Original Article

Effects of the Renin-Angiotensin Blockade on Renal Functions, Renal Norepinephrine Contents and Oxidative Stress in Cyclosporine Induced Nephrosis Rats

Suvaporn Panasjaroen Sarinee Kalandakanond-Thongsong Chollada Buranakarl*

Abstract

The experiment was conducted to investigate the effects of renin-angiotensin blockade on renal function, renal norepinephrine (NE) contents and renal oxidative stress in Cyclosporine A (CsA) induced renal impairment in rats. Rats were assigned into three groups; group 1 (control group), receiving vehicle (propylene glycol) 1 ml/kg./day, subcutaneously (s/c). for 28 days; group 2 (CsA group), receiving CsA 15 mg/kg/day, s/c for 28 days; group 3 (CsA and losartan (LST) group), receiving CsA 15 mg/kg./day, s/c with the administration of LST 10 mg/k.g./day orally for 28 days. The results showed that CsA administration alone elevated mean arterial pressure (MAP), reduced renal function, as assessed by increased plasma urea nitrogen and decreased glomerular filtration rate. CsA stimulated renal sympathetic activity as assessed by increased renal NE content and induced oxidative stress, as indicated by increased renal malondialdehyde (MDA) and decreased concentrations of renal reduced glutathione (GSH). Losartan, the angiotensin type 1 (AT_1) receptor antagonist markedly decreased MAP, improved renal function, reduced renal NE content and reduced renal MDA. It is concluded that enhanced renal sympathetic activity and oxidative stress by CsA were mediated by angiotensin II. Blocking the renin-angiotensin system can ameliorate the renal impairment caused by CsA.

Keywords : cyclosporine, losartan, norepinephrine, oxidative stress, rats

Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, Henri Dunant Road, Patumwan, Bangkok 10330, Thailand.

***Corresponding author; E-mail : bchollad@chula.ac.th**

Thai J. Vet. Med., 2008. 38(3): 9-18

บทคัดย่อ

ผลของการยับยั้งระบบเรนิน-แองจิโอเทนซิน ต่อการทำหน้าที่ของไต ปริมาณนอร์อิพิเนฟรินในไต และความเครียดออกซิเดชั่นในไต ในหนูที่ถูกเหนี่ยวนำให้เกิดความเสียหายของไตด้วยไซโคลสปอริน

สุวพร พนัสเจริญ สฤณี กลันทกานนท์ ทองทรง ชลลดา บูรณกาล*

ทำการศึกษาผลของการยับยั้งระบบเรนิน-แองจิโอเทนซินต่อการทำหน้าที่ของไต ปริมาณนอร์อิพิเนฟรินในไต และความเครียดออกซิเดชั่นในไต ในหนูที่ถูกเหนี่ยวนำให้เกิดความเสียหายของไตด้วยไซโคลสปอริน โดยแบ่งหนูออกเป็น 3 กลุ่ม ดังนี้ กลุ่มที่ 1 (กลุ่มควบคุม) ได้รับโพรไพลีนไกลคอลขนาด 1 มล. ต่อ กก. น้ำหนักตัวต่อวัน ฉีดเข้าใต้ผิวหนัง นาน 28 วัน กลุ่มที่ 2 (กลุ่มไซโคลสปอริน) ได้รับไซโคลสปอรินขนาด 15 มก. ต่อกก.น้ำหนักตัว ต่อวัน ฉีดเข้าใต้ผิวหนัง นาน 28 วัน และกลุ่มที่ 3 (กลุ่มไซโคลสปอรินและโลซาทาน) ได้รับไซโคลสปอรินขนาด 15 มก. ต่อกก. น้ำหนักตัว ต่อวันฉีดเข้าใต้ผิวหนัง และโลซาทานขนาด 10 มก. ต่อกก. น้ำหนักตัว ต่อวันโดยการกิน นาน 28 วัน จากการทดลองพบว่าไซโคลสปอรินทำให้ความดันเลือดสูงขึ้น การทำหน้าที่ของไตลดลง ซึ่งประเมินจากการเพิ่มขึ้นของระดับยูเรียในพลาสม่าและการลดลงของอัตราการกรองของไต ไซโคลสปอรินกระตุ้นการทำงานของระบบประสาทซิมพาเตติกที่ไต ซึ่งประเมินจากการเพิ่มขึ้นของปริมาณนอร์อิพิเนฟรินในไต และเหนี่ยวนำให้เกิดความเครียดออกซิเดชั่นเพิ่มขึ้น โดยการเพิ่มขึ้นของปริมาณเมลอนอัลดีไฮด์ในไต และทำให้ปริมาณรีดิวส์กลูตาธัยโอนในไตลดลง เมื่อให้โลซาทาน ซึ่งเป็นสารยับยั้งการจับของแองจิโอเทนซินทูกับตัวรับชนิด AT_1 พบว่าความดันเลือดลดลง การทำหน้าที่ของไตดีขึ้น ปริมาณนอร์อิพิเนฟรินในไตลดลง และลดปริมาณ เมลอนอัลดีไฮด์ในไต จากผลการทดลองสรุปว่าการกระตุ้นประสาทซิมพาเตติกและความเครียดออกซิเดชั่นที่ไต จากผลของไซโคลสปอริน ผ่านสารแองจิโอเทนซินทู การยับยั้งระบบเรนิน-แองจิโอเทนซินสามารถลดความเสียหายของไตในหนูที่ได้รับไซโคลสปอรินได้

