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Cyclosporin A treatment enhances angiotensin converting enzyme activity in lung and serum of rats

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Abstract—Nephrotoxicity and arterial hypertension are the most common side effects of treatment with cyclosporin A (CSA). Its effects on angiotensin converting enzyme (ACE) activity in the renal cortex, lung and serum of nephrotoxic rats have been investigated. Wistar rats were treated with CSA (20 mg kg^{-1} day^{-1} i.p.) or vehicle (olive oil containing 10% ethanol) for 14 days. On day 15, the rats were killed and ACE activity determined by radiometric assay using [^{3}H]hippuryl-glycyl-glycine as substrate. CSA treatment resulted in a decrease in creatinine clearance, urine flow and body weight and a significant increase in serum and lung ACE activities (436 ± 9 vs 391 ± 7 nmol mL^{-1} min^{-1}, $P < 0.001$; 184 ± 8 vs 142 ± 10 nmol mg^{-1} min^{-1} $P < 0.01$, respectively). In contrast, renal cortex ACE activity was reduced in the CSA-treated rats (0.35 ± 0.02 vs 0.51 ± 0.02 nmol mg^{-1} min^{-1}, $P < 0.01$). ACE activities in the renal cortex and serum were not affected by treatment with gentamicin (80 mg kg^{-1} day^{-1}) for 11 days. In rats treated simultaneously with CSA and captopril (50 mg kg^{-1} day^{-1}) ACE activity in the serum, lung and renal cortex was inhibited by 95, 93 and 92%, respectively. These changes in ACE activity were associated with a decreased systolic blood pressure in the rats receiving CSA and captopril. Therefore, ACE activity in the serum and lung of CSA-treated rats was increased, while its activity in the renal cortex was reduced. This increased activity may support the suggestion that CSA induces hypertension through an angiotensin II-dependent mechanism and/or an increased degradation of vasodilatory kinins.

Nephrotoxicity and arterial hypertension are the most common side effects of treatment with cyclosporin A (CSA). In the rat, CSA may result in a decrease in glomerular filtration rate, urine flow, and body weight (Sullivan et al 1985) and is associated with increased plasma renin substrate (Lustig et al 1989), plasma renin activity (Baxter et al 1982) and an increased renin release from the renal cortex (Baxter et al 1984). Therefore, CSA-induced hypertension in the rat was thought to be due to activation of the renin-angiotensin system (Lustig et al 1987). However, the effect of CSA on angiotensin converting enzyme (ACE), a key enzyme in the renin-angiotensin system, has not been described.

ACE (EC 3.4.15.1) mediates the cleavage of the dipeptide His-Leu from the decapeptide angiotensin I, generating the powerful vasoconstricting angiotensin II. It is also involved in the degradation of kinins (Ferreira & Vane 1967). An increasing number of studies suggest the existence of a local angiotensin II generating system which operates independently of the circulating renin-angiotensin system. In rat tissues, ACE activity has been found in kidney, lung, blood vessels, brain and ovary (Campbell 1987). In the rabbit kidney, ACE has been found only in the glomerulus and in two parts of the proximal tubule: the convoluted proximal tubule and the pars recta (Marchetti et al 1987). The administration of high doses of CSA to rats has been reported to produce proximal tubular vacuolation and swelling (Sullivan et al 1985). Therefore, the present study was designed to investigate the effects of CSA treatment on ACE activity in the renal cortex, lung and serum of CSA-treated rats.

Materials and methods

Experiments were performed on male Wistar-rats (bred at the Beilinson Medical Center, Israel), 250–265 g, kept in group metabolic cages with free access to tap water and standard chow (Purina). After the rats had been acclimatized for two days they were injected with CSA (20 mg kg^{-1} day^{-1} i.p.) or its vehicle (10% ethanol in olive oil), for 14 days. In a few experiments, CSA and captopril (50 mg kg^{-1} day^{-1} i.p.) were co-administered. On day 14, the rats were housed in individual metabolic cages and 24 h urine collections were obtained. On day 15, the rats were anaesthetized with ether, blood was withdrawn for the determination of creatinine, blood urea nitrogen (BUN) and ACE activity. Then, the rats were killed and kidneys and lung tissues were removed for the determination of ACE activity. ACE activity was also determined in the serum, renal cortex and lung tissues of rats (n = 5) treated with gentamicin (80 mg kg^{-1} day^{-1} s.c.) for 11 days.

