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Comparison of enalapril and valsartan in cyclosporine A-induced hypertension and nephrotoxicity in spontaneously hypertensive rats on high-sodium diet

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1 We compared the effects of the angiotensin converting enzyme (ACE) inhibitor enalapril and the angiotensin AT_1 receptor antagonist valsartan in cyclosporine A (CsA)-induced hypertension and nephrotoxicity in spontaneously hypertensive rats (SHR).

2 SHR (8–9 weeks old) on high-sodium diet were given CsA (5 mg $kg^{-1}d^{-1}$ s.c.) for 6 weeks. The rats were treated concomitantly either with enalapril (30 mg $kg^{-1}d^{-1}$ p.o.) or valsartan (3 or 30 mg kg^{-1} d^{-1} p.o.). To evaluate the role of bradykinin in the action of enalapril, some rats received a bradykinin B_2 receptor antagonist icatibant (HOE 140, 500 μg kg^{-1} d^{-1} s.c.) during the last 2 weeks of enalapril treatment.

3 Blood pressure was recorded every second week by tail cuff method. Renal function was measured by serum creatinine, creatinine clearance and urinary excretion of proteins at the end of the experiment. The activity of the renal kallikrein-kinin system was estimated by urinary kallikrein excretion.

4 CsA caused hypertension, impaired renal function and induced morphological nephrotoxicity with glomerular damage and interstitial fibrosis.

5 Enalapril and the lower dose of valsartan attenuated the CsA-induced hypertension to the same extent, while the higher dose of valsartan totally abolished it. Icatibant did not reduce the antihypertensive effect of enalapril. Urinary kallikrein excretion was similar in all groups. Enalapril and valsartan equally prevented the CsA-induced deterioration of kidney function and morphology.

6 The renin-angiotensin but not the kallikrein-kinin system plays a crucial role in CsA-toxicity during high intake of sodium in SHR.

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Keywords: Cyclosporine A; sodium; enalapril; valsartan; angiotensin II; bradykinin; icatibant (HOE 140); hypertension; nephrotoxicity

Abbreviations: ACE, angiotensin converting enzyme; CsA, cyclosporine A; SHR, spontaneously hypertensive rat(s)

Introduction

Cyclosporine A (CsA) is an immunosuppressive drug widely used to prevent rejection of transplanted organs and to treat autoimmune diseases. Unfortunately, CsA treatment is often limited by adverse effects like hypertension and nephrotoxicity (for review see Mason, 1989; Faulds *et al*., 1993). An adverse interaction between CsA and a high intake of dietary sodium has been described in spontaneously hypertensive rats (SHR) (Mervaala *et al*., 1997; Pere *et al*., 1998). During high-sodium diet, CsA caused more pronounced hypertension and renal dysfunction than during moderately low-sodium intake (Mervaala *et al*., 1997). It has been suggested that activation of the renin-angiotensin system is involved in the pathogenesis of CsA-induced nephrotoxicity in sodium-depleted rats (Burdmann *et al*., 1995; Pichler *et al*., 1995). However, plasma renin activity is also elevated in CsA-treated SHR during high intake of sodium (Mervaala *et al*., 1997; 1999). In addition, we have shown that inhibition of angiotensin converting enzyme (ACE) with enalapril is effective in preventing CsA-induced vascular and renal adverse effects in SHR on high-sodium diet (Mervaala *et al*., 1999).

Enalapril reduces blood pressure by decreasing angiotensin II formation. In addition, inhibition of the breakdown of vasodilatory bradykinin may be involved in the antihypertensive effect of ACE inhibitors (for review see Linz *et al*., 1995). The latter mechanism may be important, because there is evidence that the renal kallikrein-kinin system is also associated with CsA-induced toxicity (Wang *et al*., 1997). Besides ACE inhibitors, the function of the renin-angiotensin system can be suppressed at the receptor level by blockade of angiotensin AT_1 receptors. Angiotensin AT_1 receptor antagonists are as effective as ACE inhibitors in the treatment of hypertension in different experimental models as well as in clinical studies (Holwerda *et al*., 1996; Yamamoto *et al*., 1997).

We undertook this study to compare the inhibition of the renin-angiotensin system by the ACE inhibitor enalapril and by the AT_1 receptor antagonist valsartan in CsA toxicity in SHR during high intake of sodium. In addition, to evaluate the role of bradykinin in the action of enalapril, the effects of a bradykinin B_2 receptor antagonist icatibant (HOE 140) were studied during enalapril treatment.

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Methods

Experimental protocols

Forty-eight 8–9-week-old, male spontaneously hypertensive rats (SHR) (243–314 g, Harlan Sprague Dawley, Indianapolis, IN, U.S.A.) were used. The protocol of the study was approved by the Animal Experimentation Committee of the Institute of Biomedicine, University of Helsinki, Finland.

