatment with prednisolone (28).

If the inflammation in COPD is considered to play a central pathogenic role in the condition, as in asthma, a reduction in the inflammation may accompany a clinical benefit, and if steroid responsiveness is present, it should be apparent within a short period of time. We wished to determine whether the inflammation in COPD is sensitive to the effect of inhaled or oral glucocorticoids, and whether any change is related to clinical benefit. We initially examined the effect of a 2-wk period of treatment with inhaled budesonide (800 μg twice daily) and placebo on both pulmonary function and markers of inflammation in induced sputum in patients with severe COPD. Because there may be problems with inhaled steroids reaching the lower airways in patients with severe airflow obstruction, we then went on to study the effect of oral glucocorticoids (prednisolone 30 mg daily for 2 wk) on the same parameters. In addition, we included patients with asthma as a positive control for the effects of steroids on inflammatory indices in induced sputum.

METHODS

Patients

Budesonide study. We recruited 15 patients aged 45 to 78 yr (nine males and six females) with stable COPD from the outpatient departments of the Royal Brompton Hospital, St. George's Hospital, and Chelsea and Westminster Hospital, London, and a local general practice. Inclusion criteria for entry were $FEV_1/FVC < 70\%$, $FEV_1 < 70\%$ predicted value, reversibility with inhaled albuterol of < 10% of predicted FEV_1, and a smoking history of at least 10 pack-yr. Patients with any history of asthma or variability in symptoms, and patients who had taken inhaled or oral steroids or had suffered a respiratory tract infection or exacerbation of their airways disease in the previous 6 wk were excluded.

Prednisolone study. Eleven patients with mild atopic asthma were recruited from the outpatient clinic of The Royal Brompton Hospital and from among a panel of volunteers for clinical studies, and nine subjects with COPD were recruited as described earlier. Inclusion criteria for asthmatic patients included nonsmokine subjects with stable asthma with a demonstrated reversibility following 200 μg inhaled albuterol > 15% of initial FEV_1 or > 10% predicted FEV_1. All of the subjects were atopic, with positive skin prick testing to at least one of four common aeroallergens (Grass pollen, cat dander, *Dermatophagoides pteronyssinus*, *Aspergillus fumigatus*). Subjects were excluded if they had taken inhaled or oral steroids or had suffered an exacerbation of asthma or respiratory tract infection within the previous 8 wk.

All subjects gave written informed consent, and the studies were approved by the ethics committees of the Royal Brompton Hospital.

Study Design

Both studies had a sequential single-blind, crossover design. Each study had a 3- to 7-d run-in period during which medications were kept stable, followed by a 2-wk placebo period and a 2-wk treatment period. In the inhaled steroid study the treatment period consisted of budesonide 800 mg twice daily (Astra Draco, Lund, Sweden), and in the prednisolone study, oral prednisolone 30 mg daily. The clinical parts of the studies were single-blind, but all differential cell counting and assays were carried out in a double-blind fashion. Each subject kept a diary card during the study, detailing daily morning peak flow and use of reliever inhaler (in all cases albuterol). Subjects with COPD were also instructed to grade daily shortness of breath score as fellows, 0 = none, 1 = mild with no effect on routine activity, 2 = moderate (affecting routine activity), and 3 = severe. Asthmatic subjects kept a daily score of wheeze, 0 = none, 1 = mild with no effect on routine activity, 2 = moderate, affecting routine activity, and 3 = severe.

At the end of the run-in and treatment periods, subjects attended the laboratory. At each visit, spirometric data were recorded at baseline and at 15 min following inhalation of 200 mg albuterol given via metered dose inhaler (MDI) with a spacing device (Volumatic, Allen and Hanburys, Greenford, Middlesex, UK). Diary cards were collected and a sputum induction was done. The sputum sample was analyzed for total and differential cell counts, and assayed for TNF-α, ECP, EPO, MPO, and HNL concentrations.

