

Effects of prednisolone on angiotensin converting enzyme activity

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ABSTRACT Plasma angiotensin converting enzyme was measured in 23 asthmatic subjects before and after administration of prednisolone, 20 mg daily, for seven days. Plasma specimens from seven patients with asthma, seven with sarcoidosis and 14 normal subjects were also assayed before and after the addition of prednisolone *in vitro*. A plasma free extract of normal lung was also prepared and assayed before and after prednisolone treatment. Mean angiotensin converting enzyme activity was significantly greater in the asthmatic patients ($40.3 \text{ nmol min}^{-1} \text{ ml plasma}^{-1}$) than in the control population ($35.1 \text{ nmol min}^{-1} \text{ ml plasma}^{-1}$), though remaining within the 95% reference range. A fall in plasma angiotensin converting enzyme levels was seen after the addition of prednisolone in asthmatics both *in vivo* and *in vitro*. Similar *in vitro* falls were seen in patients with sarcoidosis but not in controls or in normal lung extract. No changes in angiotensin converting enzyme activity were seen after a single dose of 20 mg prednisolone in normal volunteers. Patients with asthma therefore appear to have higher mean angiotensin converting enzyme activities than the normal population and these fall after the addition of prednisolone whether this is added *in vivo* or *in vitro*.

Angiotensin converting enzyme (EC 3.4.15.1) is a dipeptidylcarboxypeptidase responsible for formation of angiotensin II from its direct precursor, angiotensin I, and also for inactivation of bradykinin and analogous vasodepressor peptides.¹ The enzyme is found on the luminal surface of the small blood vessels of the lung and other tissues and in cells of the monocyte-macrophage series.² The activity of angiotensin converting enzyme in serum or plasma can be measured by the ability of the enzyme to cleave a variety of synthetic (usually tripeptide) substrates under controlled conditions of pH, temperature, and time.

A diagnostic role for angiotensin converting enzyme measurement was first reported by Lieberman,^{3,4} who found that levels were increased in patients with active pulmonary sarcoidosis, a finding since confirmed by other authors.⁵⁻⁹ Measurement of angiotensin converting enzyme activity in serum or plasma has also been used to monitor the progress of the disease^{10,11} and its management with cor-

ticosteroid treatment¹²⁻¹⁷—angiotensin converting enzyme levels returning to normal as the features of sarcoidosis resolve. Turton and his colleagues,¹⁸ however, reported a fall in angiotensin converting enzyme activity in patients with other chest diseases when treated with prednisolone; their patients did not have raised levels before treatment, and the possibility that the fall in angiotensin converting enzyme was due to a direct effect of the steroid on the enzyme rather than the therapeutic effects of prednisolone could not be discounted.

In this study we have investigated the effects of prednisolone on plasma angiotensin converting enzyme activities both *in vivo* and *in vitro* in patients with asthma and sarcoidosis and in normal controls. In addition, angiotensin converting enzyme was assayed from an extract of normal lung in a plasma free medium before and after the addition of prednisolone.

Methods

SUBJECTS STUDIED

Blood samples were taken from the antecubital vein from a control population comprising 111 patients at the Royal Infirmary Edinburgh. These subjects

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had no evidence of pulmonary sarcoidosis or of any other clinical condition reported to be associated with abnormal angiotensin converting enzyme levels.¹⁵ All samples were taken into lithium-heparin (15 U/ml blood) and the plasma was stored at -20°C until required for assay.

Blood was collected and stored in the same way from 23 patients with asthma, none of whom was taking oral steroids, and from seven patients with active, untreated sarcoidosis.

STUDIES WITH PREDNISOLONE

Single dose study in normal subjects Blood was taken from five healthy volunteers. Further samples were taken six, 24 and 48 hours after ingestion of 20 mg prednisolone. All samples were stored at -20°C until required for assay.

Study over seven days in patients with acute asthma Thirteen of the 23 asthmatic patients had moderately severe acute asthma. Blood samples were taken from these patients before steroid treatment and again after they had taken 5 mg prednisolone four times daily for seven days. Plasma specimens were subdivided into portions and stored at -20°C until needed for assay.