All the rats received high-sodium diet (Na 2.6%, Mg 0.2%, K 0.8%, Ca 1.0%, P 0.75% of the dry weight of the chow; R36, Finnewos Aqua, Helsinki, Finland). In the beginning of the study, blood pressure- and body weight-matched SHR were divided into five different drug regimens for 6 weeks ($n=9-10$ in each) (1) Control group (vehicle s.c.), (2) CsA group (5 mg kg^{-1} d^{-1} s.c., 4 μmol kg^{-1} d^{-1}), (3) CsA group receiving concomitantly enalapril 30 mg kg^{-1} d^{-1} p.o. (80 μmol kg^{-1} d^{-1}), (4) CsA group receiving concomitantly valsartan 3 mg kg^{-1} d^{-1} p.o. (7 μmol kg^{-1} d^{-1}), (5) CsA group receiving concomitantly valsartan 30 mg kg^{-1} d^{-1} p.o. (70 μmol kg^{-1} d^{-1}). Enalapril and valsartan were mixed in the food (22.5 or 225 mg kg^{-1}) to produce the above mentioned daily doses. The dose of enalapril was chosen on the basis of our previous studies to antagonize the effects of CsA in SHR (Mervaala *et al*., 1999). The higher dose of valsartan (30 mg kg^{-1} d^{-1}) has been reported to produce a marked antihypertensive effect, whereas the lower dose (3 mg kg^{-1} d^{-1}) caused a slight antihypertensive effect during long-term administration (Yamamoto *et al*., 1997). CsA was administered subcutaneously at a dose of 5 mg kg^{-1} d^{-1} which in our previous studies (Mervaala *et al*., 1997) resulted in plasma concentrations similar to those measured in CsA-treated patients. The control rats received the same volume (1 ml kg^{-1}) of the vehicle. The rats had free access to tap water and chow, and the animals were weighed daily during the experiment.

In another set of experiments, twelve 8-week-old SHR (246–286 g) receiving the high-sodium diet and CsA (5 mg kg^{-1} d^{-1} s.c.) were treated with enalapril (30 mg kg^{-1} d^{-1} p.o.). After 4 weeks the rats were divided into two groups: (1) Enalapril + bradykinin B_2 receptor antagonist icatibant (500 μg kg^{-1} d^{-1}, 400 nmol kg^{-1} d^{-1}) by a subcutaneous minipump for 2 weeks; and (2) Enalapril + saline by minipump for 2 weeks. The rats were anaesthetized with pentobarbital (50 mg kg^{-1} i.p.) during the implantation of the subcutaneous osmotic minipump (Alzet®, model 2002, Alza Corp., Palo Alto, CA, U.S.A.). The dose of icatibant was chosen according to previous studies which showed that long-term treatment with the drug at 500 μg kg^{-1} d^{-1} inhibited the cardiovascular effects of bradykinin in rats (Bao *et al*., 1992; O'Sullivan & Harrap, 1995). Two weeks' administration was chosen because of the limited capacity of the minipump and to avoid repeated operation procedures.

Measurement of systolic blood pressure and heart rate

Systolic blood pressure and heart rate were measured every second week using a tail cuff blood pressure analyser (Apollo-2AB Blood Pressure Analyser, Model 179-2AB, IITC Life Science, Woodland Hills, CA, U.S.A.). After the implantation of the minipump, systolic blood pressure was measured weekly. Measurements were performed on the same day of the week and at the same time of the day by the same person (Markus Lassila).

Collection of samples

During the last week of the study, the rats were housed individually in metabolic cages for 24 h. They had free access to tap water and food. The consumption of food and tap water was measured by weighing the chow and the water bottles, respectively. Urine was collected, urine volumes were measured, and samples were stored at −80°C until the biochemical determinations were performed.

At the end of the experiments, the animals were made unconscious with CO_2/O_2 (AGA, Riihimäki, Finland) and decapitated 24 h after the last CsA administration. Blood samples for plasma renin activity were taken into chilled tubes using EDTA (4.5 mM) as an anticoagulant, and for serum creatinine determinations into glass tubes without an anticoagulant. The heart was excised, great vessels, atria and the free wall of the right ventricle were dissected, and the left ventricular mass was weighed. The kidneys were washed with ice-cold saline and weighed. The left ventricle and kidney weights were expressed as a ratio relative to body weight.

Hormonal and biochemical determinations

Plasma renin activity was determined by a radioimmunoassay of angiotensin I (Medix Angiotensin I test®, Medix Biochemica, Kauniainen, Finland). Total protein concentration of urine was determined by the method of Lowry *et al*. (1951) after precipitation of proteins with 10% trichloroacetic acid. Urine and serum creatinine were analysed by the Jaffe method (Bartels *et al*., 1972) (BM/Hitachi 917 analyzer, Boehringer Mannheim, Germany/Hitachi Ltd., Tokyo, Japan) without deproteinization. Creatinine clearance as an index of glomerular filtration rate was calculated using serum concentration of creatinine and 24-h urinary excretion of creatinine. Urine magnesium concentration was measured using atom absorption spectrophotometer (BM/Hitachi 917 analyzer, Boehringer Mannheim, Germany/Hitachi Ltd., Tokyo, Japan). Urine sodium and potassium were determined by flame photometer using an ion selective electrode-compensator (human serum pool, IL model 943, Instrumentarium Laboratory, Milan, Italy). Urine kallikrein activity was measured by amidolytic assay using a chromogenic tripeptide substrate H-D-valyl-L-leucyl-L-arginine-p-nitroaniline dihydrochloride (Amundsen *et al*., 1979). The urine excretions as well as creatinine clearance were expressed per 100 g of body weight.