Sputum Induction and Processing

Sputum induction was done 15 min after inhalation of 200 μg albuterol via an MDI. The aim of the procedure was explained to the subject, who was instructed to mouthwash thoroughly with water prior to the induction. Subjects inhaled 3.5% saline at room temperature, nebulized via an ultrasonic nebulizer (De Vilbiss 99, De Vilbiss, Heston, UK) at maximum output. Subjects were encouraged to cough deeply at 5 min and at 3-min intervals thereafter. Sputum was collected into two polypropylene pots and saliva was discarded into a bowl. The initial sample of sputum was discarded. Following the sputum induction, spirometry was repeated. If the FEV_1 had fallen, the subject was required to wait until it had returned to baseline value. The sputum samples were kept at 4° C for not more than 2 h prior to further processing.

The volume of the sample was recorded and the sample was diluted with 2 ml of Hanks' balanced salt solution (HBSS) containing 1% dithiothreitol (DTT) (Sigma Chemicals, Poole, UK), and gently vortexed at room temperature. When homogeneous, samples were further diluted with HBSS and again vortexed briefly. They were then spun at 300 × *g* for 10 min, and the cell pellet was resuspended. the supernatant was decanted, aliquoted, and stored at −70° C for later assay for cytokines and cell activation markers. Total cell counts were done on a hemocytometer using Kimura stain, and slides were made with a cytospin (Shandon, Runcorn, UK) and stained with May-Grünwald-Giemsa stain for differential cell counts, which were done by an observer blind to the clinical characteristics of the subject.

Sputum Assays

TNF-α assay. TNF-α concentrations were measured with an amplified sandwich-type enzyme-linked immunosorbent assay (ELISA). Ninety-six-well microtiter plates (Greiner Labortecnik Ltd., Dursley, Gloucestershire, UK) were coated with 100 μl of mouse monoclonal anti-TNF-α antibody (Serotec, Oxford, UK), at a 1:400 dilution and left for 2 h at 37° C. Plates were then washed with phosphate-buffered saline (PBS) containing 0.05% vol/vol Tween and immediately treated with bovine serum albumin (BSA) 5% vol/vol for 30 min at 37° C. After further washing. TNF-α standards, quality controls, and samples were added to the plates and left for 18 h at 4° C. The plates were washed and incubated for 2 h at room temperature with 100 μl of rabbit anti-human TNF-α polyclonal antibody, washed again, and incubated for a further

TABLE 1

LUNG FUNCTION IN PATIENTS WITH COPD AFTER PLACEBO AND BUDESONIDE TREATMENT

Patient No.	FEV_1		FEV_1 pb		FVC		PEFR	
	Placebo	Budes.	Placebo	Budes.	Placebo	Budes.	Placebo	Budes.
1	0.78	0.57	0.77	0.73	1.98	1.98	ur	ur
2	0.68	0.65	0.57	0.73	1.69	1.8	ur	ur
3	0.64	0.65	0.76	0.76	1.45	1.49	ur	ur
4	0.59	0.69	0.83	0.78	0.98	1.42	ur	ur
5	2.63	2.37	2.94	2.95	4.48	4.35	436	464
6	0.58	0.62	0.76	0.75	1.32	1.44	ur	ur
7	1.63	1.58	1.68	1.74	3.04	2.35	253	223
8	0.79	0.98	0.77	0.98	2.16	1.98	191	181
9	0.84	0.9	0.89	0.9	2.28	2.1	234	235
10	2.05	2.05	2.25	2.2	2.73	2.8	435	461
11	0.87	0.9	1.05	1.27	2.28	2.45	139	128
12	0.91	0.96	0.88	1.02	4.14	4.2	172	167
13	0.47	0.4	0.48	0.52	1.04	1.2	146	120
Mean	1.04	1.02	1.13	1.18	2.34	2.30	250	247
SEM	0.19	0.17	0.21	0.20	0.32	0.30	45.6	52.4

Definition of abbreviations: FEV_1 = forced expiratory volume in first second; FEV_1 pb = FEV_1 following inhaled albuterol 200 μg; FVC = forced vital capacity; PEFR = mean morning peak expiratory flow rate during treatment; Budes. = budesonide; ur = unrecordable peak flow rate.