IN VITRO STUDIES

Fourteen plasma samples were chosen at random from the controls and seven from the group of acute asthmatics. These samples and the plasma from all seven untreated patients with sarcoidosis were assayed for angiotensin converting enzyme activity and then divided into two aliquots. Prednisolone was added to one portion, which was immediately assayed again for angiotensin converting enzyme activity. The assay was repeated at 24, 48, and 72 hours after addition of prednisolone. The untreated portion of each plasma sample was assayed on each occasion to detect any time dependent change in angiotensin converting enzyme activity that was not due to the presence of the steroid. All specimens were kept at 4°C for the duration of the study. This experiment was carried out with three concentrations of prednisolone—0.5, 1, and 1.5 $\mu\text{g/ml}$ plasma.

PREPARATION OF LUNG EXTRACT

A sample of normal lung tissue, which had been obtained at postmortem examination and stored at -40°C , was homogenised and fractionated according to the protocol of Bakhle.¹⁹ Five of the fractions obtained showed measurable angiotensin converting enzyme activity. Samples of these fractions were treated with prednisolone and assayed, again according to the above protocol.

ASSAY FOR ANGIOTENSIN CONVERTING ENZYME ACTIVITY

Angiotensin converting enzyme activity was measured by allowing the plasma sample (10 μl) to cleave a tripeptide substrate (240 μl of a 5 mmol/l hippuryl-L-histidyl-L-leucine substrate in 0.1 mmol/l phosphate, pH 8.3) for 15 minutes at 37°C . The reaction was terminated by addition of 1.45 ml of 0.28 mmol/l sodium hydroxide. The histidyl-leucine product was then reacted with *o*-phthalaldehyde (2 g/l) for 10 minutes to form a fluorescent adduct. This reaction was terminated by the addition of 20 μl of 3 mmol/l HCl and the fluorescence ($\lambda_{\text{ex}} = 360 \text{ nm}$; $\lambda_{\text{em}} = 500 \text{ nm}$) of the product measured on a Perkin-Elmer LS5 fluorimeter after the tubes had been centrifuged to remove protein *o*-phthalaldehyde complexes. This method is based on that of Friedland and Silverstein.²⁰

Angiotensin converting enzyme activity was assayed in 25 samples that had been previously assayed independently by the same technique in the department of biochemistry, Royal Northern Hospital, London. The results were analysed by linear regression analysis. The mean difference was 3.24 and the standard deviation of that difference $7.06 \text{ nmol min}^{-1} \text{ ml plasma}^{-1}$ ($y = 2.38 + 1.01x$, $Sy.x = 7.16$).

STATISTICAL ANALYSES

The group data were tested by the χ^2 test and the distribution was shown not to differ significantly from normal (fig 1). Groups were compared with *t* tests, paired or unpaired, and linear regression analyses as appropriate.

Results

Comparison of the distribution of angiotensin converting enzyme activities in the control group and in the patients with asthma by independent *t* test showed that the asthmatics had a significantly higher mean angiotensin converting enzyme activity, although the data were distributed within the 95% reference range of the normal group (fig 1 and table). Patients with active sarcoidosis showed grossly increased angiotensin converting enzyme activities (table) by comparison with both the normal and the asthmatic groups.

STUDIES WITH PREDNISOLONE

In vivo In the single dose study with normal volunteers angiotensin converting enzyme levels six, 24, and 48 hours after they had taken prednisolone were not significantly different ($p > 0.05$) from pretreatment values. Mean angiotensin converting

Mean angiotensin converting enzyme activities of groups studied

Group	n	Mean (SD) (nmol min ⁻¹ ml plasma ⁻¹)	p*
Control	111	35.1 (11.1)	
Asthmatic	23	40.3 (10.7)	0.024
Sarcoid	7	84.4 (11.4)	0.00001

**t* test for significance of differences between patients and controls.

enzyme activity fell from 36.27 to 26.08 nmol min⁻¹ ml plasma⁻¹ in the 13 asthmatic patients studied after one week of prednisolone treatment (5 mg four times a day) (fig 2). This fall was significant (paired *t* test, *p* < 0.001).