คำสำคัญ : ไซโคลสปอริน โลซาทาน นอร์อิพิเนฟริน ความเครียดออกซิเดชั่น หนู

ภาควิชาสรีรวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ถนนอังรีดูนังต์ ปทุมวัน กรุงเทพ ฯ 10330
***ผู้รับผิดชอบบทความ**

Introduction

Cyclosporine (CsA) has been proven to be useful in several diseases. However, the clinical usage of CsA is often limited by nephrotoxicity and hypertension. A number of adverse effects of CsA on renal function have been described, including severe impairment in the glomerular filtration rate (GFR), a reduction in renal blood flow (RBF) related to renal vasoconstriction (Li et al., 2004).

Several mechanisms underlying chronic CsA nephrotoxicity have been proposed. Cyclosporine may involve the sympathetic nervous system via increased afferent sympathetic nerve activity (Zhang and Victor, 2000). Decreased GFR and RBF by cyclosporine is greatly attenuated by either renal nerve denervation or the administration of an alpha adrenergic blocker or a central sympatholytic drug (Murray et al., 1985; Murray and Paller, 1986).

Several evidences suggest the involvement of renin angiotensin system (RAS) in CsA induced nephrotoxicity (Mason et al., 1991). Increased renin content in kidney tissues and hyperplasia of granular cells in the juxtaglomerular apparatus have been observed in CsA-treated rats (Nitta et al., 1987; Tufro-McReddie et al., 1993). Avdonin et al. (1999) reported that chronic CsA administration upregulates angiotensin type-1 (AT_1)

receptors in vascular and renal tissue. Activation of the RAS in the failing kidneys may be responsible for the increased sympathetic nerve discharge. Ligtenberg et al. (1999) reported decreased muscle sympathetic nerve activity in patients with chronic renal failure after treatment with an angiotensin-converting enzyme inhibitor. It is possible that CsA activates sympathetic nerve activity via the augmentation of AII production.

CsA increases reactive oxygen species (ROS) production by increasing renal sympathetic nerve activity resulting from vasoconstriction. These alterations may be mediated via RAS since it was recently shown that AT_1-receptor antagonist could prevent increase in vascular superoxide and kidney TBARS as well as increase in blood pressure in CsA-treated rats (Nishiyama et al., 2003).

The objectives of this study are to investigate whether firstly, CsA impairs renal function with a concomitant increase in renal sympathetic nerve activity by measuring intrarenal NE concentration, secondly, whether CsA can induce an increase in lipid peroxidation and, thirdly, whether these changes are mediated through RAS which can be ameliorated by AT_1-receptor antagonist, losartan.

Materials and Method

Experimental animals: Male Sprague-Dawley rats weighing between 200 to 250 g were obtained from the National Laboratory Animal Center, Mahidol University, Thailand. The animals were housed individually and were maintained at 25±2°C under a controlled light and dark cycle (L:D = 12:12). All the rats were fed *ad libitum* with standard rat chow (CP, Thailand) and allowed free access to tap water. All procedures were done with the approval of the Animal Use Committee at the Faculty of Veterinary Science, Chulalongkorn University. The animals were divided into three groups. Group 1 (control, n = 21), rats received a daily subcutaneous injection of propylene glycol (1 ml/kg.) and gavage with water (1 ml/kg) as a control for 28 days. Group 2 (CsA, n = 28), rats received a daily subcutaneous injection of CsA (Sandimmun®, Novartis Pharma AG, Basle, Switzerland) at a dose of 15 mg/kg and gavage with water (1 ml/kg.) for 28 days. Group 3 (CsA + LST, n = 21), rats received a daily subcutaneous injection of CsA at a dose of 15 mg/kg and LST (Cozaar®, Merck Sharp & Dohme Ltd., England) at a dose of 10 mg/kg by gavaged for 28 days.

Body weight (BW) and feed intake were recorded daily throughout the experimental period. At days 0, 14 and 28, the rats were placed in individual metabolic cage for 24-hr urine collection for the measurement of urine volume, urinary protein and electrolyte concentrations (Na, K, Cl), osmolarity and MDA concentrations. Plasma was collected by cutting the tip of the rat's tail vein for the measurement of plasma urea nitrogen (PUN) and creatinine. Renal clearance was performed in 32 animals from three groups on day 29. Other animals were euthanized to evaluate catecholamine content and oxidative stress in the kidneys.