Data are for 13 patients with COPD.

2 h at room temperature with an alkaline phosphatase-conjugated donkey anti-rabbit polyclonal IgG antibody (diluted 1:2,000). Excess antibody was again washed off and plates were developed with a p-nitrophenyl phosphate assay kit (No. 50–80–00; KPL/Dynatech Laboratories Ltd., Billinghurst, Sussex, UK). The optical density of the wells was read using a plate photometer. The detection limit of the assay is 470 pmol.

IL-8 assay. IL-8 concentrations were measured with a competitive radioimmunoassay (RIA) (29). Human recombinant IL-8 was radiolabeled with ^{125}I. Samples were mixed with an equal volume of 22% polyethylene glycol/1% protamine sulfate, incubated for 1 h at 4° C, and centrifuged at 5,420 × *g* for 10 min at 4° C. The resulting supernatant fluid (100 μl) was mixed with 50 μl [^{125}I]-human IL-8 (0.5 ng). After 24 h incubation at room temperature, 50 μl of donkey anti-goat IgG antibody (1:30 dilution) was added and incubated for 16 h at room temperature. After addition of 1 ml PBS containing 0.1% sodium azide and immediate centrifugation, the supernatant fluid was removed by suction and antibody-bound radioactivity was counted in a gamma counter. All samples were assayed in duplicate with human recombinant IL-8 standards. The lower limit of detection was 197 pmol IL-8, and nonspecific binding was 5.0%.

ECP assay. ECP concentrations were measured with a commercially available RIA, a generous gift from Pharmacia Diagnostics AB, Upsala, Sweden.

EPO assay. Anti-EPO monoclonal antibody was coupled to ImmunoCAP (Pharmacia Diagnostics). Samples and standards were added and incubated for 30 min at room temperature. After washing, anti-EPO labeled with β-galactosidase was added. Following incubation for 2.5 h at room temperature, suspensions were washed and the substrate 4-methylumbelliferyl-β-D-galactoside was added. Fluorescence was measured after 10 min, a standard curve was constructed, and EPO concentrations were determined. Cross-reactivity with ECP was < 0.3% and with MPO < 0.01%.

MPO assay. MPO concentrations were determined with a commercially available RIA (Pharmacia Diagnostics) according to the manufacturer's instructions. Cross-reactivity with ECP and MPO was < 0.1%.

HNL assay. Monoclonal antibodies (mAb) were raised in rats immunized with HNL. One mAb was coupled to ImmunoCAP (Pharmacia Diagnostics). Samples and standards were added and incubated for 30 min at room temperature. After washing, β-galactosidase-labeled anti-HNL was added. Following incubation for 2.5 h at room temperature, suspensions were washed and 4-methylumbelliferyl-β-D-galactoside was added. Fluorescence was measured and HNL concentrations were determined.

Statistical Analysis

Data are expressed as the mean ± SEM. Statistical analysis of comparisons between groups was performed with Student's *t* test for parametric data and with Wilcoxon's rank sum test for nonparametric data. Two-tailed tests were performed, and a p value of < 0.05 was considered significant.

RESULTS

Budesonide Study

Clinical parameters. Patients had severe airflow limitation, with a mean FEV_1 of 35.1 ± 1.3% predicted. All subjects were nonatopic, with negative results on skin prick testing to four common aeroallergens. Mean cigarette smoking history was 48 ± 2 pack-yr, and six subjects were current smokers. Two subjects withdrew from the study (one due to work commitments and another who did not wish to have a repeat sputum induction). All subjects produced an adequate specimen of sputum. Mean FEV_1 was 0.93 ± 0.18 L at baseline, 1.04 ± 0.19 L with placebo, and 1.02 ± 0.17 L with budesonide (i.e., no difference between placebo and active treatment periods). Post-albuterol FEV_1 was 1.09 ± 0.27 L, 1.13 ± 0.21 L, and 1.18 ± 0.20 L at baseline, on placebo, and active treatment periods, respectively (Table 1).