Tissues were cleaned from adhering connective tissue and homogenized (5 mL g^{-1} tissue) in 0·05 M HEPES buffer containing NaCl (0·15 M), Na_2SO_4 (0·6 M), and NaN_3 (0·1%) at pH 8·0, using a Politron homogenizer (Kinematica, Luzern, Switzerland). The homogenates were centrifuged at 600 *g* for 10 min (Jouan refrigerated centrifuge C.411) and ACE activity was determined in the supernatant.

Tissue and serum ACE activity was assayed by a radiometric assay method using [^{3}H]hippuryl-glycyl-glycine as substrate as described by Cohen & Kurz (1982). Briefly, serum and lung samples were incubated with 800 nmol of substrate (25 000 counts min^{-1} 234·6 μg) and renal cortex with 100 nmol (65 000 counts min^{-1} 29·3 μg) for 60 min at 37°C in a final volume of 100 μL. The reaction was stopped with 1·0 mL of HCl (0·1 M) and the medium was extracted with 1·5 mL ethyl acetate, centrifuged and radiolabelled hippuric acid present in the organic phase was counted by liquid scintillation spectrometry (LKB Wallac, (1217 Rackbeta). ACE activity was expressed in nmol hippuric acid formed min^{-1} (mg protein)$^{-1}$ or mL^{-1}. The assays were made in duplicates within 4 h of death. Protein in tissue samples was determined by the method of Lowry et al (1951).

Plasma and urine creatinine were measured using a Beckman creatinine analyzer and BUN was measured using SMA analyzer (Technicon, Tarrytown, USA).

Systolic blood pressure was measured by tail cuff manometry with a programmed electrosphygmomanometer (PE-300, Nacro Bio Systems, Houston, TX, USA) after maintaining the animals for 20 min at 37°C.

CSA as a powder was a generous gift from Sandoz Company (Basel, Switzerland). Captopril was kindly donated by the Squibb Institute (Hounslow, UK). [^{3}H]Hippuryl-glycyl-glycine (461 mCi $mmol^{-1}$) was purchased from Amersham (Buckinghamshire, UK) and hippuryl-glycyl-glycine was obtained from Sigma (Petah-Tikva, Israel).

Statistical analysis. Results are expressed as mean ± s.e.m. The data were analysed by unpaired Student's *t*-test. A *P* value less than 0·05 was considered significant.

Results

CSA administration to Wistar-rats (20 mg kg^{-1} day^{-1}) for 14 days resulted in a significant decrease in creatinine clearance (1.23 ± 0.05 vs 1.49 ± 0.11 mL min^{-1}, $P < 0.01$), body weight (260 ± 6 vs 281 ± 7 g, $P < 0.05$) and an increase in BUN

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Table 1. Inhibition of serum and lung ACE activity by captopril and EDTA. Sera and lungs from untreated rats (n = 5) were preincubated in the presence of EDTA and captopril for 10 min before the addition of substrate and ACE activity was determined as described in Materials and methods.

	ACE	
	Serum (nmol mL^{-1} min^{-1})	Lung (nmol mg^{-1} min^{-1})
Control (n = 5)	331 ± 14	180 ± 14
EDTA (2 mM, n = 5)	12·6 ± 2·2	1·8 ± 0·5
Captopril (2 μM, n = 5)	8·7 ± 1·9	3·6 ± 1·0

(27·1 ± 2·2 vs 17·8 ± 1·8 mg%, $P < 0·01$). ACE activity in the serum and lung of CSA-treated rats was significantly increased by 11 and 29%, respectively, whereas its activity in the renal cortex was decreased by 31% (Fig. 1). Preincubation of the sera and lung supernatants with captopril (2 μM) or EDTA (2 mM) for 10 min reduced the activity of the enzyme by more than 95% (Table 1).

