Effect of corticosteroids on sputum sol-phase protease inhibitors in chronic obstructive pulmonary disease

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ABSTRACT Corticosteroids caused a reduction in the ratio of sol-phase sputum concentration to serum concentration of albumin in 12 patients with chronic obstructive bronchitis, suggesting a reduction in protein transudation. Alpha-1-antitrypsin values followed the same pattern as those of albumin in both the control and treatment periods, confirming the similar behaviour of the two proteins. The $\alpha_1$-antichymotrypsin ratios were on average three times higher than those of albumin in the control period, confirming the presence of local mechanisms in the lung for preferentially concentrating this protein. The sputum-to-serum ratio of $\alpha_1$-antichymotrypsin, however, rose during steroid treatment with the result that there was a selective increase in this protease inhibitor, which may be of potential benefit to such patients.

Uncontrolled enzyme activity within the lung is potentially harmful to the bronchial mucosa and cilia and has been implicated in the pathogenesis of emphysema. The serum and pulmonary secretions, however, contain several enzyme inhibitors that may protect the tissues from enzyme-induced damage. The main serum and alveolar inhibitor of proteolytic enzymes is $\alpha_1$-antitrypsin and severe deficiency of this protein is associated with the development of pulmonary emphysema. This association has led to the hypothesis that emphysema in subjects with normal serum $\alpha_1$-antitrypsin concentrations arises as a result of an imbalance between the enzymes and inhibitors within the lung such that enzyme activity persists. Any factor therefore that disturbs the balance between enzymes and inhibitors within the lung may be a major determinant of subsequent disease. Recently it has been shown that danazol can increase the $\alpha_1$-antitrypsin concentration in serum and that this is of potential benefit in protecting the lung. The effect of other, more commonly prescribed therapeutic agents, however, has received little attention.

Corticosteroids are frequently given to patients with chronic bronchitis and emphysema in the absence of reversible airflow obstruction, despite the general lack of evidence of therapeutic benefit. Chranowski and his associates, however, suggested that steroids may have had a beneficial effect on lung destruction in a study of a small group of patients with emphysema.

The purpose of the present study was to investigate the effect of corticosteroid treatment on the concentration of two proteolytic enzyme inhibitors, $\alpha_1$-antitrypsin and $\alpha_1$-antichymotrypsin, in both the serum and the secretions from a group of patients with chronic obstructive bronchitis. On theoretical grounds we were particularly interested to see whether the anti-inflammatory properties of steroids might reduce protein (and hence $\alpha_1$-antitrypsin) transudation from serum to the lung secretions.

Methods

Fourteen patients with chronic obstructive bronchitis were studied in the stable clinical state (average age 56·2 years, SD ± 11·0 years). All were current smokers and nine were men. All had severe airflow obstruction (mean FEV$_1$, 0·93 l, SD ± 0·27 l) that showed no acute response to bronchodilator treatment.

The patients were admitted to hospital for the period of study at least four weeks after any previous infective episode. Placebo was given for five
days followed by prednisolone (40 mg daily) for one
week. Sputum was collected daily over a six-hour
period for the first and last five days of the study.
The sputum was centrifuged at 100 000 g for 90
minutes and the sol phase was removed and stored
at −70°C until analysis. Venous blood was taken on
each sample day during the period of sputum collec-
tion and the serum was also stored at −70°C.

All proteins were measured by standard rocket
immunoelectrophoresis techniques with mono-
specific antisera prepared in the immunodiagnostic
research laboratory, University of Birmingham. The
results were expressed as percentages of a standard
reference serum (100% = 44 400 mg/l albumin,
2040 mg/l α₁-antitrypsin, and 429 mg/l α₁-anti-
chymotrypsin). The main statistical analysis was
performed on the sputum-to-serum protein con-
centration ratios as this overcomes individual dif-
ferences in serum concentrations of the acute-phase
proteins such as α₁-antitrypsin and α₁-anti-
chymotrypsin.9 The significance of any change was
assessed by the Wilcoxon rank sum test for paired
data with a single-tailed test for albumin and α₁-
antitrypsin and a two-tailed test for α₁-anti-
chymotrypsin.

Results

Two patients developed acute chest infections
during the period of study and their results were omit-
ted from further analysis. The remaining data were
incomplete on days 5 and 12 and these results were
not included in statistical analysis (data for eight
patients on day 5 are shown in the table and figures).

No significant change in FEV₁ was noted during
the study and treatment had no effect on the mean
serum values of any protein. The average serum and
sputum protein concentrations and sputum-to-
serum protein concentration ratios are summarised
in the table.

Albumin Individual sputum-to-serum ratios var-
died from 0·11 × 10⁻² to 1·28 × 10⁻². During the
placebo period, however, the average daily value for
the group as a whole was relatively constant and
similar to our previous results.10 The average daily
ratios for the whole group did not differ significantly
from one another during the placebo period. Data
for the first day of collection during steroid treat-
ment (day 8, the third day of treatment) were similar
to the control data. By the following day (day 9, the
fourth day) the average sputum-to-serum albumin
ratio had fallen but the difference just failed to reach
significance (p < 0·1, >0·05). The results obtained
for the fifth and sixth days of treatment (table),
however, were significantly lower than the first four
days of placebo treatment, when complete data were
available (p < 0·05).

Alpha-1-antitrypsin The individual data for
α₁-antitrypsin were more variable in the placebo
period than the data for albumin but tended to fol-
low a similar trend, values falling during cortico-
steroid treatment. The lowest sputum-to-serum

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Fig 1 Sputum-to-serum concentration ratios for
α₁-antichymotrypsin (solid circles) and albumin (open
circles) during placebo (days 1–5) and steroid
treatment (days 8–11). Each point represents the average results for all
patients for the collection days (value for day 5 is from 8
patients only). The bars represent ±1 SEM.

