EFFECT OF CYCLOSPORIN A ON TISSUE LIPID PEROXIDATION AND MEMBRANE BOUND PHOSPHATASES IN HYPEROXALURIC RAT AND THE PROTECTION BY VITAMIN E PRETREATMENT

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SUMMARY: The effect of cyclosporin A, a highly effective immunosuppressant, was investigated on hyperoxaluric rats with and without vitamin E pretreatment. Hyperoxaluria was induced by oral feeding of 3% ammonium oxalate in water for 3 days. Cyclosporin A (50 mg/kg body wt.) was administered for 3 days. Pretreatment with vitamin E (50 mg/100 g body wt., once a week for 3 weeks) was carried out before the administration of cyclosporin A and ammonium oxalate. Nonenzymatic ascorbate-induced lipid peroxidation was increased to 1.55-fold in either cyclosporin A-administered or hyperoxaluric rat kidney and liver when compared to control. The lipid peroxidation was further elevated to 1.9-fold when both cyclosporin A and ammonium oxalate were coadministered. The activities of renal and hepatic ATPase, glucose-6-phosphatase as well as the concentrations of thiols were decreased significantly ($p < 0.001$) when cyclosporin A was administered under hyperoxaluric condition. On pretreatment with vitamin E the cyclosporin A-induced biochemical changes observed in the presence of hyperoxaluria were abolished.

INTRODUCTION

Cyclosporin A (CsA) is a powerful immunosuppressive drug and has proved to be a potent agent in a wide variety of experimental models of tissue transplantation and in clinical organ transplantation. All modern protocols of immunosuppression include CsA (1). Despite the selective advantage, clinical studies with post-transplant patients have demonstrated that CsA is toxic to kidneys as well as to the liver (2) and the nephrotoxicity and hepatotoxicity consti-

tute the main dose-limiting factor in the utilization of the drug. The precise toxic mechanism is not clear even though, alterations in mitochondrial functions (3), covalent binding of CsA-metabolites to proteins (4), elevated thromboxane synthesis (5), and lipid peroxidation (LPO)(6) have been reported in the CsA-mediated cell damage. Lipid peroxidation reaction has been proposed to be one of the major mechanisms for CsA toxicity and cell injury, based on the partial reversibility by some antiperoxidative agents (7).

CsA enhances renal calcium oxalate deposition in hyperoxaluria by enhancing absorption of oxalate in the intestine (8) as well as retention of oxalate and calcium in the renal cells (9), but the effect of CsA administration on renal and hepatic cell membrane functions in hyperoxaluric condition is not known. This study was aimed, therefore, at investigating the membranal enzymes and LPO status due to CsA administration under hyperoxaluria and the protective effect of vitamin E (Vit. E) pretreatment under this condition.

MATERIALS AND METHODS

Experimental design

Animals: Adult male albino Wistar strain rats weighing 180-200 g were divided into four groups each containing 8-10 rats.

Vitamin E pretreatment: Animals received one ip injection of Vit. E (500 mg kg⁻¹ body wt. w/w) in mineral oil per week for 3 weeks (10).

Drug: Commercially available CsA (Sandimmune; Sandoz Ltd., Basel, Switzerland) was dissolved in olive oil (67 mg ml⁻¹ w/v). About 0.15 ml was given by daily gavage.

Group 1 received CsA (50 mg kg⁻¹ body wt. w/w) for 3 days; Group 2 received isovolumetric olive oil for 3 days; Group 3 received CsA (50 mg kg⁻¹) for 3 days along with 3% ammonium oxalate (AmOx) in drinking water; Group 4 received isovolumetric olive oil for 3 days along with 3% AmOx in drinking water. The experiments were conducted with and without Vit. E pretreatment.

At the end of the experimental period animals were killed by cervical dislocation and the liver and kidney tissues were quickly dissected into ice-cold saline.

Homogenization: The weighed tissues were homogenized in Tris-HCl buffer (pH 7.4, 0.01 M) with a Potter Elvehjem homogenizer.

Tissue parameters: The thiobarbituric acid-reactive substances (TBARS) formed served as an index of LPO and was measured by the method of

Devasagayam et al (11). Nonenzymatic LPO in the presence of promoters, ascorbic acid, ferrous sulfate (FeSO_4) and t-butyl hydroperoxide (tBH), 2 mM each was studied.