Renal function study: Renal clearance was performed in 9, 14 and 9 rats, in three groups, respectively on day 29 using inulin and para-aminohippurate (PAH). The procedures for and calculation of the measurements of renal clearance and blood pressure were performed as previously described (Buranakarl et al., 2003) except that the urine was collected from a catheter passed into the urinary bladder. At the end of the renal clearance study, blood was collected from the right femoral artery to measure creatinine, PUN, osmolality and electrolytes (Na, K, Cl).

In each group, some rats (n = 12, 14 and 12 in group 1, 2 and 3, respectively) were anesthetized with halothane (Rhodia Organique Fine Ltd., UK.). Blood was collected from cardiac puncture to measure plasma MDA and NE. The right kidney was removed and stored at -80°C for the determination of catecholamines. Catecholamine content was determined by reversed-phase high- performance liquid chromatography (HPLC) detection as previously described (Viera-Coelho et al.,

1999). The left kidney was removed immediately and stored at -80°C for the analysis of GSH, MDA and catalase (CAT) activity.

Analytical procedures: The concentrations of plasma urea nitrogen and creatinine were measured by automate analyzer (Humalyzer 2000 Human, H.E. supply Ltd. TART.). The sodium and potassium concentrations were determined by flame photometer (Flame photometer 410C, Ciba Corning Diagnostic Instruments, Halstead, USA). The chloride concentration was determined by chloridometer (Chloride Analyzer 925, Ciba Corning Inc., USA). The osmolarity was measured using an osmometer (Osmometer 3D3, Advanced instruments, Inc., MA, USA). The fractional excretions of electrolytes were calculated from the renal electrolyte to inulin clearance ratio. The inulin and PAH concentrations were determined by an antrone method (Young and Raisz, 1952) and the method of Brun (1951), respectively. Extractions of catecholamine in plasma and the kidneys were performed according to previously published procedures by Anton and Sayre (1962) and Eldrup and Richter (2000) respectively, using the HPLC system with an electrochemical detector (Bioanalytical systems, West Lafayette, IN, USA.). Kidney malondialdehyde was assayed in the form of thiobarbituric acid reacting substances (TBARS) as described by Ohkawa et al. (1979). The Kidney GSH and CAT activity were determined by the methods of Beutler et al., (1963) and Aebi (1983), respectively.

Statistical analysis: All data are expressed as mean± standard error (SE). To compare between groups, a one-way analysis of variance (One-way ANOVA) or ANOVA on rank were used. To compare within the same group, one way repeated measures ANOVA or ANOVA with repeated measures on ranks were used. The post-hoc analyses with Student-Newman-Keuls or Dunn's tests were used to compare the data between all pairwise. Differences between means were considered statistically significant at $p<0.05$. Sigma-stat software was used for the statistical analysis.

Results

Body weight and feed intake: Mean weight and feed intake were similar before treatment. At day 28 of treatment, the percentage change in BW of group 1 was higher than groups 2 and 3 (58.2±2.3, 34.7±2.4 and 40.9±1.3%, respectively). No significant changes in average feed intake were found at day 27 in any group (18.36±1.24, 20.99±1.27 and 21.9±1.21 g per day, respectively).

Plasma creatinine and PUN concentrations: The creatinine concentrations did not differ among groups at any period of measurement. The PUN was significantly higher in group 2 both on day 14 and 28 (27.88±1.80 and 35.98±3.05 mg%, n = 28, respectively) compared with group 1 (15.53±0.44 and 19.76±1.08 mg%, n = 21). In group 3, PUN had the tendency to be lower than group 2 on day 14 and day 28 (19.26±1.22 and 26.72±1.52 mg%, n = 21, respectively).

Plasma osmolality and plasma electrolyte concentration: There was significantly increased in plasma osmolality, sodium and potassium in group 2 as compared to group 1 (323.1±2.6, 140.6±1.0, 4.86±0.15, n = 28 vs 311.5±1.4 mOsm/L, 133.9±1.2 mEq/L, 4.32±0.15 mEq/L, n = 21, respectively). The LST treatment did not modify these effects. Plasma chloride concentration was not different among groups.

Renal haemodynamics: By comparing with control group 1, the glomerular filtration rate decreased significantly in group 2 but improved in group 3 (Table 1). The effective renal plasma flow (ERPF) and the effective renal blood flow (ERBF) were not changed in group 2 but slightly increased in group 3. Losartan significantly increased ERBF compared with group 2.

Cyclosporine treatment decreased FF significantly as compared with group 1. Co-treatment with LST did not affect the FF. The RVR of group 2 appeared to be higher than group 1. The RVR significantly decreased in group 3 as compared to group 2 although it was not different from group 1 The packed cell volume (PCV) of group 2 was significantly lower compared with groups 1 and 3.

The MAP was significantly increased in group 2 compared with group 1. Co-treatment with LST significantly decreased MAP compared with group 2 (Figure 1). There was no significant change in the urine flow rate in all groups.