Fig 2 The sputum-to-serum concentration ratio for
α₁-antichymotrypsin corrected for the corresponding
albumin ratio during placebo (days 1–5) and steroid
treatment (days 8–11). Each point is the average value for a
day's collection and the bars represent ±1 SEM. The dotted
line indicates expected values if α₁-antichymotrypsin in
sputum sol phase were solely derived from serum by simple
transudation (the difference in molecular size between
albumin and α₁-antichymotrypsin* being taken into
account). Values above this line suggest a “local”
mechanism for preferentially concentrating
α₁-antichymotrypsin.
Serum and sol-phase sputum protein concentrations and concentration ratios in 12 patients with chronic obstructive pulmonary disease (mean values with 1 standard deviation in parenthesis)

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<td>4</td>
<td>5*</td>
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<td>(1-20)</td>
<td>(4-82)</td>
<td>(5-05)</td>
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*Data on only eight patients included.

*Percentage of standard serum (see under "Methods").

## Discussion

Albumin is thought to enter the bronchial secretions by diffusion alone and changes in its sputum-to-serum ratio may reflect changes in the degree of inflammation in the bronchial tree. During corticosteroid treatment the sputum-to-serum concentration ratios of albumin fell, which would be consistent with a reduction in inflammation. Alpha-1-antitrypsin is of almost identical molecular size to albumin and behaves similarly, entering the bronchial secretions which would reflect albumin concentration ratios. The values for alpha-1-antitrypsin and albumin during the placebo period and similar changes during corticosteroid treatment. Again the results suggest a reduction in protein transudation because of a decrease in mucosal inflammation. An alternative explanation might be an increase in dilutional factors, as a result of contamination with greater quantities of saliva or of increased fluid content of the sol-phase during corticosteroid treatment. The results for alpha-1-antitrypsin and albumin are emphasised by "correction" for the corresponding albumin ratios (table and fig 2).

The difference in behaviour between alpha-1-antitrypsin and albumin is emphasised by "correction" for the corresponding albumin ratios (table and fig 2).

The difference in behaviour between alpha-1-antitrypsin and albumin is emphasised by "correction" for the corresponding albumin ratios

**alpha-1-antitrypsin and albumin** are emphasised by "correction" for the corresponding albumin ratios.

**alpha-1-antitrypsin** and albumin are emphasised by "correction" for the corresponding albumin ratios.
antichymotrypsin, however, make this possibility less likely.

Alpha-1-antichymotrypsin is a slightly larger molecule than albumin and because the ratio of sol-phase sputum concentration to serum concentration of proteins derived solely from serum is affected by the protein size it should have a lower ratio than albumin. The current results show a sputum-to-serum ratio about three times higher for \( \alpha_1 \)-antichymotrypsin than for albumin and confirm our previous findings of a preferential concentration of this protein in the secretion as a result of local mechanisms. The results are summarised in the table and in figure 1.

If the changes for \( \alpha_1 \)-antitrypsin and albumin were purely dilutional, a similar effect would be seen for all proteins and the results for \( \alpha_1 \)-antichymotrypsin should also fall. The opposite occurred, however, with a rise in sputum-to-serum concentration ratios during corticosteroid treatment (fig 1 and table). The different behaviour of \( \alpha_1 \)-antichymotrypsin and albumin is emphasised in figure 2, where the sputum-to-serum concentration ratios of \( \alpha_1 \)-antichymotrypsin are divided by the corresponding albumin results. The overall effect is a greater selective increase in \( \alpha_1 \)-antichymotrypsin concentration in the secretions during steroid treatment. This may reflect an increase in "local production" or selective transport, or alternatively a reduction in pulmonary catabolism of \( \alpha_1 \)-antichymotrypsin during corticosteroid treatment. Further studies will be necessary, however, to clarify these points.

The time course of the response to corticosteroids that we observed is of interest. When such patients have reversible airflow obstruction the maximum physiological response is usually found in the first eight days of treatment. The present study shows that in the absence of such a physiological response a change in the sol-phase protein content may occur as early as three days from the start of treatment and is clearly demonstrable by the fourth day. It therefore seems likely that when a response to steroid treatment, either physiological or biochemical, is sought in patients with chronic bronchitis and emphysema the therapeutic trial need last no more than 7–10 days. Nevertheless, the effects of the biochemical response on slowly progressive diseases such as bronchitis and emphysema could only be determined by continued study on a large group of patients over many years.

In conclusion, sputum-to-serum concentrations for albumin and \( \alpha_1 \)-antitrypsin fell during corticosteroid treatment, which is consistent with reduced transudation from serum. The reduction in transudation may decrease the effective inhibitor screen to proteolytic enzymes such as elastase within the lung and may be potentially harmful. The importance of this observation is, however, difficult to assess fully without consideration of the effect of corticosteroids on the proteolytic enzymes or the complexing between enzyme and inhibitor within the lung. Corticosteroids may theoretically reduce the activity of these enzymes within lung secretions by stabilisation of lysosomal membranes of neutrophils taking part in the inflammatory response. Further studies on the balance between the enzyme and inhibitor are warranted.

The sputum-to-serum concentration ratios of \( \alpha_1 \)-antichymotrypsin rose during corticosteroid treatment. The implications of this finding are not clear, but \( \alpha_1 \)-antichymotrypsin is known to inhibit cathepsin G and bacterial proteases and steroid treatment may increase protection against these proteolytic enzymes, which could be of benefit to some patients, particularly those with recurrent bacterial infections.

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References


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