Microsomal enzyme glucose-6-phosphatase (G6Pase) (12), membrane-bound ATPases (13) and thiols (14) were analyzed.

Statistical analysis: Student's 't' test was used for statistical analysis and the 'p' value was arrived at to assess the statistical significance of the changes observed. Pearson's correlation coefficient 'r' was arrived at to assess the degree of linear association among the different variables taken two at a time.

RESULTS

A significant elevation ($p < 0.001$) in the TBARS formation was seen in the presence of promoters of LPO (ascorbate, ferrous sulphate and tBH). The basal LPO as well as LPO in the presence of promoters was high in both CsA alone- and AmOx alone-treated rat kidney and liver when compared to that of control (Table I). This susceptibility to LPO was further elevated significantly ($p < 0.001$) in the tissues of rats administered with CsA in hyperoxaluric condition.

The Vit. E-pretreated animals of all the experimental groups showed complete protection against tissue peroxidation even in the presence of promoters.

Total thiol, protein and nonprotein thiols were reduced by 20% in both CsA alone- and AmOx alone-administered rat kidney. Further, decrease of total and protein thiol (30% each) and nonprotein thiol level (45%) was observed when CsA was coadministered with AmOx (9).

The protein thiol concentration of liver was reduced by 30% to 40% in CsA- as well as AmOx-treated groups (Fig. 1). This concentration was further reduced to 45% when CsA was given to hyperoxaluric rats. In contrast, nonprotein SH was not significantly altered. The thiol content was unaffected under the above conditions upon pretreatment with Vit. E. The concentration of thiols negatively correlated with LPO.

The activities of total, $\text{Na}^+ + \text{K}^+$ -, Mg^{2+} - and Ca^{2+} -dependent ATPases significantly decreased ($p < 0.001$) in both AmOx- as well as CsA-treated rat kidney and liver (Table II) and the decrease in their activity was highly significant ($p < 0.001$) in hyperoxaluric rats when coadministered with CsA. ATPase showed negative correlation with LPO. Positive correlation was observed with protein

Fig. 1. Effect of CsA administration on the levels of thiols in the liver of rats pretreated with and without Vit. E.

(Values are mean + S.D. for eight animals in each group) Values are statistically different compared to a: control; b:CsA; c:AmOx. Values are statistically significant when, $x_p < 0.001$; $y_p < 0.01$; $z_p < 0.05$. Correlation coefficient r_1 Vs LPO. -Vit E : Vitamin-E untreated; +Vit. E : Vitamin E-pretreated.

thiol. The above changes were not observed in the Vit. E-pretreated rats on challenging with CsA and AmOx.

The activity of microsomal enzyme G6Pase was reduced to 45-52% in both CsA alone- or AmOx-administered rat liver and kidney when compared to that of control. The reduction in enzyme activity was more pronounced (55-63%) when CsA and AmOx were co-administered. Pretreatment with Vit. E abolished the above effects.

Table I. Effect of CsA administration on the basal and non-enzymatic lipid peroxidation in the kidney and liver of rats pretreated with and without Vit. E