Eight of the 13 patients had recordable peak flows. Mean peak flow in these patients was 250 ± 45.6 L/min in the placebo period and 247 ± 52.4 L/min during treatment with budesonide.

Use of reliever medication was 2.3 ± 0.3 puffs/d on placebo and 2.13 ± 0.4 on budesonide, (p = NS). Shortness-of-breath scores were 1.18 ± 0.3 on placebo and 1.34 ± 0.4 on budesonide, (p = NS).

Inflammatory indices. Total and differential cell counts did not change from baseline on placebo or active treatment (Table 2). The eosinophil number was skewed by one patient (Subject 3) whose eosinophil count was initially high (9.6%) and fell to zero following budesonide.

There was no significant change in ECP, EPO, MPO, or HNL after placebo or budesonide treatment periods. Similarly, the concentration of TNF-α remained unchanged throughout the study.

Prednisolone Study

One asthmatic subject withdrew from the study during the placebo limb due to work commitments, and one subject with COPD withdrew due to failure to fully understand how to fill in the diary card. Results therefore relate to 10 asthmatic sub-

TABLE 2

INFLAMMATORY INDICES IN INDUCED SPUTUM IN COPD AFTER TREATMENT WITH BUDESONIDE AND PLACEBO

	Baseline	Placebo	Budesonide	p Value
Total cell count/ml	6.3 ± 2.0	5.7 ± 2.0	4.8 ± 1.9	NS
Macrophages, %	28.5 ± 6.0	27.6 ± 3.7	27.5 ± 5.1	NS
Neutrophils, %	67.9 ± 6.3	69.9 ± 4.5	69.9 ± 5.1	NS
Eosinophils, %	2.7 ± 1.2	2.0 ± 1.1	1.0 ± 0.4	NS
Lymphocytes, %	0.9 ± 0.6	1.4 ± 0.1	1.6 ± 0.3	NS
TNF-α, pg/ml	760.2 ± 119	463.5 ± 35.7	715.5 ± 90.8	NS
IL-8, nM	3.3 ± 1.6	3.5 ± 2.0	2.1 ± 1.1	NS
ECP, μg/L	835.8 ± 0.40	730.3 ± 0.29	910.5 ± 0.21	NS
EPO, μg/L	262.4 ± 53.2	85.4 ± 15.1	41.9 ± 5.1	NS
MPO, mg/L	8.05 ± 1.49*	4.17 ± 0.58	2.97 ± 0.39	NS
HNL, mg/L	10.48 ± 1.02	8.98 ± 0.67	8.47 ± 0.68	NS

Definition of abbreviations: TNF-α = tumor necrosis factor-α; IL-8 = interleukin-8; ECP = eosinophil cationic protein; EPO = eosinophil peroxidase; MPO = myeloperoxidase; HNL = human neutrophil lipocalin.

Mean ± SEM values of 13 patients are shown.

jects and eight subjects with COPD. The mean age of the asthmatic patients was 29.8 ± 3.4 yr and their mean FEV_1 was 95.9 ± 5.7% predicted. The patients with COPD had a mean age 64.1 ± 5.1 yr and FEV_1 of 48.0 ± 6.8% predicted.