Renal histology

Cross-sections of the left kidney with renal artery were collected and fixed for 24–48 h with 10% formaline. The samples were dehydrated and embedded in paraffin by using the standard protocol. Sections of the renal tissue were deparaffinized, hydrated and stained with Masson's trichrome. The slides were blindly graded by the authors (P. Finckenberg and A.-K. Pere). Interstitial, tubular and glomerular changes were looked for. The slides were scored according to arterioglomerular changes using the method described by Pere *et al*. (1998): 100 consecutive glomeruli and their afferent arterioli from each kidney slide were assigned for severity of changes using scores from 0 to 3: (0) Normal arterioglomerular unit with open capillary lumina and a normal afferent arteriole; (1) Slight thickening of the media of the afferent arteriole. Slight proliferation of the mesangial cells and a slight increase in the mesangial matrix, but persisting capillary lumens in the glomerulus; (2) More severe medial thickening than in score

1. Clearly narrowed capillary lumens. Necrosis of the media of the wall of the afferent arteriole and partly collapsed capillaries in the glomerulus. Occasional segmental necrosis of the glomerular tuft and (3) Fibrinoid necrosis of the arteriolar wall, hemorrhagic necrosis of the glomerular tuft with some plump, but still persisting mesangial cells.

In order to emphasise the degree of changes, we used a damage index which was calculated by assessment of 100 consecutive arterioglomerular units in each kidney and counting the number of affected glomeruli in each score group, e.g.: $a \times 0 + b \times 1 + c \times 2 + d \times 3$ ($a + b + c + d = 100$ glomeruli, $0-3$ = the degree of change, i.e. score) (Pere *et al*., 1998).

Drugs

CsA (Sandimmun® infusion concentrate 50 mg ml^{-1}) and valsartan were generous gifts from Novartis Ltd (Basel, Switzerland). Enalapril was kindly donated by Leiras Ltd (Turku, Finland). Icatibant (HOE 140; d-Arg[Hyp^3, Thi^5, Oic^8]-bradykinin) was a generous gift from Dr Klaus Wirth, Aventis AG (Frankfurt, Germany). CsA was diluted in a lipid solution (Intralipid®, Kabi Pharmacia, Stockholm, Sweden). Icatibant was dissolved in physiological saline.

Statistical analysis

Data for systolic blood pressure were analysed by 2-way ANOVA with repeated measures for overall treatment effect. Other data were analysed by 1-way ANOVA. The Tukey's test was used for multiple pairwise comparisons of the treatment groups. Plasma renin activity was analysed by the Kruskal–Wallis ANOVA and the Mann–Whitney *U*-test. Bonferroni correction ($p' = p^*k$) was used to the resultant P values to allow pairwise comparisons of multiple groups (Ludbrook, 1994). Data for experiment with icatibant were analysed by the Student's *t*-test. $P < 0.05$ was considered significant. The results are expressed as means ± s.e.mean.

Results

Body weight, urine volume, food and water consumption

CsA decreased the body weight gain during the 6 weeks' treatment period ($P < 0.01$ vs control; Table 1). There were no differences in the body weight between CsA group and CsA groups receiving enalapril or valsartan.

There were no significant differences in the intake of food between the experimental groups, but the food intake tended to be smaller in CsA-treated animals (Table 2). Intake of water was somewhat lower in rats receiving enalapril or valsartan compared to CsA group, but the difference was not significant (Table 2). The urine volume was not affected by CsA alone, but it was significantly smaller in rats receiving simultaneously enalapril or valsartan at 30 mg kg^{-1} d^{-1} compared to the control rats (Table 2).

The body weight gain, food or water consumption or urine volume were not affected by icatibant compared to saline during CsA and enalapril treatment (Table 3).

Blood pressure and heart rate

During the first 4 weeks CsA caused a marked rise in systolic blood pressure (Figure 1) with a concomitant increase in heart rate (Table 1) ($P < 0.001$ vs control group). The hypertensive

Table 1 Effects of CsA, enalapril and valsartan on body weight gain, left ventricle and right kidney wet weight, and development of heart rate of SHR on high-sodium diet ($n = 9-10$)

Variable	*Control*	*CsA*	*CsA + ena30*	*CsA + val3*	*CsA + val30*	P, *ANOVA*
Body weight, g						
Week 0	284 ± 5	288 ± 6	284 ± 4	289 ± 5	288 ± 7	0.923
Week 6	350 ± 8	301 ± 9##	311 ± 7##	318 ± 6	320 ± 9	< 0.01
Left ventricle wet weight, g kg^{-1}	3.24 ± 0.06	3.50 ± 0.07	2.96 ± 0.10#***	2.95 ± 0.06#***	2.73 ± 0.05###***	< 0.001
Right kidney wet weight, g kg^{-1}	3.28 ± 0.06	3.40 ± 0.11	3.25 ± 0.10	3.11 ± 0.07	3.24 ± 0.07	0.230
Heart rate, beats min^{-1}						
Week 0	354 ± 11	359 ± 10	351 ± 13	361 ± 12	374 ± 14	0.692
Week 2	351 ± 12	399 ± 13	379 ± 19	384 ± 12	388 ± 11	0.198
Week 4	364 ± 12	431 ± 7###	412 ± 10##	410 ± 10#	395 ± 9	< 0.001
Week 6	363 ± 11	422 ± 6##	408 ± 12#	401 ± 8	393 ± 9	< 0.01

CsA, cyclosporine A; ena30, enalapril 30 mg kg^{-1} d^{-1}; val3, valsartan 3 mg kg^{-1} d^{-1}; val30, valsartan 30 mg kg^{-1} d^{-1}. Values are means ± s.e.mean. #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs control; *$P < 0.05$, ***$P < 0.001$ vs CsA.

