Serum Angiotensin Converting Enzyme Activity Measured by Angiotensin I Hydrolysis

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Accepted for publication on May 10, 1995

ABSTRACT. A sensitive and specific assay method to determine serum angiotensin converting enzyme (ACE) was developed. Although hippuryl-his-leu is commonly used as substrate, Angiotensin I (AI) was the substrate used to measure ACE activity in this study. AI hydrolysis was measured by radioimmunoassay of angiotensin II. ACE activity was expressed as the amount of AII formed from AI per minute. There was a positive correlation ($p<0.01$, $r=0.81$) between the ACE activities determined by AI hydrolysis and by hippuryl-his-leu hydrolysis. The ACE activity in eighty-three normal subjects was 4.08 ± 1.04 nmol/ml/min (normal range, from 2.00 to 6.16 nmol/ml/min). ACE activity was significantly suppressed after acute administration of captopril to seven normal subjects (0.40 ± 0.12 nmol/ml/min, $p<0.01$), and significantly elevated in five with active pulmonary sarcoidosis (10.08 ± 4.44 nmol/ml/min, $p<0.05$), compared with normal controls.

Key words: angiotensin converting enzyme — angiotensin converting enzyme activity — angiotensin converting enzyme inhibitor — sarcoidosis

Angiotensin converting enzyme (ACE) is localized in the vascular endothelium, and epithelial cells of the kidney and gut. ACE is a dipeptidyl carboxypeptidase that induces release of histydylleucine from the C-terminal of Angiotensin I (AI) converting it to Angiotensin II (AII), the vasoactive agent in the renin-angiotensin system. ACE is also hydrolyzes the vasodilator peptide, bradykinin. The most commonly used assay method measures ACE activity using synthetic substrates to determine the rate of hippuryl-his-leu hydrolysis.[1,2] Such measurements may not reflect the true enzymic activity since the kinetic parameter of ACE for this synthetic substance differs from that for AI, the natural peptide substrate.[3] In the present study, we developed an assay method in which AI was used as substrate to determine ACE activity, and measured ACE activity in the sera of normal subjects, normal subjects administered ACE inhibitor and patients with active pulmonary sarcoidosis.

MATERIALS AND METHODS

AI, AII and Na-EDTA were purchased from Sigma (St. Louis, USA). ACE activity was measured from hydrolysis of the substrate, AI. 25 μl of 3.2 mM substrate and 12.5 μl of 50 mM Hepes buffer (pH 7.5), containing 50 mM

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NaCl, 1 mM $ZnSO_4$, were incubated 2 min, at 37°C, with 12.5 μl serum samples, the reaction was then stopped by addition of 12.5 μl of 100 mM Na-EDTA, and the reaction tube was immediately incubated at 4°C until assay. The amount of AII produced from the substrate, AI, was determined by radioimmunoassay (RIA), as described below.

^{125}I-AII (30,000 cpm) (Amersham UK) in 50 μl of 50 mM Tris buffer (pH 7.5) containing 0.3% bovine serum albumin (RIA buffer) and 100 μl of antiserum (Amersham UK) were added to 100 μl of the diluted samples. The incubation was carried out at 4°C for 16-24 hours. Bound and free AII were separated with 1 ml of dextran (Nakarai, Kyoto, Japan)-coated charcoal (Sigma, St. Louis, USA) (charcoal 10 mg/ml, dextran 0.2 mg/ml, suspended in RIA buffer). After centrifugation at 2000×g for 20 min at 4°C, the supernatant was counted for 1 min in a gamma counter (Aloka, Tokyo, Japan).

Since the antiserum slightly cross-reacted with AI (1.5%), AI hydrolysis reactions with and without Na-EDTA were also performed in parallel, and the amount of immunoreactive AI was subtracted from the amount of AII determined. ACE activity was defined as the amount of enzyme-catalyzed formation of AII from AI per ml serum per minute. Kinetic parameters for AI hydrolysis under standard assay conditions were determined by Lineweaver-Burk analysis. Assays were conducted in duplicate. ACE activity determined from hippuryl-his-leu hydrolysis was measured as described previously.[4)]

Eighty-three healthy subjects (67 male, 16 female, aged 19-51 years old), as normal controls, and five patients with active pulmonary sarcoidosis were studied. The effect of ACE inhibitor on ACE activity was also studied in seven normal subjects. Blood samples were drawn for ACE activity estimations before and at 2 hours after a single oral 25 mg dose of captopril. The serum samples were stored at −20°C until assay.