In vitro The changes in plasma angiotensin converting enzyme activity seen after the addition of prednisolone are shown in fig 3. In the normal controls no changes were seen either immediately after the addition of prednisolone or after 72 hours of incubation at 4°C. In the plasma free lung extract angiotensin converting enzyme activity also remained constant over 72 hours in the presence of prednisolone. In the seven patients with moderately severe acute asthma there was a significant fall in mean angiotensin converting enzyme activity after 24 hours, from 35.00 to 25.03 nmol min⁻¹ ml plasma⁻¹ (paired *t* test, *p* < 0.01). The samples from the patients with asthma showed a further significant reduction in angiotensin converting enzyme values

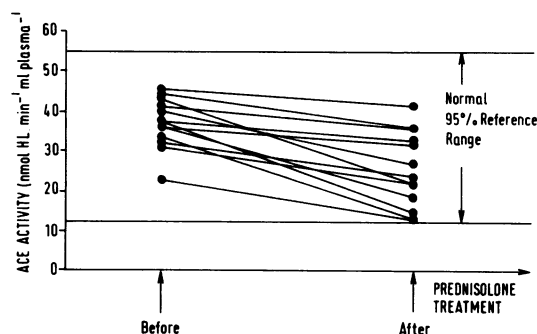


Fig 2 Plasma angiotensin converting enzyme (ACE) activity in 13 asthmatic patients before and after oral prednisolone treatment.

from 24 to 72 hours after the addition of prednisolone (*p* < 0.03). A significant fall was also seen in the seven patients with pulmonary sarcoidosis 24 hours after the addition of prednisolone (*p* < 0.01), but there was no further reduction between 24 and 72 hours (*p* > 0.05). The changes in angiotensin converting enzyme activities in these patients with asthma and sarcoidosis were independent of the concentrations of prednisolone (0.5, 1, and 1.5 µg/ml) used in these studies.

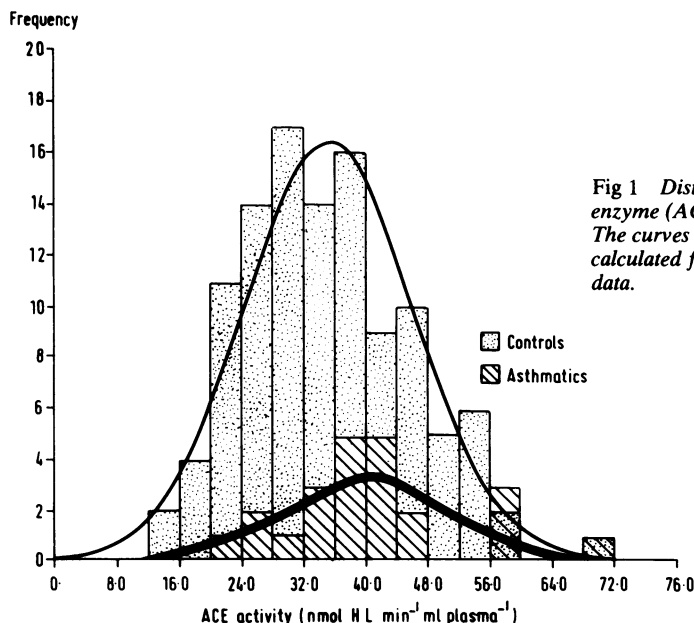


Fig 1 Distribution of plasma angiotensin converting enzyme (ACE) activity in the control and asthmatic groups. The curves represent the theoretical normal distributions calculated from the means and standard deviations of the data.