Clinical parameters. Lung function and diary card scores for both patient groups are shown in Table 3. There was a significant increase in FEV_1 and morning peak flow following prednisolone treatment in asthmatic patients, and the use of reliever inhaler was reduced significantly from 1.2 puffs/d during the placebo period to 0.5 puffs/d during treatment with prednisolone ($p < 0.05$). In contrast, there were no significant changes in lung function, peak flow, or shortness-of-breath score in the subjects with COPD. There was no difference in use of reliever inhaler with placebo and prednisolone treatment (2.9 ± 0.4 and 2.8 ± 0.3 puffs/d with placebo and prednisolone, respectively).

Differential cell counts. Differential cell counts are shown in Tables 4 and 5. Neutrophil counts were significantly higher in the COPD patients (mean 61.56 ± 2.5%, compared with 17.95 ± 1.20% in the asthma patients at baseline. Eosinophils were significantly higher in the asthma patients, at 6.66% ± 0.98%, than in the patients with COPD, at 0.58 ± 0.11%. ($p < 0.0001$). Changes in eosinophil numbers with treatment are shown in Figure 1. In the asthma patients there was a statistically significant decrease in eosinophils following treatment with prednisolone as compared with placebo, whereas in the COPD patients eosinophil numbers did not change after either the treatment or placebo period. In both groups of patients, numbers of other cell types were not altered following treatment with prednisolone.

Markers of cell activation. ECP concentrations were similarly elevated in both subject groups. In subjects with asthma, there was a significant decrease in ECP concentrations after prednisolone ($p < 0.05$), with no effect after placebo. In the patients with COPD, however, the concentration of ECP did not change following either treatment. Similarly, there was a significant decrease in EPO after steroid treatment in the asthma patients but this was not significant in COPD (Figure 2).

Although there was a decrease in concentrations of MPO and HNL in both patient groups after prednisolone, this did not reach significance (Tables 4 and 5).

There was no change in TNF-α concentrations in either the asthmatic or the COPD group of subjects following treatment with prednisolone as compared with placebo (Tables 4 and 5).

DISCUSSION

Our study has confirmed the irreversibility of chronic airflow limitation and lack of response to short-term steroid therapy. In

TABLE 3

SPIROMETRIC VALUES FOR PATIENTS WITH ASTHMA AND COPD FOLLOWING PLACEBO AND PREDNISOLONE

Patient No.	FEV_1		FEV_1 pb		FVC		PEFR	
	Placebo	Pred.	Placebo	Pred.	Placebo	Pred.	Placebo	Pred.
Asthma								
1	4.2	4.8	4.77	4.95	5.45	5.5	623	634
2	3.31	3.41	3.57	3.61	3.97	3.96	490	500
3	4.7	5.11	4.88	5.18	5.75	5.95	590	663
4	2.66	2.45	2.95	2.86	3.42	3.5	353	364
5	4.62	4.94	4.9	5.2	5.73	5.26	644	660
6	3.46	3.5	3.89	3.92	4.16	4.17	493	582
7	2.86	3.12	3.18	3.55	3.93	4.35	398	510
8	5.61	5.69	5.75	5.8	6.42	6.5	729	724
9	3.38	3.4	3.65	3.99	4.76	4.7	501	525
10	2.73	3.13	2.65	3.29	3.43	3.52	396	408
Mean	3.75	3.96*	4.02	4.24*	4.702	4.741	521	557*
SEM	0.32	0.32	0.34	0.41	0.36	0.34	41	39
COPD								
11	0.72	0.71	0.75	0.95	1.63	2.1	232	234
12	0.6	0.46	0.46	0.31	1.95	2.17	ur	ur
13	1.88	2	2.10	2.19	2.62	2.8	443	453
14	0.84	0.59	0.73	0.77	2.98	3.4	146	146
15	0.98	0.9	1.02	0.98	3.2	3.88	205	197
16	0.98	0.92	1.11	0.92	1.98	1.84	213	226
17	1.79	2.5	1.65	2.44	2.25	3.05	450	478
18	2.38	2.28	2.65	2.57	3.65	3.4	389	373
Mean	1.27	1.30	1.31	1.39	2.53	2.83	297.2	301.5
SEM	0.25	0.32	0.29	0.33	0.27	0.28	48.5	50.8

Definition of abbreviations: FEV_1 = forced expiratory volume in first second; FEV_1 pb = FEV_1 following inhaled albuterol 200 μg; ur = unrecordable peak flow rate; Pred. = prednisolone; Data are for 10 patients with asthma and eight patients with COPD.