The results are expressed as the means±SD. Statistical analysis was performed by an unpaired or paired Student's *t* test.

RESULTS

Serum ACE activity was estimated using the rate of AII formed from AI. The AII formation rate was linear, at a serum sample volume of 2 to 50 μl and incubation time ranging from 1 to 30 minutes (Fig 1). Lineweaver-Burk analyses are presented in Fig 2. The Km (Michaelis constant) of ACE based on AI hydrolysis was 12.5 μM. Comparisons of ACE activity measured by AI or hippuryl-his-leu hydrolysis in 30 samples were shown in Fig 3. A significant ($p<0.05$) positive correlation was observed between the two methods.

The ACE activity level determined from AI hydrolysis in 83 normal subjects was 4.08±1.04 nmol/ml/min. Therefore the normal range is from 2.00 to 6.16 nmol/ml/min (mean±2SD). The minimum detectable quantity was 0.10 nmol/ml/min in this assay. The mean intra- and interassay coefficients of variation were 6.89% and 7.57%, respectively.

ACE activity was significantly ($p<0.01$) suppressed to 0.40±0.12 nmol/ml/min by administration of 25 mg captopril, a potent competitive ACE inhibitor. On the other hand in five patients with active pulmonary sarcoidosis, the ACE activity level was significantly ($p<0.05$) elevated to 10.08±4.44 nmol/ml/min, compared to normal subjects (Fig 4).

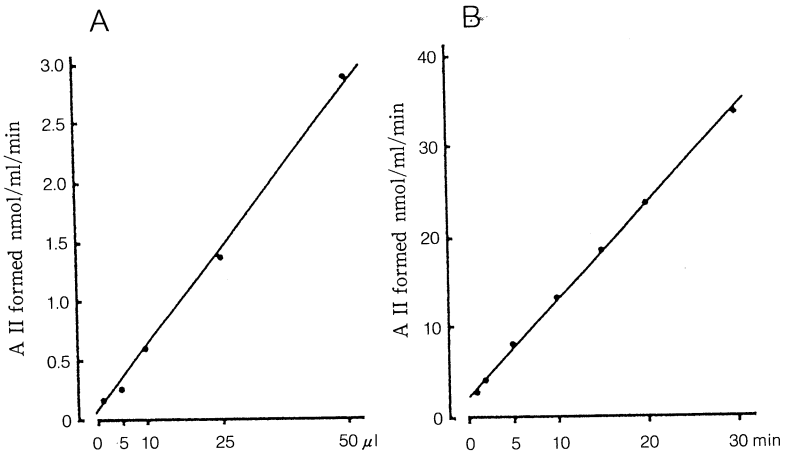

Fig 1. Effect of serum sample volume on angiotensin II formation (A) and reaction time course (of angiotensin II formation) (B)

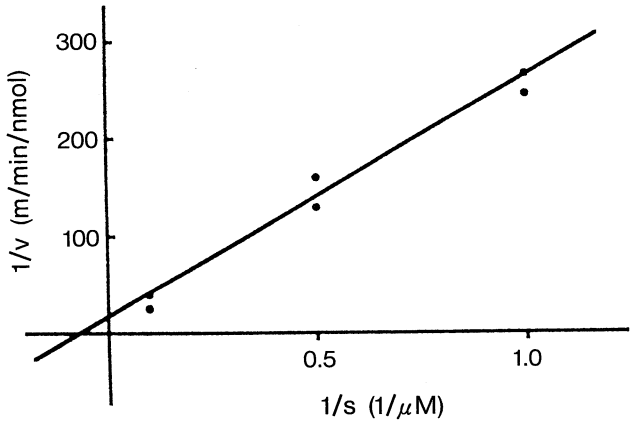

Fig 2. Lineweaver-Burk analysis of angiotensin converting enzyme
Reaction velocity (v) at various concentrations of the substrate (s), angiotensin I were determined.

