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EFFECTS OF COMBINATION OF CYCLOSPORINE WITH LOSARTAN OR ENALAPRIL ON KIDNEY FUNCTION IN UREMIC RATS

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Long-term treatment with cyclosporine in solid organ transplantation has been shown to be associated with the development of hypertension and nephrotoxicity. Angiotensin-converting enzyme inhibitors have well-known nephroprotective properties and may prevent cyclosporine A (CYA)-induced hypertension. Angiotensin receptor 1 antagonists have similar properties. The purpose of this study was to investigate if losartan or enalapril could be administered with CYA to reduce its nephrotoxic effect in uremic rats. The studies were performed on the following groups of rats: group I – control; group II – control rats + losartan; group III – control rats + CYA; group IV – uremic rats; group V – uremic rats + losartan; group VI – uremic rats + CYA; group VII – uremic rats + losartan + CYA, group VIII – control rats + enalapril; group IX – control rats + enalapril + CYA; group X – uremic rats + enalapril; group XI – uremic rats + enalapril + CYA. Pretreatment with CYA, losartan or enalapril in uremic rats resulted in a significant increase in urea and creatinine levels and a decrease in hematocrit. The same effect was observed when uremic rats were given CYA + losartan or CYA + enalapril. Pretreatment with losartan was associated with the increase in the level of CYA much higher than with CYA treatment alone. Similarly, pretreatment with enalapril resulted in a significant increase in CYA concentration in both groups of rats given CYA: uremic and non-uremic. Results of our study show that the treatment with cyclosporine and a combination of losartan or enalapril results in an increase in creatinine and urea levels and a decrease in hematocrit. Therefore, physicians should exercise caution, when they give losartan and enalapril to kidney allograft recipients treated with cyclosporine, particularly with impaired allograft function.

Key words: *cyclosporine, enalapril, losartan, uremia*

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Abbreviations: ACE – *angiotensin-converting enzyme,* AT_1 – *angiotensin receptor 1,* CYA – *cyclosporine A,* LOS – *losartan,* NO – *nitric oxide*

INTRODUCTION

Cyclosporine A (CYA) is a potent immunosuppressive drug that selectively inhibits transcription of interleukin-2 and several other cytokines, mainly in T-helper lymphocytes [2]. Its introduction has dramatically improved the outcome of solid organ transplantation and CYA is also used with increasing frequency for the treatment of autoimmune diseases. However, the long-term treatment with CYA in solid organ transplantation has been shown to be associated with the development of hypertension and nephrotoxicity [8]. The pathophysiology of CYA-induced acute nephrotoxicity is not fully understood but glomerular hypoperfusion following CYA administration has been shown [11, 14]. Several mechanisms, including endothelin-mediated systemic and renal vasoconstriction, sodium retension, impaired vasodilatation secondary to reduction in nitric oxide (NO), and altered cytosolic calcium translocation have been proposed to underline CYA-induced hypertension [12, 15].

The renin-angiotensin system plays a major role in the physiological regulation of the kidney function, including the control of renal microvascular and tubular function. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor 1 (AT_1) antagonist – losartan (LOS) are widely used for the treatment of hypertension, but caution is advised because these drugs may induce reversible renal failure [5, 9]. Although this has been ascribed in some cases to nephrotoxicity, hypertension, hypersensitivity reaction and interstitial nephritis, most cases have been associated with stenosis of the renal arteries or arterioles occurring in either native or transplanted kidneys [5, 9]. The AT_1 receptor recently has been shown to play an important role in the stimulation by angiotensin II of a number of renal vasodilator substances, including bradykinin and NO [4]. On the other hand, ACE inhibitors have well-known nephroprotective properties and may prevent CYA-induced hypertension and deterioration of kidney function in spontaneously hypertensive rats [10]. The purpose of this study was to investigate if losartan (LOS) or enalapril could be administered with CYA to reduce its nephrotoxic effect in uremic rats.