* $p < 0.05$ compared with placebo value.

TABLE 4

TOTAL AND DIFFERENTIAL CELL COUNTS, CELL ACTIVATION MARKERS, AND CONCENTRATIONS OF TNF-α IN ASTHMATIC SUBJECTS AT BASELINE AND AT END OF TREATMENT PERIODS (PREDNISOLONE STUDY)

	Baseline	Placebo	Prednisolone	p Value
Total cell count/ml	1.48 ± 0.12	1.34 ± 0.12	0.81 ± 0.07	NS
Macrophages, %	74.11 ± 1.68	60.15 ± 2.16	67.19 ± 1.77	NS
Neutrophils, %	17.95 ± 1.20	31.63 ± 1.68	30.89 ± 1.68	NS
Eosinophils, %	6.66 ± 0.98	7.28 ± 1.04	0.99 ± 0.25	$p < 0.05$
Lymphocytes, %	0.42 ± 0.04	0.17 ± 0.04	0.15 ± 0.04	NS
TNF-α, pg/ml	1,743 ± 193.6	1,760 ± 228	2,049 ± 246	NS
ECP, μg/L	687.0 ± 87.1	871.6 ± 121.8	80.2 ± 14.2	$p < 0.05$
EPO, μg/L	59.2 ± 6.2	86.4 ± 5.5	16.4 ± 3.2	$p < 0.05$
MPO, mg/L	0.6 ± 0.1	1.0 ± 0.1	0.2 ± 0.05	NS
HNL, mg/L	3.4 ± 0.3	1.7 ± 0.1	1.8 ± 0.2	NS

Definition of abbreviations: TNF-α = tumor necrosis factor-α; ECP = eosinophil cationic protein; EPO = eosinophil peroxidase; MPO = myeloperoxidase; HNL = human neutrophil lipocalin.

the patients with COPD, there were no significant improvements in any of the clinical parameters studied. Although the study could not be fully double-blind for logistical reasons, the cell counts and protein assays were all done in a double-blind fashion. The previous demonstration of a neutrophil influx and high concentrations of TNF-α and cell activation markers in the airways indicates an active inflammatory process in COPD, suggesting that steroids may be beneficial. TNF-α has been implicated in the pathogenesis of asthma, and we have previously found high concentrations in COPD, suggesting that this cytokine is also involved in the inflammation in COPD. Although steroids do not change the airway function and symptoms in these patients, it is possible that an effect on inflammatory indices may be observed and that this could have long-term clinical effects. It has previously been shown that ECP concentrations in asthma are reduced following a 2-wk treatment period with prednisolone (30). This study confirms these findings and shows that another marker of eosinophil activation, EPO, can be reduced by treatment with steroids. It has been suggested that steroids work in asthma through reduction of cytokine gene expression, thus reducing the cytokine effects on adhesion molecule expression and chemotaxis, resulting in a reduced inflammatory influx. This study shows no effect of prednisolone on levels of TNF-α in asthma, despite *in vitro* evidence that TNF-α production from monocytes is reduced by dexamethasone and budesonide (31). Similarly, IL-8 was not inhibited after inhaled glucocorticoids in patients with COPD, despite the fact that glucocorticoids inhibit IL-8 transcription in human airway epithelial cells *in vitro* (32). TNF-α and IL-8 may be less sensitive to inhibition by steroids than other cytokines such as regulated on activation, normal T cell expressed and secreted (RANTES) and IL-5, which are thought to play an important part in eosinophil chemotaxis in asthma. Eosinophil survival is also reduced following treatment as a result of increased eosinophil apoptosis, probably due to inhibitory effects of glucocorticoids on growth factors such as IL-5 and GM-CSF (33). Because there is no evidence that glucocorticoids actually reduce degranulation of eosinophils, the reduction in granule proteins is secondary to reduced numbers of eosinophils rather than to reduced production of ECP and EPO by the cell. The lack of reduction of neutrophil numbers and markers clearly demonstrates a difference in steroid effects on eosinophils and neutrophils. Steroids are known to cause a circulating neutrophilia, and it has been shown that neutrophil apoptosis is reduced *in vitro* by treatment with glucocorticoids (34), thus prolonging the survival of neutrophils in tissues and reducing their clearance by macrophages. The initial study with inhaled glucocorticoids gives the impression that inhaled steroids are of little value in modifying inflammation in COPD, but because these patients had severe airflow limitation, we considered that the lack of effect might have been due to poor deposition of the drug in the airways. The second study, using prednisolone as the active treatment, was therefore undertaken and a group of asthmatic patients recruited as a positive control to demonstrate that our method of sputum induction was a sensitive tool with which to detect the effect of intervention.

