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Protection of cyclosporin A-induced biochemical changes by vitamin E pretreatment in hyperoxaluric rat kidney

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The study was aimed to determine whether cyclosporin A administration increases oxalate retention in renal cells under hyperoxaluria and, if so, whether pretreatment with vitamin E abolishes this effect.

Feeding cyclosporin A along with normal rat chow for 3 days resulted in increased oxalate retention in kidneys when compared with control. Pretreatment of vitamin E to cyclosporin A-administered rats prevented this increased oxalate retention, but it was only partial when cyclosporin A was coadministered with ammonium oxalate. Calcium accumulated by 260% of the control in both oxalate A- and ammonium oxalate-administered group. However, the enhanced lipid peroxidation, as well as oxalate-synthesizing enzymes due to cyclosporin A administration with or without hyperoxaluria, had been abolished completely, upon vitamin E pretreatment. Only when cyclosporin A and ammonium oxalate were coadministered, a highly significant decrease in the cytosolic enzyme aspartate transaminase was observed, whereas alanine transaminase activity decreased considerably with cyclosporin or ammonium oxalate when given either together or separately. The concentrations of antioxidants and thiol components were significantly reduced in cyclosporin A-treated rats with or without ammonium oxalate coadministration. All these changes were restored to normal on pretreatment with vitamin E. In conclusion, the impact on oxalate and calcium retention, the susceptiblity to lipid peroxidation and the related biochemical changes were absent upon vitamin E pretreatment and, thereby, favors protection from cyclosporin A induced cell injury under hyperoxaluric condition. © Elsevier Science Inc. 1997 (J. Nutr. Biochem. 8:32–37, 1997.)

Keywords: cyclosporin A; lipid peroxidation; nephrotoxicity, vitamin E; antioxidants; hyperoxaluria

Introduction

Cyclosporin A (CsA) has been widely used as a potent and selective immunosuppressant.[1] However, it has been associated with nephrotoxicity,[2] hepatotoxicity,[3] and neurotoxicity.[4] Alterations in mitochondrial functions,[5] covalent binding of CsA metabolites to proteins,[6] elevated thromboxane synthesis,[7] and lipid peroxidation[8] have all been implicated in the CsA-mediated cell damage. Whereas its precise toxic mechanisms remain to be investigated, lipid peroxidation ascribable to oxygen radicals produced in the kidney has been proposed to be one of the major mechanisms for CsA nephrotoxicity and cell injury, which are partly reversed by some antioxidants.[9] Cell injury has been suggested as one of the mechanisms for calcium oxalate retention,[10] which is a disorder caused by persistent hyperoxaluria. The possible involvement of free radicals and mediated lipid peroxidation reactions in the retention of calcium oxalate has been reported in our earlier studies.[11] There is relatively little known about the effect of CsA on renal oxalate metabolism. The objectives of this study were 2-fold: 1) To determine whether CsA administration influences oxalate retention in renal cells in presence and ab-

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sence of hyperoxaluria and 2) To determine whether vitamin E pretreatment protects cells from CsA-mediated alterations.

Methods and materials

Adult male albino Wistar rats weighing 200 to 220 g were purchased from Fredrick Institute of Plant Protection and Toxicology (Madras, India) and housed in the ventilated animal room and no special arrangements were made for heating and cooling. Animals were divided into two major groups, each containing 40 rats. One of the groups was pretreated with vitamin E (Vit. E); one intraperitoneal injection of Vit. E (50 mg/100g body weight) in mineral oil per week for 3 weeks. Both Vit. E pretreated and untreated groups were then subdivided into four minor groups each containing 10 rats. Group 1 received the vehicle (olive oil) alone for 3 days; group 2 received CsA (50 mg/kg). CsA (Sandimmun, Sandoz, Ltd., Basle, Switzerland) dissolved in olive oil, was administered daily by gavage for 3 days; group 3 received the vehicle for 3 days, along with 3% ammonium oxalate (AmOx) in drinking water; group 4 received CsA (50 mg/kg) for 3 days, along with 3% AmOx in drinking water. The dosage of the administration of CsA, AmOx, and Vit. E was based on the protocols used by Massicot et al. (1994),[12] Kumar et al. (1991),[13] and Dillard et al. (1982),[14] respectively. AmOx feeding was carried out only for 3 days, beyond which calcium oxalate crystal deposition on its own in the kidney of rats. So to study the triggering effect of CsA, 3 days of administration of AmOx was carried out. When CsA dosage was increased above the 50 mg/kg body weight, the food intake by the animals was reduced markedly during coadministration with AmOx.