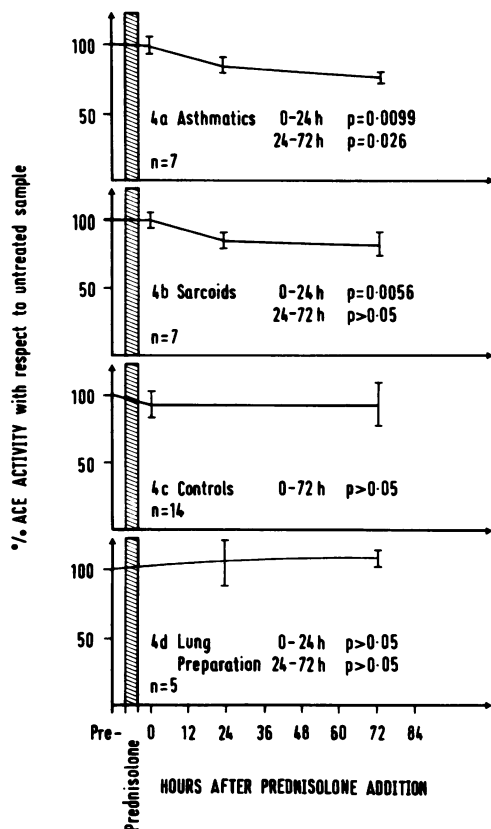


Fig 3 Change in angiotensin converting enzyme (ACE) activity in plasma from patients with asthma, patients with sarcoidosis, and control subjects after *in vitro* addition of a single dose of prednisolone to the specimen of plasma. The lowest graph shows change in angiotensin converting enzyme activity in lung extract after the addition of prednisolone.

Discussion

In our group of 23 asthmatic patients, the mean angiotensin converting enzyme activity was significantly higher than in a normal control population, though remaining within the 95% reference range (fig 1). The reasons for this are not clear but angiotensin converting enzyme measurement may represent a non-specific indicator of the severity of inflammation in the bronchi or lungs. Further studies of angiotensin converting enzyme activity in asthmatic patients after exercise challenge and bronchial provocation testing may help to explain this phenomenon.

The plasma angiotensin converting enzyme activities found in the control subjects given a single

20 mg dose of prednisolone cannot be compared directly with the values obtained in the asthmatic patients after the same dose of prednisolone taken for seven days. Although peak plasma prednisolone concentrations are achieved about two hours after an oral dose^{21,22} it seems probable that prolonged therapeutic concentrations of steroid in the plasma are required to suppress angiotensin converting enzyme activity *in vivo*.

The fall in angiotensin converting enzyme levels seen in asthmatic patients taking prednisolone is similar to that reported for other non-sarcoid lung diseases.¹⁸ There was also a consistent fall after plasma from patients with either asthma or sarcoidosis had been treated with prednisolone *in vitro*. This suggests that steroids have a direct effect on angiotensin converting enzyme activity independent of any action on the underlying disorder. These factors cannot be readily distinguished, which complicates interpretation of serial measurements of angiotensin converting enzyme activity in patients receiving steroids. These reductions in enzyme activity *in vitro* were also independent of the concentration of prednisolone used within the range of 0.5–1.5 µg/ml, which is consistent with partial inhibition of the enzyme by the steroid. Such reductions in angiotensin converting enzyme activity *in vitro* were not seen in plasma from controls or in normal lung extract. This independent effect of prednisolone on the activity of the enzyme in the plasma of patients with asthma or sarcoidosis may indicate a fundamental difference in the enzyme in these disorders or, possibly, the presence of a cofactor in the plasma in such patients. These results must also cast considerable doubt on the belief that angiotensin converting enzyme measurement is a specific and reliable index of the activity of pulmonary sarcoidosis.

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References

- 1 Igic R, Erdos EG, Yeh HS, Sorrels K, Nakajima T. Angiotensin I converting enzyme of the lung. *Circ Res* 1973;30/31,suppl 2:51–61.