ECP and EPO concentrations were high in the patients with

TABLE 5

TOTAL AND DIFFERENTIAL CELL COUNTS, CELL ACTIVATION MARKERS AND CONCENTRATIONS OF TNF-α IN PATIENTS WITH COPD AT BASELINE AND AT END OF TREATMENT PERIODS (PREDNISOLONE STUDY)

	Baseline	Placebo	Pred.	p Value
Total cell count/ml	5.62 ± 1.32	5.25 ± 0.78	5.78 ± 0.88	NS
Macrophages, %	37.76 ± 2.49	34.70 ± 1.74	29.50 ± 3.47	NS
Neutrophils, %	61.56 ± 2.5	65.0 ± 1.73	70.21 ± 3.44	NS
Eosinophils, %	0.58 ± 0.11	0.68 ± 0.10	0.20 ± 0.02	NS
Lymphocytes, %	0.1 ± 0.02	0.25 ± 0.06	0.03 ± 0.01	NS
TNF-α, pg/ml	550 ± 20	496 ± 36	624 ± 47	NS
ECP, μg/L	575.6 ± 87.6	677.2 ± 202.2	989.2 ± 234.8	NS
EPO, μg/L	70.0 ± 13.7	33.1 ± 5.8	90.0 ± 2.5	NS
MPO, mg/L	10.3 ± 3.1	8.6 ± 2.1	5.0 ± 1.2	NS
HNL, mg/L	17.8 ± 3.9	11.7 ± 1.9	11.5 ± 1.4	NS

Definition of abbreviations: TNF-α = tumor necrosis factor-α; ECP = eosinophil cationic protein; EPO = eosinophil peroxidase; MPO = myeloperoxidase; HNL = human neutrophil lipocallin; Pred. = prednisolone.

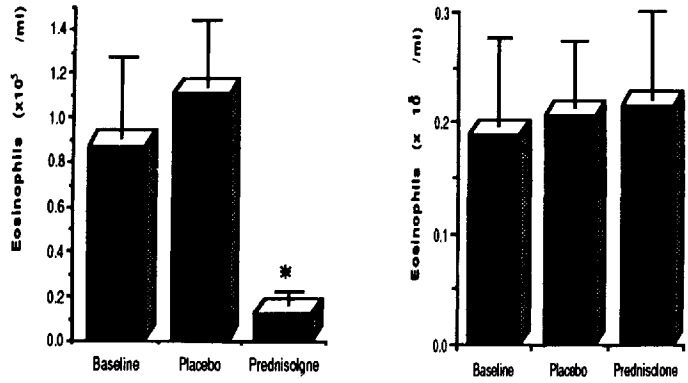

Figure 1. Changes in eosinophil numbers following treatment periods in asthmatic patients (*left panel*) and subjects with COPD (*right panel*). *$p < 0.05$.