Tubular functions: No difference was found in osmolar clearance and free water clearance in all groups. In group 2, fractional excretion of Na was significantly decreased compared to group 1 (0.275±0.052, n = 14 vs 0.347±0.035 %, n = 9) but there was no difference from group 3 (0.281±0.030 % , n = 9). At day 0 and day 14, urinary electrolyte excretions (Na^+, K^+, Cl^-) were not significantly different among groups. Urinary sodium excretion at day 28 of treatment was significantly decreased in group 2 compared with group 1(625±82, n = 28 vs 909±88 μEq /day, n = 21). Reduced Na excretion was also found in group 3 (708±90 μEq /day, n = 21) but was not significant from group 1 and 2. There was no significant change in urinary potassium and chloride excretions among groups.

Urinary protein excretion and urinary protein creatinine ratio (UPC): At day 28, urinary protein excretion in group 2 was similar to group 1 (24.98±8.47, n = 24 vs 20.22±1.47 mg/day, n = 17). Concomitant treatment with LST significantly decreased urinary protein excretion compared with groups 1 and 3 (11.67±1.99 mg/day, n = 18). At day 28, UPC ratio was significantly decreased in group 3 (1.36±0.19) compared with groups 1 and 2 (2.15±0.11 and 2.07±0.19, respectively).

Oxidative stress: Although the values tended to be higher from day 0 to day 28. No significant differences in urinary MDA excretion and urine MDA creatinine ratio were found among groups at any period (day 0, 14 and 28) of the experiment.

The kidney MDA and plasma MDA concentrations were significantly increased in group 2 compared with group 1. Co-treatment with LST inhibited CsA-induced lipid peroxidation and resulted in a significant decrease in MDA level in the kidney (Figure 2). Cyclosporine produced a significant reduction in renal GSH concentrations compared with the control group. There was no significant difference of renal CAT activity between groups.

Kidney and plasma catecholamine contents: No differences in plasma noerpinephrine (NE), epinephrine (E) and dopamine (DA) concentrations were found among groups. Cyclosporine treatment caused significant increases in kidney NE and E contents as compared to group 1 (Figure 3). Concomitant treatment with LST prevented an increase in the kidney NE and E contents. Kidney DA concentrations were not different among groups.

Table 1 Renal hemodynamics in three groups of rats at 28 days

	Control (n=9)	**CsA (n=14)**	**CsA + LST (n=9)**
GFR (μl/g/min)	3.89±0.16[a]	2.88±0.23[b]	3.31±0.29[ab]
ERPF (μl/g/min)	18.69±1.74	17.46±1.46	21.92±1.59
ERBF (μl/g/min)	32.24±3.03[ab]	28.43±2.42[b]	39.13±3.21[a]
FF (%)	22.70±2.22[a]	17.14±1.03[b]	15.41±1.34[b]
RVR (mmHg/μl.g^{-1}.min^{-1})	3.76±0.50[ab]	5.11±0.78[a]	2.80±0.36[b]
PCV (%)	41.91±0.96[a]	38.43±0.32[b]	43.04±1.55[a]
Urine flow rate (ml/min)	0.012±0.005	0.010±0.001	0.008±0.001

All value are expressed as mean ± SE

[a,b] Means in the same row with different superscripts differ significantly ($p<0.05$) using one way ANOVA or one way ANOVA on rank.

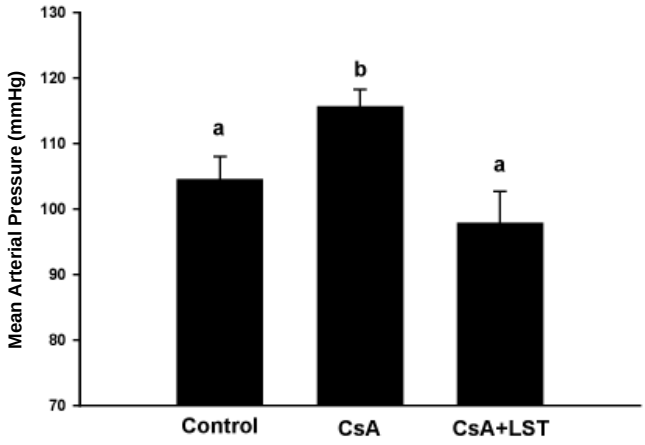

Figure 1 Effect of CsA and cotreatment with losartan (LST) on mean arterial blood pressure. The data were shown as mean±SE.[a,b] Means with different superscripts differ significantly (p<0.05) by using one way ANOVA.