Gentamicin administration to rats (80 mg kg^{-1} day^{-1}) for 11 days resulted in a significant decrease ($P < 0·02$) in creatinine clearance compared with rats injected with saline (1·21 ± 0·09 vs 1·56 ± 0·09 mL min^{-1}, respectively) and an increase in BUN

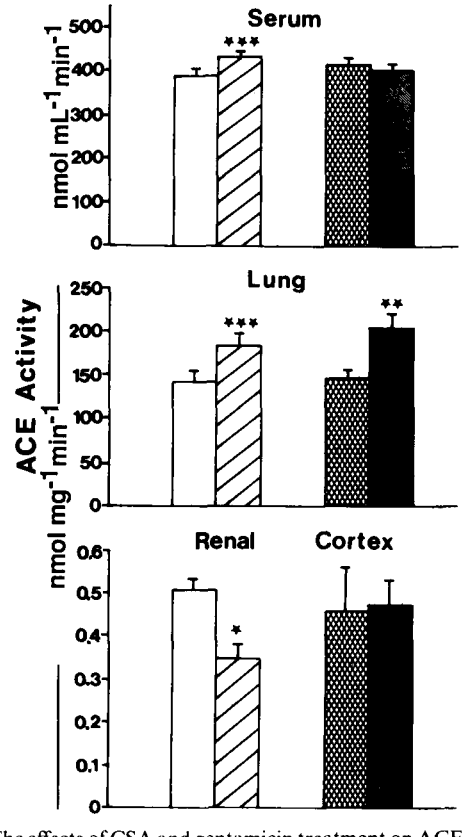

FIG. 1. The effects of CSA and gentamicin treatment on ACE activity in serum, lung and renal cortex. Rats received injections of CSA (hatched column, n = 15) or vehicle (open column, n = 10) for 14 days, saline (cross hatched column, n = 5) or gentamicin (solid column, n = 5) for 11 days and ACE in the serum, lung and cortex was determined as described in Materials and methods. Values are mean ± s.e.m. For statistical analysis the CSA group was compared with the vehicle group and the gentamicin group was compared with the saline group using unpaired Student's *t*-test. * $P < 0·02$, ** $P < 0·01$, *** $P < 0·001$.

Table 2. Effect of captopril administration on ACE activity in sera, lung and cortex of CSA-treated rats. Rats received injections of CSA (20 mg kg^{-1} day^{-1}, n = 6) or CSA (20 mg kg^{-1} day^{-1}) plus captopril (50 mg kg^{-1} day^{-1}, n = 6) for 14 days and ACE activity in the sera, lung and renal cortex was determined as described in Materials and methods. Values are mean ± s.e.m.

	ACE		
	Serum (nmol mL^{-1} min^{-1})	Lung (nmol mg^{-1} min^{-1})	Cortex (nmol mg^{-1} min^{-1})
CSA (n = 6)	416 ± 12	205 ± 11	0·28 ± 0·28
CSA + captopril (n = 6)	20 ± 3	14 ± 2	0·02 ± 0·01
Activity (%)	5	7	8

(27·8 ± 2·8 vs 19·3 ± 0·9 mg%, respectively). Despite the decreased creatinine clearance, ACE activity in the sera and cortex of gentamicin-treated rats was not significantly changed. However, its activity in lung tissue was significantly increased (Fig. 1).

ACE activity in the serum, lung and renal cortex of rats treated with CSA and captopril simultaneously for 14 days, was reduced by 95, 93 and 92%, respectively (Table 2). However, 24 h after captopril injections ceased, ACE activity in the serum returned to its previous levels while its activity in the lung and renal cortex was still inhibited by 33 and 92%, respectively.

The co-administration of CSA and captopril to the rats did not affect creatinine clearance significantly compared with the CSA-treated rats (1·32 ± 0·13 vs 1·17 ± 0·15 mL min^{-1}). However, the systolic blood pressure in the rats receiving CSA and captopril (n = 6) was significantly lower than in the CSA-treated rats (n = 6, 85 ± 8 vs 117 ± 12 mmHg, respectively, $P < 0·001$).