COPD, and correlated with eosinophil numbers, suggesting that the proteins are derived from highly activated eosinophils. In contrast to asthma, however, concentrations of ECP and EPO were unchanged following treatment with prednisolone. Given that glucocorticoids increase neutrophil survival, it is not surprising that they do little to modify inflammation in COPD, but the lack of inhibition of eosinophil activation by prednisolone in COPD is not readily explained. It does suggest that there is at least one steroid-unresponsive step in the development of this process that is different from the eosinophil activation process in asthma. This may in part explain the relative resistance of COPD to treatment with steroids.

Sputum induction was used in this study because it was a method of ensuring that subjects were able to produce a sample at each visit since not all subjects with COPD can produce sputum on demand. ASking subjects to bring a morning sample of sputum to the visit would lengthen the period of time between production and processing, which could result in samples containing variable numbers of degenerate cells.

It is possible that a 14-d treatment period was too short to detect a change in inflammatory indices, but in the asthma patients, a clinical benefit and an antiinflammatory effect was seen within this period, and a 2-wk period is commonly used in clinical practice when a trial of steroids is administered to patients with COPD. Two weeks of oral corticosteroid therapy have been shown to modify ECP concentrations in asthma. We are continuing to study the effect of corticosteroids in COPD, using a longer treatment period and a more potent inhaled steroid. In summary, we have demonstrated that in patients with COPD, the intense inflammatory process is also unresponsive to therapy with either a 2-wk course of high-dose inhaled steroids or of high-dose oral prednisolone. This confirms the clinical impression that these drugs are of little value in COPD, at least in the short term. Since it is clear that the inflammatory mechanisms in COPD differ significantly from those in asthma, further study should be directed at the elucidation of these mechanisms with a view to discovering more specific therapy for this condition.

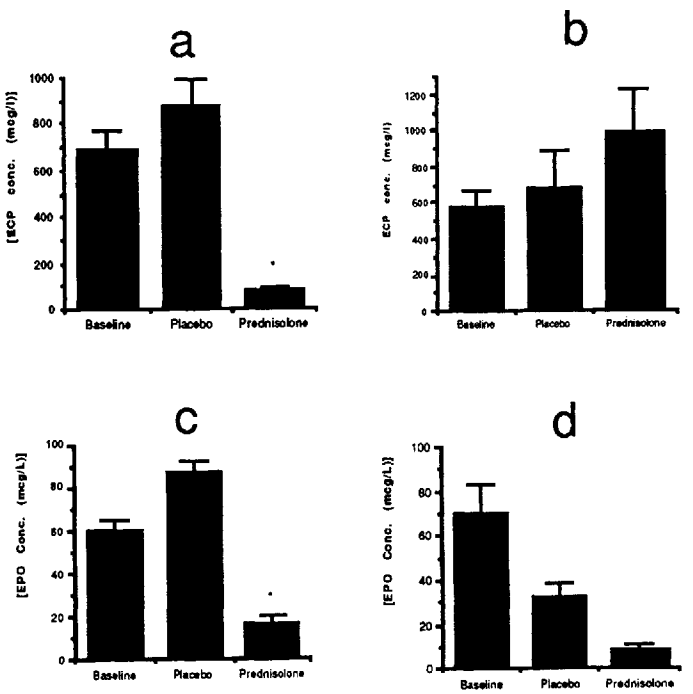

Figure 2. Changes in concentration of eosinophil cationic protein (ECP) in patients with asthma (*a*) and COPD (*b*), and in eosinophil peroxidase (EPO) in patients with asthma (*c*) and COPD (*d*) before and after treatment with oral prednisolone or placebo. Mean values ± SEM of 10 asthma and eight COPD patients are shown: *$p < 0.05$ for difference between two treatment groups.

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