Table 2 Effects of CsA, enalapril and valsartan on 24-h food and water intake, urine volume and urinary excretion of electrolytes, urinary kallikrein, and plasma renin activity (PRA) ($n = 9-10$)

Variable	*Control*	*CsA*	*CsA + ena30*	*CsA + val3*	*CsA + val30*	P, *ANOVA*
Food intake, g d^{-1}	38 ± 4	30 ± 4	40 ± 3	30 ± 3	30 ± 2	< 0.05
Water intake, ml d^{-1}	73 ± 10	65 ± 13	46 ± 3	51 ± 8	40 ± 8	0.073
Urine						
Volume, ml 100 g^{-1} d^{-1}	20.7 ± 2.5	19.2 ± 3.9	12.9 ± 0.6#	13.5 ± 2.1	11.7 ± 1.7#	< 0.05
Sodium, mmol 100 g^{-1} d^{-1}	6.2 ± 0.9	3.0 ± 0.5##	3.8 ± 0.4	4.1 ± 0.6	3.6 ± 0.6#	< 0.05
Potassium, mmol 100 g^{-1} d^{-1}	0.79 ± 0.09	0.54 ± 0.04#	0.57 ± 0.04#	0.63 ± 0.04	0.60 ± 0.05	< 0.05
Magnesium, nmol 100 g^{-1} d^{-1}	78 ± 12	50 ± 9	51 ± 7	68 ± 8	52 ± 7	0.126
Kallikrein, U 100 g^{-1} d^{-1}	680 ± 61	771 ± 108	623 ± 109	764 ± 48	874 ± 74	0.265
PRA, ng Ang I ml^{-1} h^{-1}	0.5 ± 0.1	24 ± 4###	56 ± 11###	36 ± 7###	49 ± 13###	< 0.001

CsA, cyclosporine A; ena30, enalapril 30 mg kg^{-1} d^{-1}; val3, valsartan 3 mg kg^{-1} d^{-1}; val30, valsartan 30 mg kg^{-1} d^{-1}. Values are means ± s.e.mean. #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs control.

Table 3 Effects of bradykinin B_2 receptor antagonist icatibant (500 μg kg^{-1} d^{-1}) on CsA (5 mg kg^{-1} d^{-1}) and enalapril (30 mg kg^{-1} d^{-1}) treated SHR on high-sodium diet ($n=5-7$)

Variable	*Enalapril*	*Enalapril + icatibant*	P, t-*test*
Body weight, g			
Week 0	263±4		0.739
Week 6	306±8	306±8	0.826
Left ventricle wet weight, g kg^{-1}	3.06±0.04	3.09±0.04	0.599
Right kidney wet weight, g kg^{-1}	3.41±0.04	3.53±0.09	0.360
Heart rate, beats min^{-1}			
Week 0	363±9		
Week 2	382±10		
Week 4	413±7		
Week 5	400±14	405±4	0.730
Week 6	378±13	401±6	0.161
Food intake, g d^{-1}	48±2	45±2	0.180
Water intake, ml d^{-1}	33±9	45±3	0.260
Urine			
Volume, ml 100 g^{-1} d^{-1}	13.9±2.0	13.8±1.1	0.984
Sodium, mmol 100 g^{-1} d^{-1}	4.1±0.7	3.1±0.5	0.263
Potassium, mmol 100 g^{-1} d^{-1}	0.48±0.14	0.49±0.09	0.961
Magnesium, nmol 100 g^{-1} d^{-1}	66±13	33±10	0.092
Kallikrein, U 100 g^{-1} d^{-1}	635±229	458±54	0.399
PRA, ng Angiotensin I ml^{-1} h^{-1}	46±19	69±10	0.315
Serum creatinine, μmol l^{-1}	55±0.9	54±0.8	0.449
Creatinine clearance, ml min^{-1} 100 g^{-1}	0.44±0.08	0.38±0.04	0.483
Urinary protein excretion, mg 100 g^{-1} d^{-1}	14.5±2.4	11.96±0.68	0.257

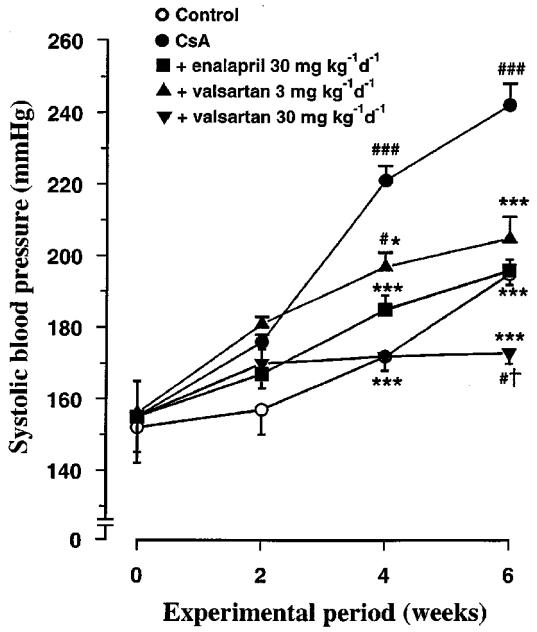

Figure 1 Effect of enalapril (30 mg kg^{-1} d^{-1}) and valsartan (3 and 30 mg kg^{-1} d^{-1}) on systolic blood pressure in cyclosporin A (CsA)-treated SHR during high-sodium diet ($n=9-10$): #$P<0.05$, ###$P<0.001$ vs control; *$P<0.05$, ***$P<0.001$ vs CsA, †$P<0.05$ vs enalapril 30 mg kg^{-1} d^{-1} or vs valsartan 3 mg kg^{-1} d^{-1}.