Animals were given normal rat chow [M/s. Hindustan Lever Ltd., Bombay, India). The pelleted feed contained protein (21%), lipids (5%), crude fiber (4%), ash (8%), calcium (1%), phosphorus (0.6%), nitrogen-free extract (55%), and provided metabolizable energy of 3600 K cal/kg and was enriched by vitamins, minerals, and tracc elements] and water ad libitum.

Animals were killed by cervical dislocation. Kidneys were removed immediately and placed in ice-cold saline. The weighed tissues were homogenized in Tris-HCl buffer (pH 7.4, 0.01 M).

Tissue lipid peroxidation (LPO) was estimated in terms of thiobarbituric acid-reactive substances (TBARS) described by Devasagayam (1986).[15] Oxalate was estimated in the kidney homogenate after removal of the lipid by extraction with benzene-petroleum ether (1:1 v/v) mixture followed by extraction with 1.25N HCl as described by Hodgkinson and Williams (1972).[16] For the determination of calcium the homogenate was digested with perchloric acid and the concentration was analysed using atomic absorption spectroscopy (Perkin-Elmer, USA) after appropriate dilution.[17] Xanthine oxidase (XO)[18] and lactate dehydrogenase (LDH)[19] were determined in the homogenate as described. Alanine transminase (ALT) and aspartate transaminase (AST) were estimated in the homogenate by the method of Reitman and Frankel (1957).[20]

The antioxidants concentrations of ascorbic acid (AA)[21] and Vit. E[22] were determined as described. Reduced glutathione (GSH) content was estimated by the method of Moron et al.[23] This is based on the reaction of GSH with 5-5′-dithio bis (2-nitrobenzoic acid) to form 2-nitro-5-mercapto benzoic acid, which has an absorption maximum at 412 nM. Total thiols, protein thiol (PT) and non-protein thiol (NPT) were measured according to the methods of Sedlak.[24]

Glutathione metabolizing enzymes glutathione reductase (GR)[25] gamma glutamyl transpeptidase (γ-GT),[26] glutathione-s-transferase (GST),[27] glucose-6-PO_4 dehydrogenase (G6PD),[25] and glutathione peroxidase (GPx)[28] were also analyzed as described. Histopathological studies were carried out after staining the formalin-fixed kidney sections with eosin and methylene blue.

Statistical analysis

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

Results

The effect of cyclosporin A treatment on histopathological changes were studied. Briefly, the cyclosporin A-administered rat kidney showed dilated tubular damage. The kidneys of fats that received both CsA and AmOx showed severe tubular damage with necrosis and congested vessels. Calcium oxalate crystal deposition was noted only in this group. On Vit. E pretreatment, less congested vessels with less tubular damage and near normal tubules were noted. Crystal deposition was also not observed.[29]

The renal oxalate concentration in the CsA or AmOx alone administered groups was increased to 1.4 fold and 1.9 fold, respectively when compared with that of the control (*Table 1*). The oxalate concentration was increased to 2.9 fold of the control value, whereas calcium increased by 2.6 fold when CsA was administered along with AmOx. Upon Vit. E pretreatment, the oxalate level remained significantly high in hyperoxaluric rats independent of CsA administration. However, the oxalate level was restored to near normal in the CsA-alone treated group. The basal LPO in terms of TBARS of renal cells was increased in CsA alone (122%) or AmOx alone (131%) or combined treated (144%) rats. Vit. E pretreatment abolished this effect.