Particulars	Vit-E untreated				Vit.E pretreated			
	Control	CsA	AmOx	CsA + AmOx	Control	CsA	AmOx	CsA + AmOx
Kidney								
Basal	1.28 ± 0.18	1.51 ^{rs} ± 0.17	1.50 ^{rs} ± 0.18	1.77 ^{rsabc} ± 0.17	1.25 ± 0.17	1.31 ± 0.18	1.29 ± 0.17	1.35 ± 0.17
FeSo ₄	11.78 ± 1.40	14.8 ^{rs} ± 1.70	14.1 ^{rs} ± 1.60	17.1 ^{rsabc} ± 2.2	11.7 ± 1.50	11.8 ± 1.30	11.8 ± 1.30	12.2 ± 1.70
Ascorbate	5.38 ± 0.40	8.94 ^{rs} ± 0.90	8.4 ^{rs} ± 0.80	10.6 ^{rsabc} ± 1	5.30 ± 0.27	5.39 ± 0.33	5.5 ± 0.30	6.12 ^{rsabc} ± 0.40
t-butyl Hydro Peroxide	8.24 ± 0.90	10.8 ^{rs} ± 0.70	9.56 ^{rsab} ± 0.80	13.1 ^{rsabc} ± 0.8	8.30 ± 0.09	8.36 ± 0.90	8.43 ± 1.0	8.60 ± 1.10
Liver								
Basal	1.40 ± 0.16	1.72 ^{rs} ± 0.17	1.81 ^{rsab} ± 0.16	2.03 ^{rsabc} ± 0.18	1.39 ± 0.12	1.47 ± 0.11	1.43 ± 0.13	1.53 ^{rs} ± 0.12
FeSo ₄	15.2 ± 1.20	21.6 ^{rs} ± 1.40	19.5 ^{rsabc} ± 1.20	24.0 ^{rsabc} ± 1.60	16.15 ± 1.10	16.7 ± 1.10	16.2 ± 1.10	18.2 ^{rsabc} ± 1.30
Ascorbate	7.01 ± 1.30	11.2 ^{rs} ± 1.80	9.5 ^{rsab} ± 1.60	12.6 ^{rsabc} ± 1.90	6.72 ± 1.10	6.90 ± 1.00	6.90 ± 1.00	7.12 ± 1.50
t-butyl Hydro Peroxide	11.0 ± 1.20	12.98 ^{rs} ± 1.20	12.85 ^{rs} ± 1.20	14.9 ^{rsabc} ± 1.40	10.95 ± 1.10	11.01 ± 1.20	11.01 ± 1.10	11.25 ± 1.20

Values are mean ±S.D. for eight animals and are expressed as nmol TBARS mg-1 protein. Values are statistically different compared to acontrol; bCsA; cAmOx. Values are statistically significant when, xp<0.001; yp<0.01; zp<0.05.

Table II. Effect of CsA administration on the activities of ATPases and G6Pase in the kidney of hyperoxaluric rats pretreated with and without Vit. E

Particulars	Vit.E untreated				Vit.E pretreated					
	Control	CsA	AmOx	CsA + AmOx	(r) Vs		Control	CsA	AmOx	CsA + AmOx
					LPO	PT*				
					Kidney					
G6Pase	1.79 ± 0.15	0.91 ^{aa} ± 0.08	0.99 ^{aaab} ± 0.08	0.77 ^{aaayb} ± 0.07	-0.76 ^z	+0.82 ^z	1.70 ± 0.15	1.70 ± 0.16	1.69 ± 0.15	1.67 ± 0.15
Ca ²⁺ ATPase	1.07 ± 0.09	0.82 ^{aa} ± 0.07	0.79 ^{aa} ± 0.08	0.62 ^{aaabc} ± 0.08	-0.43 ^z	+0.88 ^z	1.05 ± 0.10	1.02 ± 0.10	1.03 ± 0.09	1.01 ^{aaabc} ± 0.08
Mg ²⁺ ATPase	1.90 ± 0.13	1.83 ± 0.13	1.63 ^{aaayb} ± 0.12	1.59 ^{aaab} ± 0.10	-0.58 ^z	+0.81 ^z	1.90 ± 0.12	1.88 ± 0.12	1.85 ± 0.11	1.86 ± 0.12
Na ⁺ K ⁺ ATPase	1.32 ± 0.11	1.01 ^{aa} ± 0.09	1.11 ^{aaab} ± 0.09	0.92 ^{aaaybc} ± 0.10	-0.65 ^z	+0.95 ^z	1.30 ± 0.1	1.12 ^{aa} ± 0.09	1.8 ^b ± 0.10	1.06 ^{aaac} ± 0.09
Total ATPase	4.30 ± 0.25	3.60 ^{aa} ± 0.28	3.55 ^{aa} ± 0.27	3.25 ^{aaabc} ± 0.28	-0.67 ^z	+0.8 ^z	4.35 ± 0.22	4.30 ± 0.23	4.28 ± 0.21	4.20 ± 0.22
					Liver					
G6Pase	1.43 ± 0.12	0.62 ^{aa} ± 0.06	0.64 ^{aa} ± 0.06	0.53 ^{aaaybc} ± 0.05	-0.77 ^z	+0.96 ^z	1.43 ± 0.13	1.42 ± 0.12	1.41 ± 0.11	1.40 ± 0.12
Ca ²⁺ ATPase	0.65 ± 0.06	0.46 ^{aa} ± 0.07	0.45 ^{aa} ± 0.07	0.35 ^{aaaybc} ± 0.06	-0.79 ^z	+0.97 ^z	0.66 ± 0.06	0.66 ± 0.07	0.65 ± 0.09	0.63 ± 0.1
Mg ²⁺ ATPase	0.96 ± 0.10	0.92 ± 0.09	0.82 ^{aaab} ± 0.09	0.75 ^{aaayb} ± 0.09	-0.67 ^z	+0.79 ^z	0.95 ± 0.08	0.93 ± 0.08	0.94 ± 0.07	0.91 ± 0.08
Na ⁺ K ⁺ ATPase	1.11 ± 0.08	0.83 ^{aa} ± 0.07	0.95 ^{ayab} ± 0.09	0.66 ^{aaayb} ± 0.1	-0.9 ^z	+0.87 ^z	1.11 ± 0.08	1.01 ± 0.08	1.10 ± 0.09	0.90 ± 0.08
Total ATPase	2.63 ± 0.12	2.20 ^{aa} ± 0.11	2.17 ^{aa} ± 0.12	1.92 ^{aaabc} ± 0.13	-0.84 ^z	+0.97 ^z	2.65 ± 0.11	2.55 ± 0.12	2.61 ± 0.12	2.48 ^{aaac} ± 0.12