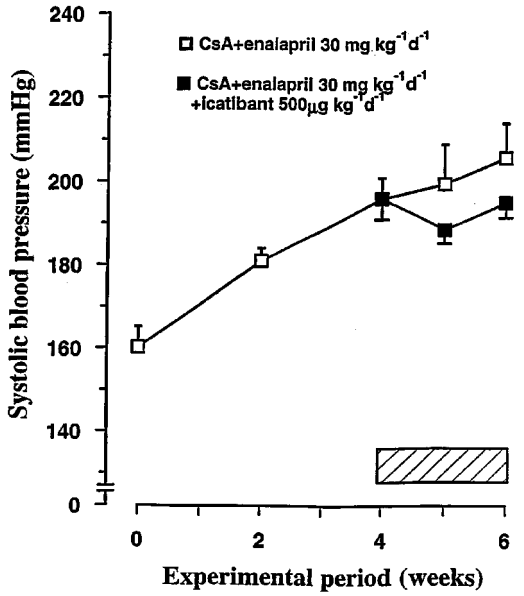

Figure 2 Effects of bradykinin B_2 receptor antagonist icatibant (500 μg kg^{-1} d^{-1}) on systolic blood pressure of SHR during high-sodium diet and cyclosporin A (5 mg kg^{-1} d^{-1}) (CsA) and enalapril treatment. Hatched bar indicates icatibant ($n=7$) or saline ($n=5$) treatment period.

effect was further augmented towards the end of the experiment; at 6 weeks of treatment CsA-induced increase in blood pressure was 47 mmHg larger than in the control group ($P<0.001$).

Both enalapril (30 mg kg^{-1} d^{-1}) and valsartan (3 and 30 mg kg^{-1} d^{-1}) prevented the CsA-induced elevation of blood pressure (Figure 1). Enalapril and the lower dose of valsartan (3 mg kg^{-1} d^{-1}) attenuated the development of hypertension to the same extent, while the higher dose of valsartan (30 mg kg^{-1} d^{-1}) totally abolished it. At week 6, the higher dose of valsartan resulted in 69 mmHg lower blood pressure compared to the CsA group ($P<0.001$), 23 mmHg lower compared to the enalapril group ($P<0.05$), 32 mmHg lower compared to the lower dose of valsartan ($P<0.001$) and 22 mmHg lower compared to the control group ($P<0.05$).

Treatment with the bradykinin B_2 receptor antagonist icatibant seemed to have a slight but not significant systolic blood pressure-lowering effect during enalapril administration in CsA-treated rats (11 mmHg at weeks 5 and 6, $P>0.05$; Figure 2).

Left ventricular hypertrophy

CsA had a tendency to increase the left ventricular mass expressed as ratio to the body weight ($P=0.098$; Table 1).

Enalapril and both doses of valsartan reduced the left ventricular mass when compared to control and CsA groups. Icatibant did not affect the left ventricular weight during CsA and enalapril treatment (Table 3).

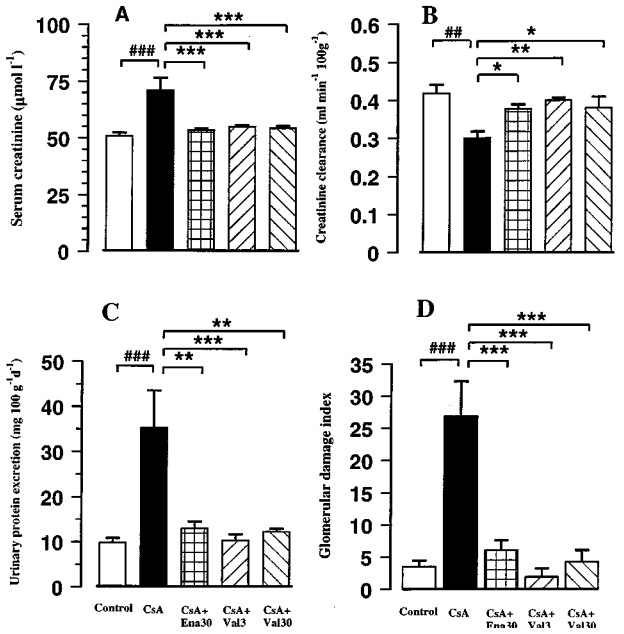

Figure 3 Influence of different drug regimens on serum creatinine (A), creatinine clearance (B), urinary protein excretion (C), and glomerular damage index (D) in SHR during high-sodium diet ($n=9-10$). CsA, cyclosporine A; Ena30, enalapril 30 mg kg^{-1} d^{-1}; Val3, valsartan 3 mg kg^{-1} d^{-1}, Val30; valsartan 30 mg kg^{-1} d^{-1}, ###$P<0.001$ vs control; *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs CsA.

Renal functions

There were no differences in the kidney wet weight-to-body weight ratio between the treatment groups (Table 1). CsA decreased creatinine clearance and increased serum creatinine concentration by 40%, and increased the 24-h urinary protein excretion by 260% (Figure 3). Enalapril and both doses of valsartan prevented the CsA-induced changes in all of these variables (Figure 3).

Icatibant did not affect the kidney weight, creatinine clearance, serum creatinine concentration or urinary excretion of protein during CsA and enalapril treatment (Table 3).