To see whether the observed increase in renal oxalate in CsA-treated rats was a cumulative effect of oxalate synthesis and intestinal absorption, further studies were undertaken. Oral administration of ^{14}C-oxalate to CsA-treated group showed a 14% increase in the oxalate absorption when the total plasma ^{14}C-oxalate was taken into account during the test period of 7 hr.[28]

XO activity was increased to 125% in CsA-treated group when compared with the control. This activity was further elevated in the presence of hyperoxaluria (*Table 1*). The activity of LDH using glyoxylate as substrate was increased to 125%, 168%, and 183% in CsA, AmOx, and CsA + AmOx-administered groups, respectively. The correlation value '*r*' was positive and highly significant when XO and LDH activities were compared with LPO or oxalate.

To determine whether cellular injury was incurred by CsA treatment, two cytosolic enzymes (AST and ALT) were studied. The activity of AST was not significantly altered either in CsA alone or AmOx alone administered groups. Interestingly, when CsA and AmOx were coadministered, a highly significant decrease ($P < 0.001$) in AST activity was observed (*Table 1*). ALT activity was decreased by 25 to 40% of control in either CsA treated or hyperoxaluric rats and the activity was decreased further by 55% when both CsA and AmOx were administered simul-

taneously. All the enzyme activities were normalized upon pretreatment with Vit. E under the above conditions.

To see whether the observed changes were associated with the altered antioxidant system the concentrations of scavengers and thiols were determined. The concentrations of ascorbic acid, Vit. E, and GSH were significantly reduced ($P < 0.001$) in CsA-treated rats with or without AmOx coadministration (*Figure 1*).

A reduction of 15 to 20% in the glutathione metabolic enzyme activities, GST, GPx, and G6PD was observed either in CsA alone or AmOx alone administered groups. When CsA and AmOx were simultaneously administered, the enzyme activities were further decreased by 25 to 30% (*Table 2*). In contrast, the activities of GR and γ-GT were elevated to 140% in CsA administered hyperoxaluric group. Vit. E pretreatment restored the enzyme activities near to normal.

Total, protein, and non-protein thiols were reduced by 20% in both CsA alone and AmOx alone administered groups (*Figure 2*). A further decrease of 30% in total and protein thiol and of 45% in non-protein thiol level were observed when CsA was coadministered with AmOx. The concentration of the antioxidants and thiols negatively correlated with LPO or oxalate. The levels of antioxidants and thiols were restored to normal on pretreatment with Vit. E.

Discussion

Lipid peroxidation, a degradative process of polyunsaturated fatty acids, has been shown to be involved in the

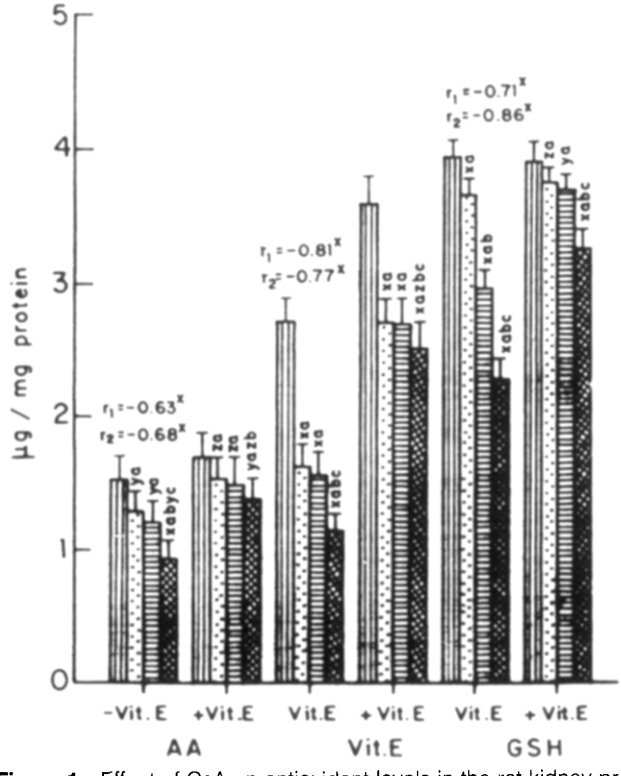

Figure 1 Effect of CsA on antioxidant levels in the rat kidney pretreated with and without Vit. E. Values are ± S.D. for eight animals and are statistically significant to [a]control; [b]CsA; [c]AmOx when $^zP < 0.05$; $^yP < 0.01$; $^xP < 0.001$. Correlation coefficient r_1 with LPO; r_2 with oxalate.—Vit. E-vitamin E untreated: + Vit. E-vitamin E pretreated. ▥Control ▨CsA ▤AmOx ▩CsA + AmOx.