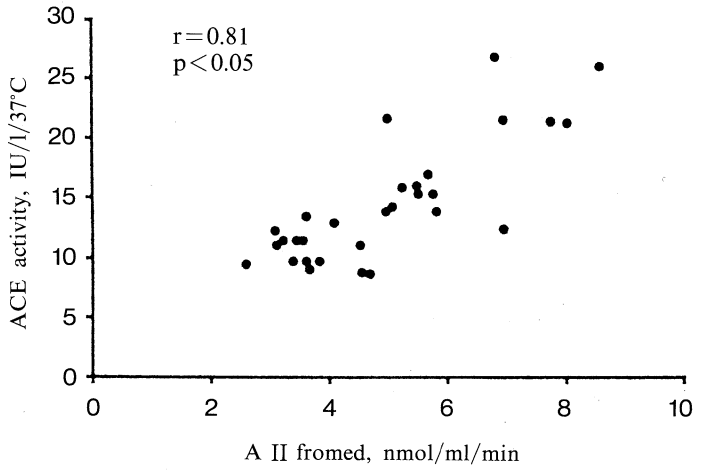

Fig 3. Comparison of parallel measurement of angiotensin converting enzyme by angiotensin I hydrolysis or hippuryl-his-leu hydrolysis in 30 samples

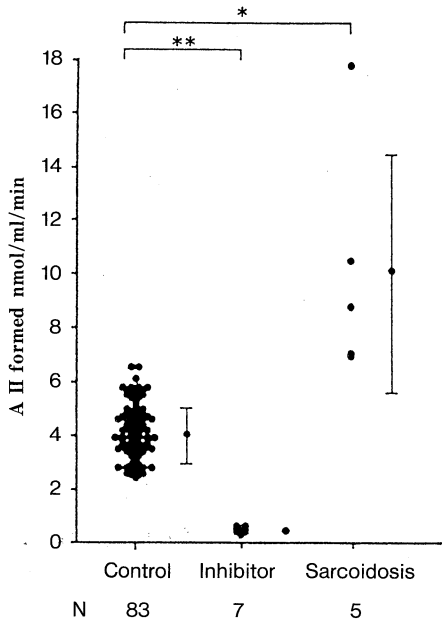

Fig 4. Serum angiotensin converting enzyme measured by angiotensin I hydrolysis in 83 normal subjects (Control), 7 normal subjects after administration of 25 mg captopril (Inhibitor) and 5 patients with active pulmonary sarcoidosis (Sarcoidosis).
Vertical bars represent the mean±SD. *$p<0.05$, **$p<0.01$ vs normal subjects

DISCUSSION

Several assays to measure ACE activity have been developed. One in which AI is used as a natural substrate,[5] and others using hippuryl-his-leu[1,2] and hippuryl-gly-gly,[6] as synthetic substrates. Hydrolysis of these substrates was assayed by ultraviolet spectrophotometric and fluorimetric techniques or radioimmunoassay. The most commonly used assay method measures ACE activity in terms of the rate of hippurate released from hippuryl-his-leu.[1,2] In the present study, we developed an assay method for ACE using AI. The major advantages of our assay over those used previously were sensitivity, reliability, simplicity. Prior dialysis of enzyme source was not required and there was no need to evaporate organic solvents before quantifying hippurate.

The rate of hydrolysis for synthetic substrates could differ from that of AI. The Km of hippuryl-his-leu is about 10^{-3} M,[7,8] while Km values for AI are much lower, of the order of 10^{-5} M.[8,9] The Km in our present study was 12.5 μM, which is similar to these observations. Therefore, the measurement of AI hydrolysis might better reflect the function of the renin-angiotensin system than that of hippuryl-his-leu hydrolysis.

The present study was demonstrated to be sensitive enough to measure ACE activity in human sera samples of 12.5 μl. ACE activity is elevated in serum of patients with active sarcoidosis, whereas those with inactive disease or patients receiving treatment have values within normal limits (data was not shown). Alehnc-Gelas *et al*[10] suggested that elevated activity in active sarcoidosis is actually induced by an increase in the number of enzyme molecules rather than by a reduction in the levels of inhibitors or by a change in the catalytic properties of the enzyme.

Following acute administration of ACE inhibitors to normal subjects, ACE activity decreased in this study. It has been suggested that ACE activity determination is a useful indicator of ACE inhibition during administration of ACE inhibitors.[11] However, ACE activity *in vitro* is not necessarily related to inhibition *in vivo*. In some studies,[12,13] the long-term administration of captopril leads to an increase in blood ACE concentration, but a rapid fall in blood ACE activity.

The present study has shown the assay method to measure serum ACE activity. This method was sensitive enough to determine enzyme activities in patients with sarcoidosis or in normal subjects after a treatment of ACE inhibitor.

ACKNOWLEDGMENTS

This work was supported in part by Research Project Grants 5-406 and 6-406 (to Nishida Seikoh) from Kawasaki Medical School.

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