Plasma renin activity and urinary kallikrein excretion

The plasma renin activity was increased in CsA-treated SHR by about 50-fold ($P<0.001$ vs control) (Table 2). Enalapril or valsartan tended to further increase plasma renin activity during CsA treatment, but the effect against the group receiving CsA alone did not reach significance. Icatibant did not affect plasma renin activity during enalapril treatment (Table 3).

CsA alone or combined with enalapril or valsartan did not affect urinary kallikrein excretion ($P=0.265$) (Table 2). Icatibant did not influence kallikrein excretion during CsA and enalapril treatment (Table 3).

Renal histology

In the control group receiving high-sodium diet, there were solely minor pathological alterations involving a small portion (4%) of the glomeruli. Neither tubular nor interstitial changes were seen. The boundaries between the medullar and cortical layers were distinct (Figure 4).

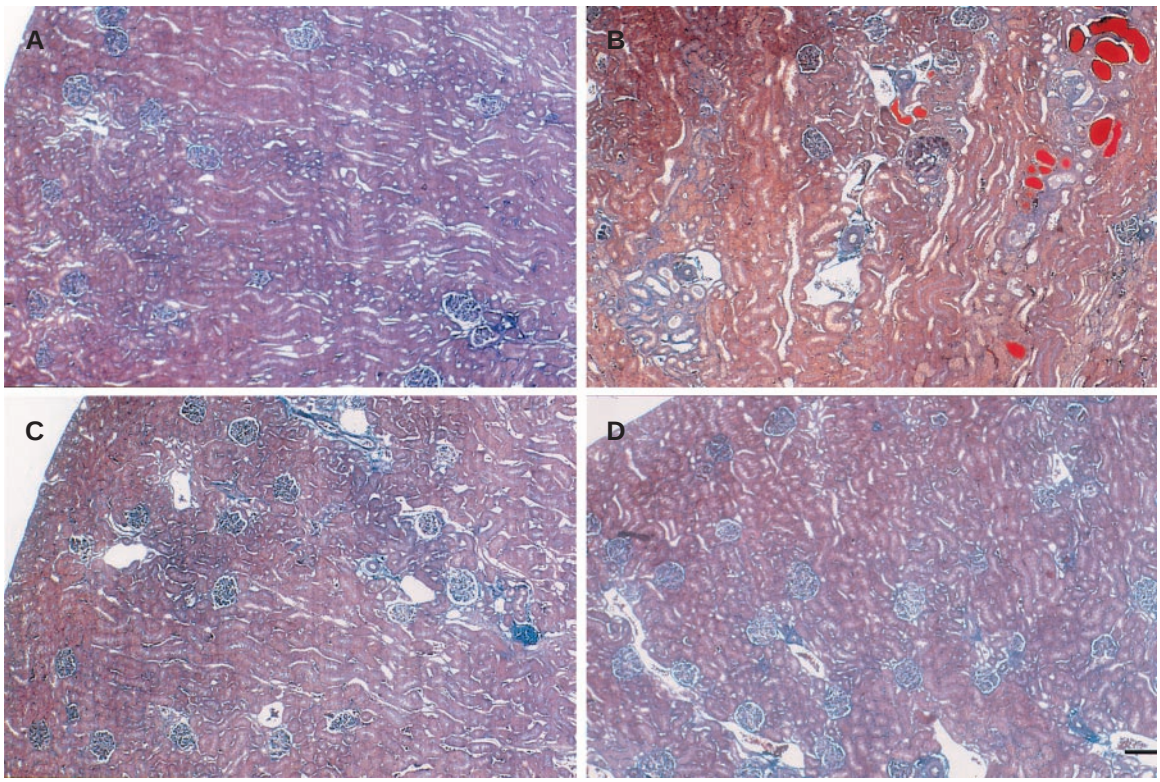

Figure 4 Sections from the SHR kidneys processed with Masson's trichrome staining. Control group has normal histology (A). CsA group has stripes of interstitial fibrosis (B). Treatment with enalapril (30 mg kg^{-1} d^{-1}) (C) or with valsartan (3 mg kg^{-1} d^{-1}) (D) prevented the development of CsA-induced alterations. (Scale bar 150 μm).

In contrast, in the CsA group one-third of the glomeruli were apparently damaged. Glomerular damage index was about eight times higher than in the control group (Figure 3). Within the affected glomeruli, the pathological changes varied from a slight mesangial matrix expansion to a severe necrosis and capillary collapse.

Interstitial fibrosis of striped pattern was also seen in the cortex of CsA-treated rats (Figure 4). Tubular atrophy and dilatation were seen in the tubuli surrounded by interstitial fibrotic tissue. Large renal arteries in the CsA group were mostly normal, only a slight thickening of the adventitia was seen, whereas varying degrees of smaller arterioles had signs of medial changes ranging from a minor smooth muscle cell vacuolization to massive smooth muscle and endothelial cell proliferation, leading to complete occlusion of the vessel. There were also an increase in the amount of connective tissue surrounding the arterioles.

All the CsA-induced pathological renal changes were completely prevented by blocking the renin-angiotensin system with enalapril and valsartan (Figures 3 and 4).

Icatibant did not affect renal histology during CsA and enalapril treatment (data not shown).

Urine electrolytes

The excretion of sodium and potassium were lower in CsA group than in the control group ($P<0.01$ and $P<0.05$, respectively; Table 2). Enalapril or valsartan did not influence sodium or potassium excretions during CsA treatment (Table 2). There were no significant differences between the groups in the 24-h urinary excretions of magnesium. Icatibant did not affect urine electrolytes during CsA and enalapril treatment (Table 3).