Table 1 Effect of CsA on oxalate concentration, LPO, oxalate synthesizing enzymes and cytosolic enzymes in the rat kidney pretreated with and without Vit. E. (Values are mean ± S.D. for eight animals and are expressed as [1]mg/g wet tissue; [2]nmoles MDA/mg protein; [3]nmole/mg protein [4] units/mg protein (one unit = change in OD of 0.01/min); [5]µ moles glyoxylate, [6&7]µ moles pyruvate/min/mg protein)

	Vit. E Untreated				Vit. E Pretreated					
					Correlation Coefficient (r)					
Particulars	Control	CsA	AmOx	Csa + AmOx	LPO	Oxalate	Control	CsA	AmOx	CSA + AmOx
Oxalate[1]	0.67 ± 0.13	0.95^{ya} ± 0.16	1.27^{xayb} ± 0.16	1.95^{abcx} ± 0.18	+0.78^x		0.67 ± 0.12	0.72 ± 0.15	1.01 ± 0.11	1.22 ± 0.13
LPO[2]	1.4 ± 0.13	1.71 ± 0.15	1.83 ± 0.14	2.02 ± 0.16			1.25 ± 0.17	1.31 ± 0.17	1.29 ± 0.17	1.35 ± 0.17
Calcium[3]	36.5 ± 3.0	43.2 ± 4.4	47.60 ± 5.6	95.00 ± 6.9	+0.71^x	+0.93^x	36.0 ± 3.8	37.5 ± 4.1	39.3 ± 4.1	48.4 ± 5.3
XO[4]	1.2 ± 0.1	1.51^{xa} ± 0.09	1.4^{xazb} ± 0.06	1.9^{xabc} ± 0.06	+0.75^x	+0.9^x	1.14 ± 0.12	1.19 ± 0.07	1.2 ± 0.09	1.3^{zabc} ± 0.1
LDH[5]	1.2 ± 0.11	1.5^{xa} ± 0.13	2.01^{xab} ± 0.14	2.2^{abxcy} ± 0.1	+0.8^x	+0.92^x	1.19 ± 0.1	1.2 ± 0.09	1.25 ± 0.09	1.37^{yabzc} ± 0.13
AST[6]	0.3 ± 0.02	0.29^{ya} ± 0.02	0.27^{xabyc} ± 0.01	0.23^{xabyc} ± 0.03	−0.62^x	−0.69^x	0.31 ± 0.01	0.3 ± 0.03	0.29^{za} ± 0.02	0.29 ± 0.04
ALT[7]	0.4 ± 0.05	0.3^{ya} ± 0.05	.24^{xazb} ± 0.04	0.18^{xaybzc} ± 0.06	−0.73^x	−0.7^x	0.41 ± 0.06	0.39 ± 0.06	0.4 ± 0.05	0.37^{za} ± 0.1

Values are statistically significant compared with [a]control, [b]CsA, [c]AmOx when $^xP < 0.001$; $^yP < 0.01$; $^zP < 0.05$.

Table 2 Effect of CsA on the activities of glutathione metabolisizing enzymes in the rat kidney pretreated with and without Vit. E (Values are mean ± S.D. for eight animals and are expressed in [1]units × 10^{-1}, [2]units/mg protein; [3]µg GSH utilized/min/mg protein; [5]µmoles p-nitroaniline/min/mg protein) one unit = change in OD of 0.01/min