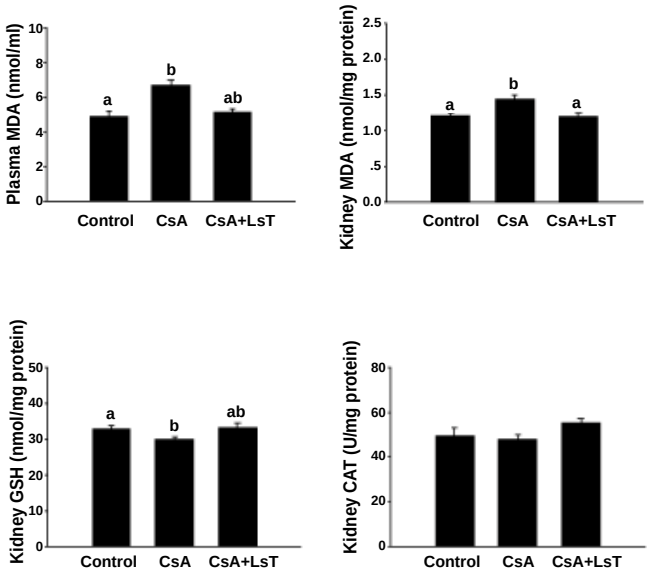

Figure 2 Effect of cyclosporine (CsA) and losartan (LST) on plasma MDA (a), kidney MDA concentration (b), kidney reduced glutathione concentration (c) and kidney catalase activity (d). The data are expressed as mean±SE.[a,b] Means with different superscripts differ significantly (p<0.05) by using one way ANOVA.

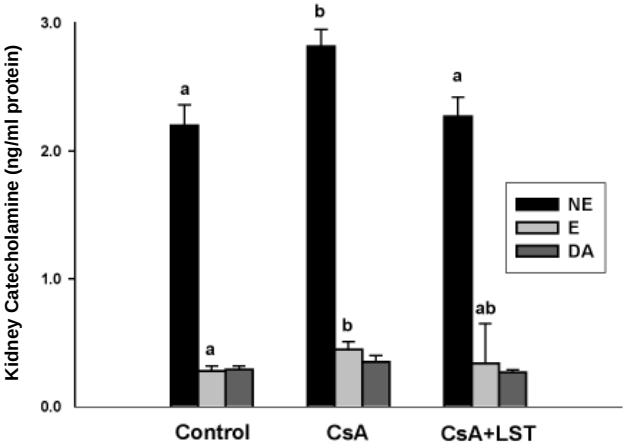

Figure 3 Kidney concentrations of norepinephrine, epinephrine and dopamine in each group. Each bar represents the mean±SE.[a,b] indicates the difference (p<0.05) between the control group, cyclosporine treated group and cyclosporine cotreatment with losartan group by using one way ANOVA.

Discussion

In the present study, CsA significantly reduced BW gain which was similar to the earlier study (Shaltout and Abdel-Rahman, 2003). This was not related to anorexia but it may be due to enhanced metabolism since no effect on feed intake was found. CsA induced renal dysfunction and increased blood pressure. An increase in PUN disproportionates to the increase in serum creatinine concentration due to the greater tubular reabsorption of urea which was found in CsA-treated renal transplant recipient responded to 4 days of volume depletion (Lascow et al., 1990). Increased PUN with a concomittent decreased GFR and ERBF has been previously reviewed in both human and animals (Mason, 1989). In this study, filtration fraction (FF) was reduced suggesting the vasoconstriction occurred more at afferent arteriole. Significant increase in MAP was also demonstrated. Myers et al. (1988) found that CsA caused renal vasoconstriction and hypertension although renal vasoconstriction may be prerenal in origin and related to hypovolemia (Devarajan et al., 1989). Hypertension caused by CsA in the present study is suggested to be due to peripheral vasoconstrictor effect of AII since it can be reversed by giving LST. Moreover, previous studies had demonstrated the CsA-induced hypertension which was mediated by the effects of AII on baroreceptor sensitivity (Ouisuwan and Buranakarl, 2005), or sodium retention (Ciresi et al., 1992). In the present study, sodium retention was demonstrated by decreased urinary and fractional excretion of Na. The decreased

FENa has been suggested to be an early marker of CsA nephrotoxicity after renal transplantation (Morales et al., 1990). The mechanism of renal haemodynamic changes may be due to AII action. Cyclosporine increases renin release and synthesis directly from JG cells (Kurtz et al., 1988). CsA treatment downregulated AT_1-receptor gene expression *in vivo* (Tufro-McReddie et al., 1993) but upregulated AT_1 receptors in human vascular smooth muscle cells (Avdonin et al., 1999). In the present study, administration of AT_1-receptor antagonist could decrease plasma creatinine and PUN levels, improved GFR and normalized blood pressure suggesting that these effects were mediated by AII. The results are supported by previous study in rats which showed that LST attenuated the inflammatory and fibrotic processes induced by CsA (Li et al., 2005). However, some studies showed that the interstitial fibrosis was prevented rather than an improvement of GFR or tubular function (Burdmann et al., 1995). The expression of osteopontin by tubular cells macrophage infiltration, transforming growth factor -1 beta expression and interstitial fibrosis were reduced without changes in GFR (Pichler et al., 1995). Thus, interstitial fibrosis can be dissociated from vascular effects of CsA.