Discussion

In the present study, CsA caused hypertension and nephrotoxicity in SHR on high-sodium diet during the developmental phase of hypertension. The renal changes included a decrease in glomerular filtration rate as evidenced by decreased creatinine clearance, proteinuria and glomerular damage, as well as tubulointerstitial fibrosis of striped pattern in renal cortex. These are typical findings of CsA toxicity in CsA-treated patients (Mason, 1989; Faulds *et al*., 1993). The dose of CsA in our study has been shown to produce CsA blood levels that are within the therapeutic range in clinical practice (Mervaala *et al*., 1997). Inhibition of the renin-angiotensin system with the ACE inhibitor enalapril or the AT_1 receptor antagonist valsartan attenuated the CsA-induced hypertension and completely prevented from the renal toxicity in SHR.

The most widely used animal model to study the renal toxicity of CsA is sodium-depleted rat, which develop renal histological changes similar to human findings (Burdmann *et al*., 1995; Pichler *et al*., 1995). However, in SHR on high-sodium diet CsA also caused hypertension that is a common adverse effect in patients receiving CsA. In contrast, no elevation in blood pressure was seen in sodium-depleted rats (Burdmann *et al*., 1995).

The exact mechanisms of CsA-induced hypertension and nephrotoxicity are not known, but there are several lines of evidence suggesting an involvement of the renin-angiotensin system. This is also supported by our study through the finding that plasma renin activity was higher in the CsA-treated SHR than in the control group. CsA has been reported to stimulate renin production and release in isolated juxtaglomerular cells (Kurtz *et al*., 1988). An increase in plasma renin activity has been demonstrated in CsA-treated rats either on sodium depletion (Burdmann *et al*., 1995), on standard chow (Abassi *et al*., 1996), or during high intake of sodium (Mervaala *et al*., 1997; 1999). In this study, enalapril and valsartan had a tendency to an additional increase in plasma renin activity in CsA-treated rats, probably due to the lack of negative feedback mechanisms during ACE inhibition and angiotensin AT_1 receptor antagonism (see Brunner *et al*., 1993 for review). CsA has also been reported to elevate plasma angiotensin II levels (Edwards *et al*., 1994). Furthermore, long-term treatment with CsA upregulates AT_1 receptors in vascular and renal tissue (Iwai *et al*., 1993; Regitz-Zagrosek *et al*., 1995) and increases vasoconstrictive effect of angiotensin II (Auch-Schwelk *et al*., 1993; Takeda *et al*., 1995).

Chronic administration of angiotensin II in rats produces renal injury similar to that observed in CsA nephropathy (Giachelli *et al*., 1994; Noble & Border, 1997). Both angiotensin II and CsA cause an overexpression of transforming growth factor (TGF-β_1), a cytokine which has been implicated in the pathophysiology of many fibrotic diseases of the kidney and other organs (Pichler *et al*., 1995; Noble & Border, 1997). In addition, tubulointerstitial expression of osteopontin, an adhesion molecule associated with renal fibrosis, is increased by both angiotensin II (Giachelli *et al*., 1994) and CsA (Pichler *et al*., 1995). Thus, activation of the renin-angiotensin system by CsA may be at least partly responsible for the interstitial fibrosis through stimulation of TGF-β_1 and osteopontin expression. This is also supported by the finding that the AT_1 receptor antagonist losartan reduced the CsA-induced interstitial fibrosis concomitantly with decreased renal TGF-β_1 and osteopontin expression (Pichler *et al*., 1995).

CsA reduces glomerular filtration rate and renal blood flow by causing vasoconstriction of the glomerular afferent arterioles (Murray *et al*., 1985; Myers *et al*., 1988). When renal blood flow is substantially reduced, angiotensin II contributes to the maintenance of glomerular filtration rate by constricting the efferent glomerular arterioles. In such conditions, dilation of the efferent arterioles by inhibitors of the renin-angiotensin system may potentiate the reduction in glomerular filtration rate. There are clinical studies in which ACE inhibitors have not affected renal function in CsA-treated patients (Sennesael *et al*., 1995; Hausberg *et al*., 1999). In addition, enalapril and losartan prevented the CsA-induced renal morphological changes but not the decrease in glomerular filtration rate in sodium-depleted rats (Burdmann *et al*., 1995; Pichler *et al*., 1995). On the contrary, a reversion of the CsA-induced decrease in glomerular function by both enalapril and valsartan was seen in our study. This may be due to higher blood pressure and glomerular perfusion pressure in SHR during high-sodium intake compared to rats on sodium-depleted diet, which had very low systemic blood pressure below the renal autoregulatory range (Burdmann *et al*., 1995). It is also possible that preservation of kidney function by the antagonists of the renin-angiotensin system is at least partly due to prevention from glomerular damage rather than direct effects on renal haemodynamics. Captopril and saralasin have previously been shown to increase glomerular filtration rate and renal blood flow in CsA-treated normotensive rats (Kaskel *et al*., 1987). Enalapril was also able to prevent the CsA-induced decline in glomerular

filtration rate in diabetic patients (Hannedouche *et al*., 1996).

In this study, CsA caused proteinuria which was reversed by inhibition of the renin-angiotensin system. CsA has been reported to induce proteinuria simultaneously with glomerular injury and increased glomerular permeability (Griffiths *et al*., 1996). Accordingly, protection from proteinuria by the renin-angiotensin system inhibition may be due to prevention of the glomerular damage. ACE inhibition has been reported to reduce proteinuria in CsA-treated renal allograft patients (Hausberg *et al*., 1999).