Discussion

CSA has become the drug of choice in the management of organ transplantation. More recently, CSA has been tested clinically as an immunosuppressive drug for autoimmune diseases such as insulin-dependent diabetes mellitus, rheumatoid arthritis and ocular inflammatory diseases. The currently used doses of CSA in recipients of heart, kidney, liver and bone marrow transplants are associated with an increased incidence of hypertension and nephrotoxicity (Luke 1987). Similarly, CSA-related hypertension occurs in non-transplant patients treated for various immunologic diseases. It was suggested that CSA-induced hypertension is caused by constriction of the renal afferent arterioles in humans and animal models (Luke 1987). Nonetheless, CSA-induced hypertension does not appear to be a renin-dependent state in man (Curtis et al 1988; Heering et al 1988). In contrast, in the rat, CSA-induced hypertension is associated with increased plasma renin substrate and activity (Perico et al 1986; Lustig et al 1987). In the present study, we have demonstrated for the first time that CSA treatment stimulates the activity of serum and lung ACE, but inhibits the ACE activity in the rat renal cortex. The increase in ACE activity in the serum and its decrease in the cortex was specific to CSA treatment and was not observed after treatment with gentamicin, although the degree of nephrotoxicity obtained by the two drugs, as estimated by creatinine clearance, was similar.

Experimental CSA nephrotoxicity is associated with glomerular disfunction but structural damage is restricted to proximal straight tubules (Remuzzi & Bertani 1989). Work done using a combined morphological functional approach (English et al 1987) hints at an afferent arteriole vasoconstriction, an effect compatible with local activation of the renin-angiotensin system, as the cause for this structural damage. It has also been

reported that ACE was only found in the glomerulus and in the proximal tubule (Marchetti et al 1987). Indeed, results obtained in-vivo and in-vitro indicate that renin release and renin activity are activated in CSA-nephrotoxicity (Baxter et al 1984; Kurtz et al 1988). The increased ACE activities in the lung and serum of rats expresses an activated renin-angiotensin state. ACE activity in the rat renal cortex is few hundreds-folds less than its activity in the serum and decreases significantly following CSA administration. Since angiotensin II in the blood and peripheral tissues, including the kidney is exchangeable (Campbell 1987), we suggest that the effects of renal angiotensin II on renal function, in CSA-nepthrotoxicity is less important, contrary to the view that emphasizes the role of the local tissue renin-angiotensin system. Furthermore, we and others (Kawaguchi et al 1985; Erman et al 1989) have shown that CSA treatment resulted in an elevation in renal production and excretion of prostacyclin and thromboxane. Prostacyclin (Levenson et al 1982) and thromboxane (Luft et al 1989) production is enhanced in response to activation of the renin-angiotensin system. The increase in 6-keto prostaglandin $F_{1\alpha}$ and thromboxane B_2 excretion may indirectly reflect high levels of renal angiotensin II. We propose that in the CSA-nephrotoxic model the decrease in renal cortex ACE may be due to the structural damage in the proximal tubule. However, hypertension induced by CSA might be caused by low doses of CSA (5 mg kg^{-1} day^{-1}) and might be not associated with perceptible changes in glomerular filtration rate (Lustig et al 1987).

Captopril treatment inhibited ACE activity in the serum, lung and renal cortex of CSA-treated rats. This was associated with a significant decrease in systolic blood pressure. However, captopril did not improve creatinine clearance. Our results support the findings of Murray et al (1985) indicating that captopril did not prevent the decrease in renal blood flow caused by CSA, and the finding of Lustig et al (1987) indicating that CSA-induced hypertension may be dissociated from CSA-nephrotoxicity. Therefore, our results do not support an important contribution of the renal failure in the pathogenesis of CSA-induced hypertension. The possibility that captopril might affect blood pressure through mechanisms other than the renin-angiotensin system such as prostanoids and/or kinins has to be considered.

ACE, also known as kininase II, metabolizes kinins. In the lung, more than 90% of the bradykinin administered into the venous side of the circulation has been inactivated by ACE (Ferreira & Vane 1967). Recently it has been shown that plasma bradykinin concentrations in rats pretreated with ACE inhibitors, were doubled following bradykinin infusion. These increased levels of bradykinin were correlated to decreases in mean arterial blood pressure (Ishida et al 1989). We suggest that the increased activity of ACE in the plasma and in the lung of CSA-treated rats would facilitate the metabolism of circulatory vasodilatory kinins and thus may contribute to the hypertensive effect induced by CSA.

In summary, ACE activity in the serum and lung of CSA-treated rats is significantly increased. Captopril inhibited this activity and reduced the blood pressure effect of CSA. These results are consistent with the notion that CSA causes hypertension by activating the renin-angiotensin system.

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