The urinary protein excretion was unchanged in the CsA-treated group although it was demonstrated earlier (Lassila et al., 2000). However, the LST group had the lowest protein excretion. Inhibition of RAS could reduce urinary protein excretion and UPC ratio as has been demonstrated in Spontanous Hypertensive Rats (Lassila et al., 2000) and in renal allograft recipients receiving CsA (Hausberg et al., 1999).

The mechanism responsible for renal impairment and hypertension by CsA has been the subject of much controversy. The possible role of NE has also been suggested. Enhanced renal sympathetic nerve activity may be responsible for renal vasoconstriction and Na retention with subsequent reduction in GFR and hypertension. In the present study, renal NE concentrations were increased significantly in CsA-induced nephrosis rats. Muscle sympathetic nerve firing was increased in cardiac transplant recipients and patients with myasthenia gravis who were treated with CsA (Scherrer et al., 1990). Cyclosporine increased the NE release from the sympathetic nerve endings of rat aorta and in renal sensory nerve ending which involved calcineurin-dependent process (Zhang and Victor, 2000; Tavares et al., 2003). Moreover, renal denervation and alpha-adrenergic blocker could reverse GFR, blood flow and reduce RVR in CsA treatment (Murray and Paller, 1986). However, van den Dorpel et al. (1996) found no change in plasma NE levels in patients with kidney transplant undergoing treatment with CsA. Kaye et al. (1993) described the arterial concentrations of NE and the rate of NE spillover into plasma in CsA treated cardiac transplant recipients as being similar to those of age-matched controls. The renal vasoconstriction associated with CsA therapy was not associated with elevated renal NE spillover. Moreover, giving CsA the same dose as this study in rats maintained on low salt diet showed no differences in either renal function or structure between denervated and sham-operated animals (Elzinga et al., 2000). Differences in results may be due to differences in dose and duration of CsA administration, the subject and the protocol of experimental researches. Moreover, it was proposed that sympathetic stimulation was mediated by enhanced activity of intrarenal RAS. This evidence was supported by reduced renal NE contents after LST treatment in the present study.

Cyclosporine administration resulted in the excess local production of hydroxy radical, leading to lipid peroxidation and nephrotoxicity (Wang and Salahudeen, 1995; Zhong et al., 1998; Hager et al., 2006). Increased renal TBARS and reduced GSH without changes in CAT activity were similar to the previous study (Wolf et al., 1994). The glomerular contraction, tubular cell regeneration, inclusion bodies, vacuolation and calcification were inhibited by specific reactive oxygen scarvenger. Cyclosporine induced ROS production in the kidneys was prevented by either renal denervation

(Zhong et al., 1999) or giving AT_1-receptor antagonist (Padi and Chopra, 2002; Satyanarayana et al., 2002). Besides reduction in both renal catecholamine and lipid peroxidation after LST suggest that AII had interrelationship with sympathetic activity and also involved in oxygen free radicals formation and subsequent lipid peroxidation in the kidney.

In conclusion, CsA caused renal dysfunction and hypertension with a concomittent increase in renal NE content and oxidative stress. These effects were alleviated after LST administration suggesting the mechanisms of CsA induced renal impairement are also mediated via AII and renal dysfunction may be prevented by blocking RAS.

Acknowledgements

This study was supported by the Graduate school and the Faculty of Veterinary Science, Chulalongkorn University

References

Aebi, H. 1983. Catalase. In: Methods of Enzymatic Analysis. H.U. Bergmeyer (ed). New York: Academic Press. 273-282.

Anton, A.H. and Sayre, D.F. 1962. A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. J. Pharmacol. Exp. Ther. 138: 360-374.

Avdonin, P., Cottet-Maire, F., Afanasjeva, G.V., Loktionova, S.A., Lhote, P. and Ruegg, U. 1999. Cyclosporine A up-regulates angiotensin II receptors and calcium response in human vasculas smooth muscle cells. Kidney Int. 55: 2407-2414.

Beutler, E., Duron, O. and Kelly, B.M. 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61: 882-888.

Brun, C. 1951. A rapid method on the determination of para-aminohippuric acid in kidney function tests. J. Lab. Clin. Med. 37: 955-958.

Buranakarl, C., Kitjawonrat, A., Pondeenana, S., Sunyasujaree, B., Kanchanapangka, S., Chaiyabutr, N. and Bovee, K.C. 2003. Comparison of dipyridamole and fosinopril on renal progression in nephrectomized rats. Nephrology 8: 80-91.

Burdmann, E.A., Andoh, T.F., Nast, C.C., Evan, A., Connors, B.A., Coffman, T.M., Lindsley, J. and Bennett, W.M. 1995. Prevention of experimental cyclosporine-induced interstitial fibrosis by losatan and enalapril. Am. J. Physiol. 269: F491-F499.

Ciresi, D.L., Lloyd, M.A., Sandberg, S.M., Heublein, D.M. and Edwards, B.S. 1992. The sodium retaining effects of cyclosporine. Kidney Int. 41: 1599-1605.