MATERIAL and METHODS

The study was carried out on male Wistar rats (200–250 g, 20 animals per group). Experimental chronic renal failure was evoked using the method of subtotal nephrectomy under pentobarbital anesthesia (50 mg/kg *ip*) [13]. The right kidney was totally resected and 60% of the left one was removed [13]. Four weeks after the surgery serum creatinine, urea and hematocrit were assayed to prove the development of renal insufficiency. Then, the administration of the studied drugs (CYA, Neoral, Novartis, Switzerland, LOS, MSD Inc., USA, enalapril, KRKA, Slovenia) started and continued for 8 weeks. LOS was given at a dose of 10 mg/kg *po*, enalapril at a dose of 10 mg/kg *po*, and CYA at a dose of 15 mg/kg *ip*. Rats were divided into the following groups: group I – control; group II – control rats + LOS; group III – control rats + CYA; group IV – uremic rats; group V – uremic rats + LOS; group VI – uremic rats + CYA; group VII – uremic rats + LOS + CYA, group VIII – control rats + enalapril; group IX – control rats + enalapril + CYA; group X – uremic rats + enalapril; group XI – uremic rats + enalapril + CYA. At the end of the therapy, mean serum creatinine and urea levels, and hematocrit were assayed by standard laboratory methods and whole blood CYA level (by TDX) was recorded. All the results are presented in Table 1 and data are expressed as means ± SD. Completely randomized analyses of variances was used for comparison among groups, followed by Dunnet's test for multiple comparisons. Statistical significance was defined at $p < 0.05$.

RESULTS

We assessed creatinine (0.34 ± 0.08 mg%), and urea (27.5 ± 3.14 mg%) levels, and hematocrit (45.4 ± 3.75%) in control rats in order to establish their normal ranges. As expected, chronic renal failure induced a marked rise in both creatinine and urea concentrations (0.68 ± 0.11 mg% and 57.3 ± 9.47 mg% vs 0.34 ± 0.08 and 27.5 ± 3.14 mg%, respectively) and a decrease in hematocrit (33.85 ± 2.34% vs 45.4 ± 3.75%) when compared to the control group. Moreover, these parameters increased (creatinine and urea) and decreased (hematocrit) in uremic rats treated with CYA (0.8 ± 0.1 mg%, 124.4 ± 35.9 mg% and 35.2 ± 3.1%, respectively), uremic rats treated with LOS (0.75 ± 0.14

Table 1. Creatinine, urea and cyclosporine A (CYA) concentrations, and hematocrit value in control and uremic rats treated with losartan or enalapril and CYA

No.	Group	Creatinine (mg/dl)	Urea (mg/dl)	Hematocrit (%)	CYA concentration (ng/ml)
I	Control	0.34 ± 0.075	27.50 ± 3.14	45.40 ± 3.75	0
II	Control + losartan	0.52 ± 0.064	33.23 ± 2.80	41.37 ± 2.58	0
III	Control + CYA	0.64 ± 0.075[a]	53.06 ± 10.25	49.73 ± 5.11	46.5 ± 6
IV	Uremia	0.68 ± 0.11	57.34 ± 9.47	33.85 ± 2.34	0
V	Uremia + losartan	0.75 ± 0.14[bd]	70.53 ± 10.71	33.59 ± 2.70[d]	0
VI	Uremia + CYA	0.80 ± 0.08[b]	124.36 ± 35.93	35.17 ± 3.10	117 ± 21.2[bc]
VII	Uremia + CYA + losartan	1.33 ± 0.18[a]	214.61 ± 72.59	25.43 ± 1.29[ce]	409 ± 36.6[c]
VIII	Control + enalapril	0.64 ± 0.05[i]	53.89 ± 5.53[i]	37.30 ± 2.00[i]	0
IX	Control + CYA + enalapril	0.92 ± 0.11[ci]	139.56 ± 12.34[ci]	34.24 ± 2.30[ci]	1461 ± 88.22 [c]
X	Uremia + enalapril	0.93 ± 0.05[f]	98.33 ± 17.47[f]	24.74 ± 1.36[f]	0
XI	Uremia + CYA + enalapril	1.07 ± 0.09[h]	232.64 ± 17.45[g]	27.21 ± 3.16[g]	2722.39 ± 518.37[g]

[a] $p < 0.001$ group I vs II, III and IV vs V, VI, VII; [b] $p < 0.001$ group VII vs V, VI; [c] $p < 0.001$ group III vs VI, VII, IX; [d] $p < 0.001$ group V vs II and VII vs V; [e] $p < 0.001$ group VII vs II; [f] $p < 0.001$ group VIII vs X; [g] $p < 0.001$ group IX vs XI; [h] $p < 0.01$ group IX vs XI, [i] $p < 0.001$ group I vs VIII, IX

mg%, 70.5 ± 10.7 mg%, 33.6± 2.7 mg%) and uremic rats treated with enalapril (0.93 ± 0.05 mg%, 98.3 ± 17.5 mg%, 24.7 ± 1.4%). They were extremely elevated (creatinine and urea) or decreased (hematocrit) in uremic rats treated with both CYA and LOS (1.33 ± 0.18 mg%, 214.6 ± 72.6 mg% and 25.4 ± 1.3 %, respectively) and CYA and enalapril (1.07 ± 0.09 mg%, 232.6 ± 17.5 mg%, 27.21 ± 3.16%) when compared to the control group.