	Vit. E Untreated				Vit. E Pretreated				
					Correlation Coefficient				
Particulars	Control	CsA	AmOx	Csa + AmOx	LPO	Oxalate	Control	CsA	AmOx	CSA + AmOx
GST[1]	1.72 ± 0.25	1.41^{ya} ± 0.16	1.5^{za} ± 0.09	1.3^{yazc} ± 0.26	-0.51^{x}	-0.35^{x}	1.72 ± 0.3	1.6 ± 0.2	1.69 ± 0.3	1.51 ± 0.4
G6PD[2]	3.8 ± 0.16	3.18^{xa} ± 0.15	3.15^{xa} ± 0.13	2.86^{xaybc} ± 0.14	-0.76^{x}	-0.71^{x}	3.79 ± 0.15	3.48^{ya} ± 0.16	3.8^{xb} ± 0.12	3.27^{xaczb} ± 0.13
GPx[3]	4.44 ± 0.51	3.75^{ya} ± 0.34	3.51^{ya} ± 0.38	2.9^{xaybc} ± 0.23	-0.67^{x}	-0.68^{x}	4.43 ± 0.7	4.35 ± 0.5	4.2 ± 0.52	4.15 ± 0.3
GR[4]	0.7 ± 0.08	0.79^{za} ± 0.09	0.9^{yazb} ± 0.1	0.98^{xayc} ± 0.09	$+0.71^{x}$	$+0.84^{x}$	0.69 ± 0.1	0.72 ± 0.09	0.79^{za} ± 0.1	0.83^{yaczb} ± 0.1
γ-GT[5]	1.25 ± 0.16	1.95^{xa} ± 0.18	1.92^{xa} ± 0.16	2.15^{xazbc} ± 0.18	$+0.72^{x}$	$+0.79^{x}$	1.55 ± 0.19	1.69^{ay} ± 0.2	1.59 ± 0.2	1.85^{yaczb} ± 0.19

Values are statistically significant compared with [a]control, [b]CsA, [c]AmOx when $^{x}P < 0.001$; $^{y}P < 0.01$; $^{z}P < 0.05$.

pathogenesis of a variety of toxic processes.[30] The release of LPO products in hyperoxaluric rats is always enhanced when coadministered with CsA suggesting membranal damage under this condition. This effect may be a cumulative effect of both CsA and oxalate because oxalate itself has been shown to induce LPO.[31] Further, the observed significant positive correlation of oxalate Vs LPO supports the above possibility that oxalate retention increases with the increased LPO. Elevated excretion of oxalate has been observed in CsA-treated rats due to increased intestinal absorption of oxalate.[29] Oxalate retention in CsA-treated rat kidney may be in addition, due to the increased formation through the enzymes LDH and XO. XO is known to produce oxygen radicals.[32] The increased activity of XO in CsA-administered rats may produce increased free radicals and thereby lipid peroxidation. Cell injury is maximum in CsA + AmOx-administered rats as revealed by changes in AST and ALT activities. Further calcium accumulation is higher in the kidney of combined treated groups than either of them. In addition, tubular damage damage with calcium oxlate deposits has been observed only under this condition.[29]

The increased LPO observed in CsA + AmOx administered rat kidney was negatively correlated with antioxidants AA, GSH, Vit. E and thiols. Vit. E, AA, and GSH are