Devarajan, P., Kaskel, F.J., Arbeit, L.A. and Moore, L.C. 1989. Cyclosporine nephrotoxicity : blood volume, sodium conservation, and renal hemodynamics. Am. J. Physiol. 256: F71- F78.

Eldrup, E. and Richter, E.A. 2000. DOPA, dopamine and DOPAC concentrations in the rat gastrointestinal tract decreasing during fasting. Am. J. Physiol. Endocrinol. Metab. 279: E815-E822.

Elzinga, L.W., Rosen, S., Burdmann, E.A., Hatton, D.C., Lindsley, J. and Bennett, W.M. 2000. The role of renal sympathetic nerves in experimental chronic cyclosporine nephropathy. Transplantation. 69: 2149-2153.

Hagar, H.H., Etter, E.E. and Arafa, M. 2006. Taurine attenuates hypertension and renal dysfunction induced by cyclosporine A in rats. Clin. Exp. Pharmacol. Physiol. 33: 189-196.

Hausberg, M., Barenbrock, M., Hohage, H., Muller, S., Heidenreich, S., Rahn, K.H. 1999. ACE inhibitor versus beta-blocker for the treatment of hypertension in renal allograft recipients. Hypertension. 33: 862-868.

Kaye, D., Thompson, J., Jenning, G. and Esler, M. 1993. Cyclosporine therapy after cardiac transplantation causes hypertension and renal vasoconstriction without sympathetic activation. Circulation. 88: 1101-1109.

Kurtz, A., Bruna, N.D. and Kuhn, K. 1988. Cyclosporine A enhances renin secretion and production in isolated juxtaglomerular cells. Kidney Int. 33: 947-953.

Lascow, D.A., Curtis, J.J., Luke, R.G., Julian, B.A., Jones, P., Deierhoi, M.H., Barber, W.H. and Diethelm, A.G. 1990. Cyclosporine-induced changes in glomerular filtration rate and urea excretion. Am. J. Med. 88: 497-502.

Lassila, M., Finckenberg, P., Pere, A.K., Krogerus, L., Ahonen, J., Vapataalo, H. and Nurminen, M.L. 2000. Comparison of enalapril and valsartan in cyclosporine A- induced hypertension and nephrotoxicity in spontaneously hypertensive rats on high-sodium diet. Br. J. Pharmacol. 130: 1339-1347.

Li, C., Lim, S.W., Sun, B.K. and Yang, C.W. 2004. Chronic cyclosporine nephrotoxicity: New insights and preventive strategies. Yonsei Med. J. 45: 1004-1016.

Li, C., Sun, B.K., Lim, S.W., Song, J.C., Kang, S.W., Kim, Y.S., Kang, D.H., Cha, J.H., Kim, J. and Yang, C.W. 2005. Combined effects of losartan and pravastatin on interstitial inflammation and fibrosis in chronic cyclosporine-induced nephropathy. Transplantation. 79: 1522-1529.

Ligtenberg, G., Blankestijn, P.J., Oey, P.L., Klein, I.H.H., Oei, L.T.D., Boomsma, F., Wieneke, G.H., Huffelen, A.C.V. and Koomans, H.A. 1999. Reduction of sympathetic hyperactivity by enalapril in patients with chronic renal failure. N. Engl. J. Med. 340: 1321-1328.

Mason, J. 1989. Pharmacology of cyclosporine (Sandimmune) VII. Pathophysiology and toxicology of cyclosporine in humans and animals. Pharmacol. Rev. 42: 423-434.

Mason, J., Muller-Schweinitzer, E., Dupont, M., Casellas, D., Mihatsch, M., Moore, M. and Kaskel, F. 1991. Cyclosporine and the renin-angiotensin system. Kidney Int. 39: S28-S32.

Morales, J.M., Andres, A., Hernandez, I., Prieto, C., Ortuno, T., Montoyo, C., Moreno, E., Rodriguez-Paternino, E., Farias, J. and Rodicio, J.L. 1990. Fractional excretion of sodium is an early predictor of cyclosporine nephrotoxicity after renal transplantation. Transplant. Proc. 22(4): 1728-1729.

Murray, B.M. and Paller, M.S. 1986. Beneficial effects of renal denervation and prazosin on GFR and renal blood flow after cyclosporine in rats. Clin. Nephrol. 25(suppl 1): S37-S39.

Murray, B.M., Paller, M.S. and Ferris, T.F. 1985. Effect of cyclosporine administration on renal hemodynamics in conscious rats. Kidney Int. 28: 767-774.

Myers, B.D., Sibley, R., Newton, L., Tomlanovich, S.J., Boshkos, C., Stinson, E., Luetscher, J.A., Whitney, D.J., Krasny, D., Coplon, N.S. and Perlroth, M.G. 1988. The long-term course of cyclosporine-associated chronic nephropathy. Kidney Int. 33: 590-600.