Values are mean ±S.D. for eight animals and are expressed as μmol Pi × 10⁻¹ min⁻¹ mg⁻¹ protein. Values are statistically different compared to acontrol; ^aAmOx. Values are statistically significant when, xp<0.001; yp<0.01; zp<0.05. (r) - correlation coefficient. *Correlation obtained from the data of Ref. (9) for kidney.

DISCUSSION

Lipid peroxidation reaction, a type of oxidative degeneration of polyunsaturated fatty acids, has been linked with altered membrane structure and enzyme inactivation (15). The highly significant elevation in nonenzymatic LPO reactions observed in hyperoxaluric rats when administered with CsA suggests the enhanced susceptibility of the membranes. Further oxalate itself is known to induce membrane damage and cell injury as well as LPO (16,17). Since Vit. E-pretreatment normalizes the LPO reaction, it suggests the involvement of the radicals in the CsA- as well as AmOx- treated rats. CsA has been shown to enhance in vitro as well as in vivo tissue LPO (7,18).

The decreased activities of membrane-bound enzymes, G6Pase and ATPase also support the fact that the severe damage has been effected by CsA treatment in hyperoxaluria. The decrease in the membrane-bound enzyme activities are found to be associated with the loss of protein thiol content.

ATPases are lipid dependent as well as SH-dependent membrane-bound enzymes and alterations in membrane lipid can lead to changes in membrane fluidity. Further enhanced susceptibility to LPO of membrane can lead to loss of protein thiol, thereby change in membrane functions. The marked loss in G6Pase, total, Mg^{2+} -, Ca^{2+} -, and Na^+K^+ - ATPase activities registered in CsA-treated urolithic rats may be due to loss of protein-SH because of increased lipid peroxidative damage. This is supported by the positive correlation between ATPase and protein thiol activities. The depletion of thiol content may be associated with low concentration of nonprotein thiol as observed here and antioxidants such as ascorbic acid, Vit. E and GSH as well as low activities of GPx as reported earlier (9).

Vitamin E pretreatment protects the membrane-bound enzymes from inactivation by restoration of antioxidants (19) and thereby arresting free radical-induced damage. Depletion of total thiol has been observed under oxidative stress (20) as well as by administration of drugs (21). G6Pase is known to be inhibited by LPO (22). Decreased levels of total thiols as well as protein thiol have been reported in the urolithic rat liver and kidney when fed with vitamin B₆ deficient diet or sodium glycolate-supplemented diet (23,24). Feeding SH-generating substance like methionine (25) or free radical scavengers like Vit. E (19) along with urolithic diet restores the cellular thiol content and the membranal functions.

In conclusion, CsA induces LPO and membrane biochemical changes, and these changes were well pronounced under hyperoxaluric conditions. Vit. E-pretreatment protects the cells from CsA as well as as oxalate-induced damage.

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