CYA concentration in uremic rats was significantly higher than those observed in non-uremic rats treated with CYA (117 ± 21.2 ng/ml vs 46.5 ± 6 ng/ml). Pretreatment with LOS was associated with the increase in the level of CYA much higher than with CYA alone (409 ± 36.6 vs 46.5 ± 6 and 117 ± 21.2 ng/ml, respectively). Similarly, pretreatment with enalapril resulted in a significant increase in CYA concentration in both groups of rats given CYA (uremic + CYA + enalapril, control + CYA + enalapril) reaching 2722.4 ± 518.4 ng/ml and 1461 ± 88.2 ng/ml, respectively, when compared to the control or uremic rats given CYA.

DISCUSSION

We have demonstrated that administration of CYA to uremic rats further increases creatinine and urea concentration when compared to uremic rats. Similar but more pronounced changes were observed after the administration of LOS or enalapril to uremic rats. Combining these two drugs (CYA and LOS or CYA and enalapril) resulted in further increase in urea and creatinine concentration in uremic rats. The same pattern of changes was observed after the administration of CYA, LOS or both drugs together to non-uremic rats as well as CYA, enalapril or both drugs together to non-uremic rats. Administration of the combination of both drugs (CYA and LOS or CYA and enalapril) to uremic or non-uremic rats caused a statistically significant increase in urea and creatinine levels when compared to the rats given only one of these drugs. LOS or CYA did not affect hematocrit in control rats, whereas administration of enalapril resulted in a significant decrease in hematocrit in the control rats. Control rats given CYA have significantly higher hematocrit than control rats given LOS or enalapril. It may be due to the fact, that CYA may induce erythrocytosis (in kidney allograft recipients this phenomenon is called post-transplant erythrocytosis) [3]. However, hematocrit in uremic rats treated with LOS did not differ significantly from hematocrit in uremic rats given CYA, whereas in uremic rats given enalapril hematocrit was significantly lower than in rats given CYA. Uremic rats given CYA and LOS had significantly lower hematocrit than uremic rats given only

one of these drugs, whereas in uremic rats given combination of CYA and enalapril, hematocrit was not significantly lower when compared to the uremic rats given only enalapril. LOS was shown to have paradoxical effects on renal function [5, 9]. On the one hand, it caused renal vasodilatation, prevented the slow deterioration of glomerular filtration rate in hypertension, reduced proteinuria and improved morbidity and mortality in diabetic nephropathy, while on the other hand, in states of low fixed renal blood flow such as those arising in bilateral artery stenosis, severe congestive heart failure, and severe sodium and volume depletion, it could worsen renal function and even precipitate acute renal failure [5, 9]. The usual interpretation suggests that in these states renal function is angiotensin-dependent [5, 9]. In our study, control or uremic rats did not show any evidence of low fixed renal blood flow. They had tap water available *ad libitum*, they were fed standard chow, they were not on diuretics, and no evidence of heart failure or bilateral renal artery stenosis was noted. Therefore, the exacerbation of renal failure remained interesting, but difficult to explain. According to hitherto existing evidence, LOS is exceptionally well-tolerated. There are no data about lowering of hematocrit or aggravating anemia by LOS in end-stage renal failure or in dialysis population. However, since CYA may contribute to posttransplant erythrocytosis [3], patients with this particular pathology might benefit from this combination. LOS given to renal allograft recipients caused a significant decrease in hematocrit and hemoglobin after 6 months of the therapy [1]. It has been suggested that LOS might blunt erythropoiesis by dampening angiotensin II-driven erythropoietin production in the kidney. Moreover, to date no clinically important LOS interactions have been described. Studying effects of enalapril on kidney function, we observed a deterioration of kidney function in control and uremic rats treated with this drug. In kidney transplant recipients given enalapril, no significant change in CYA concentration was observed but impairment in allograft function in the patients with non-optimal allograft function (renal failure) was found [7]. In spontaneously hypertensive rats on a high-sodium diet, enalapril and valsartan equally prevented the CYA-induced deterioration of kidney function [6]. Therefore, taking into consideration the results of our study such as an increase in creatinine level by a combination of LOS or enalapril with CYA, physicians should exercise caution, when they give LOS and enalapril to kidney allograft recipients treated with CYA, particularly with impaired allograft function.

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