Nishiyama, A., Kobori, H., Fukui, T., Zhang, G., Yao, L., Rahman, M., Hitomi, H., Kiyomoto, H., Shokoji, T., Kimura, S., Kohno, M. and Abe, Y. 2003. Role of angiotensin II and reactive oxygen species in cyclosporine A-dependent hypertension. Hypertension 42: 754-760.

Nitta, K., Friedman, A.L., Nicastri, A.D., Paik, S. and Friedman, E.A. 1987. Granular juxtaglomerular cell hyperplasia caused by cyclosporine. Transplantation. 44: 417-421.

Ohkawa, H., Ohisi, N. and Yagi, K. 1979. Assay for lipid peroxidation in animal tissue thiobarbituric acid reaction. Ann. Biochem. 95: 531-538.

Ouisuwan, S. and Buranakarl, C. 2005. Effect of cyclosporin A on blood pressure, baroreceptor reflex and renal function in dogs. Vet. Res. Comm. 29: 201-213.

Padi, S.S. and Chopra, K. 2002. Selective angiotensin II type 1 receptor blockade ameliorates cyclosporine nephrotoxicity. Pharmacol. Res. 45(5): 413-420.

Pichler, R.H., Franceschini, N., Young, B.A., Hugo, C., Andoh, T.F., Burdmann, E.A., Shankland, S.J., Alpers, C.E., Bennett, W.M., Couser, W.G. 1995. Pathogenesis of cyclosporine nephropathy: roles of angiotensin II and osteopontin. J. Am. Soc. Nephrol. 6: 1186-1196.

Satyanarayana, S., Padi, V. and Chopra, K. 2002. Selective angiotensin II type 1 receptor blockade ameliorates cyclosporine nephrotoxicity. Pharmacol. Res. 45: 413-420.

Scherrer, U., Vissing, S.F., Morgan, B.J., Rollins, J.A., Tindall, S.A., Ring, S., Hanson, P., Mohanty, P.K. and Victor, R.G. 1990. Cyclosporine-induced sympathetic activation and hypertension after heart transplantation. N. Engl. J. Med. 323: 693-699.

Shaltout, A. and Abdel-Rahman, A.A. 2003. Cyclosporine induces progressive attenation of baroreceptor heart rate response and cumulative pressor response in conscious unrestrained rats. J. Pharmacol. Exp. Ther. 305: 966-973.

Tavares, P., Riberio, F. and Teixeira, F. 2003. Cyclosporine effect on noradrenaline release from the sympathetic nervous endings of rat aorta. Pharmacol. Res. 47: 27-33.

Tufro-McReddie, A., Gomez, R.A., Norling, L.L., Omar, A.A., Moore, L.C. and Kaskel, F.J. 1993. Effect of CsA on the expression of renin and angiotensin type1 receptor genes in the rat kidney. Kidney Int. 43: 615-622.

van den Dorpel, M.A., van den Meiracker, A.H., Lameris, T.W., Boomsma, F., Levi, M. and Weimar, W. 1996. Cyclosporine A impairs the nocturnal blood pressure fall in renal transplant recipients. Hypertension. 28: 304-307.

Viera-Coelho, M.A., Hussain, T., Kansra, V., Serrao, M.P., Guimaraes, J.T., Pestana, M., Soares-da-Silva, P. and Lokhandwala, M.F. 1999. Aging, high salt intake, and renal dopaminergic activity in Fischer 344 rats. Hypertension. 34: 666-672.

Wang, C. and Salahudeen, A.K. 1995. Lipid peroxidation accompanies cyclosporine nephrotoxicity: Effects of vitamin E. Kidney Int. 47: 927-934.

Wolf, A., Clemann, N., Frieauff, W., Ryffel, B. and Cordier, A. 1994. Role of reactive oxygen formation in the cyclosporine-A-mediated impairment of renal functions. Transplantation Proc. 26: 2902-2907.

Young, M.K.J. and Raisz, L.G. 1952. An anthrone procedure for determination of inulin in biological fluids. Proc. Soc. Exp. Biol. Med. 80: 771-774.

Zhang, W. and Victor, R.G. 2000. Calcineurin inhibitors cause renal afferent activation in rats: a novel mechanism of cyclosporine- induced hypertension. Am. J. Hypertens. 13: 999-1004.

Zhong, Z., Arteel, G.E., Connor, H.D., Yin, M., Frankenberg, M.V., Stachlewitz, R.F., Raleigh, J.A., Mason, R.P. and Thurman, R.G. 1998. Cyclosporine A increases hypoxia and free radical production in rat kidneys: prevention by dietary glycine. Am. J. Physiol. 275: F595-F604.

Zhong, Z., Connor, H.D., Yin, M., Moss, N., Mason, R.P., Bunzendahl, H., Forman, D.T. and Thurman, R.G. 1999. Dietary glycine and renal denervation prevents cyclosporin A-induced hydroxyl radical production in rat kidney. Mol. Pharmacol. 56: 455-463.