- ² Erdos EG. The angiotensin I converting enzyme. *Fed Proc* 1977;**36**:1760-5.
- ³ Lieberman J. A new confirmatory test for sarcoidosis. Serum angiotensin-converting enzyme: effect of steroids and chronic lung disease. *Am Rev Respir Dis* 1974;**109**:743.
- ⁴ Lieberman J. Elevation of serum angiotensin-converting enzyme (ACE) levels in sarcoidosis. *Am J Med* 1975;**59**:365-72.
- ⁵ Fanburg BL, Schoenberger MD, Bachus B, Snider GL. Elevated serum angiotensin I converting enzyme in sarcoidosis. *Am Rev Respir Dis* 1976;**114**:525-8.
- ⁶ Ashutosh K, Keighley JFH. Diagnostic value of serum angiotensin-converting enzyme activity in lung diseases. *Thorax* 1976;**31**:552-7.
- ⁷ Silverstein E, Freidland J, Lyons HA, Gourin A. Elevation of angiotensin-converting enzyme in granulomatous lymph nodes and serum in sarcoidosis: clinical and possible pathogenic significance. *Ann NY Acad Sci* 1976;**278**:498-513.
- ⁸ Studdy P, Bird R, James DG, Sherlock S. Serum angiotensin-converting enzyme (SACE) in sarcoidosis and other granulomatous disorders. *Lancet* 1978;ii:1331-4.
- ⁹ Rohrbach MS, De Remee RA. Serum angiotensin-converting enzyme activity in sarcoidosis as measured by a simple radiochemical assay. *Am Rev Respir Dis* 1979;**119**:761-7.
- ¹⁰ De Remee RA, Rohrbach MS. Serum angiotensin-converting enzyme activity in evaluating the clinical course of sarcoidosis. *Ann Intern Med* 1980;**92**:361-5.
- ¹¹ Ueda E, Kawabe T, Tachibana T, Kokubu T. Serum angiotensin-converting enzyme activity as an indicator of prognosis in sarcoidosis. *Am Rev Respir Dis* 1980;**121**:667-71.
- ¹² Boomsma F, Tan KY, Schalekamp MADH. Angiotensin I-converting enzyme in the diagnosis and management of sarcoidosis. *Neth J Med* 1981;**24**:169-74.
- ¹³ Baur X, Fruhmman G, Dahlheim H. Follow-up of angiotensin-converting enzyme in serum of patients with sarcoidosis. *Respiration* 1981;**41**:133-8.
- ¹⁴ Rohrbach MS, De Remee RA. Pulmonary sarcoidosis and serum angiotensin-converting enzyme. *Mayo Clin Proc* 1982;**57**:64-6.
- ¹⁵ Rohatgi PK. Serum angiotensin-converting enzyme in pulmonary disease. *Lung* 1982;**160**:287-301.
- ¹⁶ Lieberman J. Angiotensin-converting enzyme and pulmonary sarcoidosis. *Comp Ther* 1981;**7**:28-33.
- ¹⁷ Rodriguez GE, Shin BC, Abernathy RS, Kendig EL. Serum angiotensin-converting enzyme activity in normal children and in those with sarcoidosis. *J Pediatr* 1981;**99**:68-72.
- ¹⁸ Turton CWG, Grundy E, Firth G, Mitchell D, Rigden BG, Turner-Warwick M. Value of measuring serum angiotensin I converting-enzyme and serum lysozyme in the management of sarcoidosis. *Thorax* 1979;**34**:57-62.
- ¹⁹ Bakhle YS. Conversion of angiotensin I to angiotensin II by cell-free extracts of dog lung. *Nature* 1968;**220**:919-21.
- ²⁰ Friedland J, Silverstein E. A sensitive fluorimetric assay for serum angiotensin-converting enzyme. *Am J Clin Path* 1976;**66**:416-24.
- ²¹ Wilson CG, May CS, Paterson JW. Plasma prednisolone levels in man following administration in plain and enteric-coated forms. *Br J Clin Pharmacol* 1977;**4**:351-5.
- ²² Morrison PJ, Bradbrook ID, Rogers HJ. Plasma prednisolone levels from enteric and non-enteric-coated tablets estimated by an original technique. *Br J Clin Pharmacol* 1977;**4**:597-603.