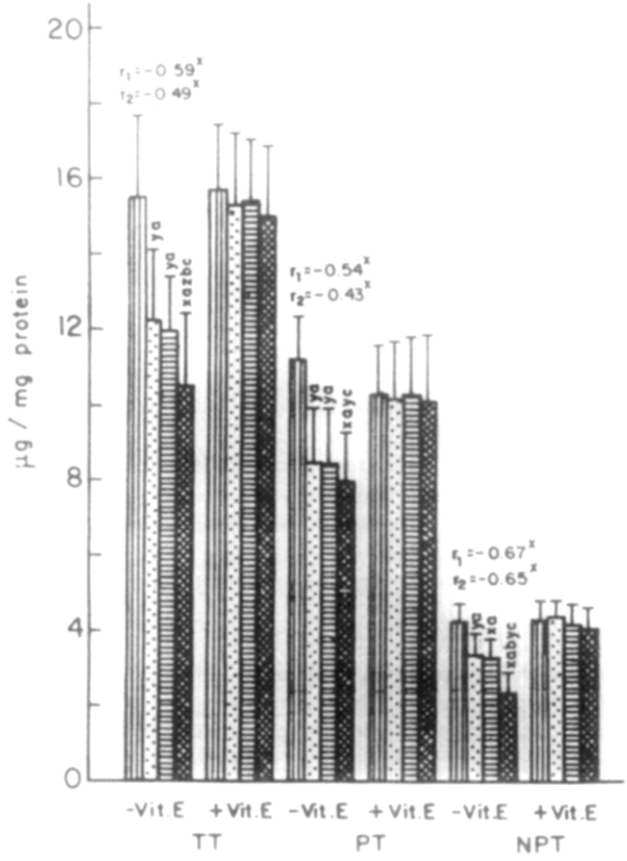

Figure 2 Effect of CsA on thiol status in the rat kidney pretreated with and without Vit. E Values are ± S.D. for eight animals and are statistically significant to [a]control; [b]CsA; [c]AmOx when $^{z}P < 0.05$; $^{y}P < 0.01$; $^{x}P < 0.001$. Correlation coefficient r_1 with LPO; r_2 with oxalate.—Vit. E-vitamin E untreated: + Vit. E-vitamin E pretreated. ▥Control ▨CsA ▤AmOx ▩CsA + AmOx.

interrelated with each other for recycling processes. Recycling of tocopheroxyl radicals to tocopherol is achieved by reaction with ascorbic acid.[33] The dehydroascorbic acid formed in the above reaction is reduced to ascorbic acid by a non-enzymatic reaction with GSH.[34] Deficiency of AA is expected to end up in depletion of tissue tocopherol resulting in elevated lipid peroxidation reactions. The increased LPO found in CsA coadministered hyperoxaluric rats in this study may be considered as a result of decreased AA and Vit. E level. It has been reported that CsA causes depletion of renal GSH to below the critical level, resulting in cell damage.[35,36] The increased activity of GR and decreased activities of GSH-utilizing enzymes, GST, and GPx suggest disturbances in glutathione metabolism. GSH maintains cell membrane sulfhydryl groups and other structural proteins in the stable form. The reduction in GSH level observed in CsA-treated group can be related to the depletion of protein thiol content. Decreased protein thiol content has also been observed in experimental stone-forming rats.[37] The decreased activity of G6PD observed in CsA-administered hyperoxaluric rats may decrease the generation of GSH because GSH generation (via GR) is dependent on the supply of NADPH by G6PD.[38] Though the activity of G6PD is lowered in CsA-administered hyperoxaluric rat kidney, the formed NADPH may be fully utilized by the elevated activity of GR. Because the GSH-utilizing enzymes GST and GPx activity are lowered, it appears that GSH is utilized for the recycling process of dehydroascorbic acid-vitamin E radical cycle,[34] which is produced in lipid peroxidation. This ultimately may decrease the protection rendered by GSH to membrane integrity against lipid peroxidative damage.

Peroxidized membranes can facilitate the entry of calcium into the cell from extracellular fluid. Conditions in which free radicals accumulate are shown to increase cellular and mitochondrial calcium in kidney. Further peroxidized microsomes are shown to increase the uptake of calcium. Thus, a high concentration of calcium can accelerate the initiation of crystallization with oxalate, which is already accumulated in conditions like CsA- and AmOx-treated conditions.

In this study, we had not observed any calcium oxalate crystals in CsA alone-treated rats. However on prolonged administration of CsA to rats, microcalcification has been reported.[42]

The biochemical changes observed in CsA-administered hyperoxaluric rat kidney are the additive effects of CsA and hyperoxaluria. Vit. E pretreatment renders complete protection against the severe cellular damage under the above condition. Though oxalate retention is still present in CsA + AmOx-administered rat kidney under Vit. E pretreated condition, there are no Calcium oxalate deposits observed suggesting protection of cell injury and membranal damage. Similar observations have been reported in rats fed with methionine supplemented calculi producing diet.[43] The protective effect of Vit. E against CsA-induced biochemical changes suggests that supplementation of vitamin E can be advocated for clinical use in preventing the renal side effects of CsA in normal as well as hyperoxaluric condition.

In conclusion, our result show enhanced retention of oxalate in hyperoxaluria under CsA-administered condition. This effect was mediated through the altered antioxidant defensive system, and these biochemical changes were not observed on pretreatment with vitamin E.

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