CsA-induced functional and morphological renal damage was prevented by enalapril and valsartan equally. No dose dependency with valsartan was seen. This suggests that the lower dose of valsartan (3 mg kg^{-1} d^{-1}) was enough for the renal protection. On the other hand, CsA-induced hypertension was totally abolished by valsartan at the higher dose of 30 mg kg^{-1} d^{-1} but not by the lower dose of valsartan or by enalapril. ACE inhibitors and AT_1 receptor antagonists have at least three distinct mechanisms which might lead to differences in their antihypertensive effect. Firstly, there is evidence that ACE is only partly responsible for angiotensin II formation because other enzymes are also able to produce angiotensin II (Okunishi *et al*., 1993; Hollenberg *et al*., 1998). AT_1 antagonists block the effects of angiotensin II regardless of whether it is generated by ACE or by some other enzymatic route. However, the alternative pathways of angiotensin II formation appear to have a minimal role in rodents (Okunishi *et al*., 1993).

The second possible difference between ACE inhibitors and AT_1 antagonists is their influence on angiotensin II receptor subtypes, AT_1 and AT_2. Most of the cardiovascular and renal effects of angiotensin II, such as vasoconstriction and decreased glomerular filtration and renal blood flow are mediated by AT_1 receptors (for review see Inagami, 1999). AT_2 receptors seem to mediate effects which are opposite to AT_1-mediated effects, including lowering of blood pressure (Inagami, 1999). AT_1 receptor antagonists increase angiotensin II formation which can activate AT_2 receptors. Thus, valsartan treatment may have led to an augmentation of blood pressure-lowering effect mediated by AT_2 receptors.

The third difference between ACE inhibitors and AT_1 receptor antagonists is that ACE inhibitors reduce the degradation of vasodilatory bradykinin, whereas AT_1 antagonists are devoid of this effect. However, bradykinin B_2 receptor antagonist icatibant did not impair the antihypertensive or nephroprotective effects of enalapril, suggesting that the effects of enalapril were not mediated to a significant degree by bradykinin. Co-treatment with icatibant even seemed to have a slight though not significant blood pressure-lowering effect during CsA and enalapril treatment. This somewhat unexpected result is in accordance with earlier studies with an ACE inhibitor- and icatibant-treated SHR (O'Sullivan & Harrap 1995). The mechanism of this effect is unknown. One possible explanation is bradykinin-induced increase in catecholamine release, which can be blocked by a B_2 receptor antagonist (Dendorfer *et al*., 1996). This effect of bradykinin has been reported to be evident during ACE inhibition (Rump *et al*., 1997).

We also assessed the role of the renal kallikrein-kinin system in CsA toxicity by measuring urinary kallikrein excretion. It has been reported in normotensive rats that the activity of the kallikrein-kinin system was elevated during CsA treatment as a compensation for drug-induced vasoconstriction and hypertension (Wang *et al*., 1997). However, a decreased activity of the kallikrein-kinin system has been found in essential hypertension as well as in rat models of genetic hypertension (for review see Majima & Katori, 1995). In our experiments, CsA alone or combined with enalapril or valsartan did not affect urinary kallikrein excretion. Thus, an essential role of the renal kallikrein-kinin system on CsA toxicity on SHR during high intake of sodium seems not likely.

In addition to angiotensin II, other vasoactive substances may be involved in CsA toxicity. One of these is endothelin, which has been suggested to contribute to CsA-induced vasoconstriction of the renal afferent arterioles (Lanese & Conger, 1993). An increased production of endothelin has been reported after the administration of CsA (Abassi *et al*., 1996). Moreover, endothelin receptor antagonists attenuated CsA-induced vasoconstriction (Lanese & Conger, 1993) and hypertension (Takeda *et al*., 1995; Oriji & Keiser, 1998). Endothelin receptor antagonists also prevented CsA-induced renal dysfunction (Kon *et al*., 1995; Abassi *et al*. 1996). However, the tubulointerstitial fibrosis associated with CsA was not suppressed by an endothelin receptor antagonist (Kon *et al*., 1995), whereas enalapril was able to prevent the development of interstitial fibrosis (Kon *et al*., 1995); Burdmann *et al*., 1995). The renal damage by CsA was also totally prevented by the inhibitors of the renin-angiotensin system in our study.

CsA did not cause marked left ventricular hypertrophy although blood pressure was dramatically elevated compared to the control group. Left ventricular hypertrophy-index was markedly lower in enalapril as well as in the both valsartan groups compared to the control and CsA groups even though only higher dose of valsartan decreased blood pressure compared to the control group. CsA as a calcineurin inhibitor has been suggested to offer some protection against the development of left ventricular hypertrophy (Sussman *et al*., 1998). ACE inhibitors have been reported to prevent left ventricular hypertrophy in SHR during high-sodium diet (Mervaala *et al*., 1994). Taken together, it seems logical that inhibitors of the renin-angiotensin system reduced left ventricular hypertrophy in the presence as well as in the absence of CsA-administration.

In summary, CsA produced clinically relevant adverse effects such as hypertension and kidney dysfunction as well as morphological nephrotoxicity in SHR during high-sodium diet. Valsartan was slightly better than enalapril in preventing from CsA-induced hypertension in SHR on high-sodium diet, whereas both drugs similarly protected against CsA nephropathy. The renin-angiotensin but not the renal kallikrein-kinin system seems to play a central role in CsA- and sodium-induced toxicity.

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