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Effect of Cyclosporin on Liver Antioxidants and the Protective Role of Vitamin E in Hyperoxaluria in Rats

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Abstract

This study aimed to evaluate whether administration of cyclosporin to hyperoxaluric rats affects liver antioxidant status, and whether pretreatment with vitamin E reverses the effect.

Male Wistar rats were divided into two major groups of 40. One group was given vitamin E. Both major groups were then divided into four subgroups which received vehicle (olive oil), cyclosporin in olive oil (50 mg kg^{-1}), 3% ammonium oxalate or cyclosporin + 3% ammonium oxalate for three days. The activities of liver lactate dehydrogenase, glycolic acid oxidase and xanthine oxidase, and the level of malondialdehyde, an indicator of lipid peroxidation, increased when cyclosporin was administered to hyperoxaluric rats. The levels of antioxidants ascorbic acid, vitamin E and reduced glutathione and the activities of glutathione-metabolizing enzymes were altered significantly when hyperoxaluric rats were treated with cyclosporin. All these enzymes and antioxidants showed highly significant correlation values, r. These changes were restored to near normal by pretreatment with vitamin E.

These findings suggest that cyclosporin-induced hepatotoxicity is aggravated in hyperoxaluria. This was almost totally prevented by pretreatment with vitamin E.

Cyclosporin is a potent immunosuppressive drug used in organ transplantation in man (Starzl et al 1986). Its usage, however, is restricted by its nephrotoxicity and hepatotoxicity (Myers et al 1984), the cellular mechanism of which remains unclear (Le Thai et al 1988). It has been postulated that cyclosporin toxicity might be a result of alteration of plasma membrane permeability or interference with cellular energy derived from mitochondria (Schwertz et al 1985). Drug-induced toxicity might also be related to direct cell injury (Strezeleski et al 1988). Some of these alterations have been reversed by antioxidant supplementation (Kumano et al 1989).

Hyperoxaluria is another condition in which lipid peroxidation is shown to be enhanced in both liver and kidney (Selvam & Ravichandran 1991), but the effect of cyclosporin on hyperoxaluria has not been studied. The purpose of this study was to determine the effect of cyclosporin on the antioxidant status in the hyperoxaluric rat liver and its reversibility by pretreatment with vitamin E.

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Materials and Methods

Adult male albino Wistar rats, 200–220 g, were divided into two major groups of 40 rats each. One group received a single intraperitoneal injection of vitamin E (50 mg/100 g) in mineral oil per week for three weeks. Vitamin E pretreated and untreated groups were then both subdivided into four groups of 10 rats. Group 1 received the vehicle (olive oil) only for three days; group 2 received cyclosporin (Sandoz, Basle, Switzerland; 50 mg kg^{-1}) in olive oil, administered daily by gavage for three days; group 3 received the vehicle for three days and 3% ammonium oxalate in drinking water; group 4 received cyclosporin (50 mg kg^{-1}) for three days and 3% ammonium oxalate in drinking water. The doses of cyclosporin, ammonium oxalate and vitamin E were based on the protocols used by Massicot et al (1994), Kumar et al (1991) and Dillard et al (1982), respectively. In the study of the triggering effect of cyclosporin, ammonium oxalate

feeding was performed for three days only because beyond this time deposition of calcium oxalate crystals occurs in the kidneys of the rats. When the cyclosporin dosage was increased above 50 mg kg^{-1}, food intake by the animals decreased markedly during co-administration with ammonium oxalate.

At the end of the experimental period animals were killed by cervical dislocation and the liver tissue was quickly dissected into ice-cold saline. The weighed tissues (1 g) were homogenized in 10 mL Tris-HCl buffer (pH 7·4, 0·01 M) at 4°C, by means of a Potter Elvejhem homogenizer, to produce a 10% homogenate.

Tissue lipid peroxidation, oxalate, and the activities of the enzymes xanthine oxidase, the oxalate metabolizing enzyme lactate dehydrogenase, alanine transaminase and aspartate transaminase were estimated as described elsewhere (Adhirai & Selvam 1997). The activity of glycolic acid oxidase was also estimated (Lui & Roels 1970).

The levels of antioxidants, ascorbic acid, vitamin E and reduced glutathione were determined. The activities of the glutathione metabolizing enzymes glutathione reductase, γ-glutamyl transpeptidase, glutathione peroxidase, glucose-6 phosphate dehydrogenase and glutathione-*S*-transferase were analysed as reported elsewhere (Adhirai & Selvam 1997).

Statistical analysis

Student's *t*-test was used for statistical analysis. Pearson's correlation coefficient, r, was used to assess the linear correlation between pairs of results.

Results

Liver lipid peroxidation increased to approximately 123 and 136%, respectively, in rats treated with either cyclosporin alone or ammonium oxalate alone. This was further increased to 155% when cyclosporin was administered to hyperoxaluric rats. Pretreatment with vitamin E resulted in protection against this increased lipid peroxidation (Table 1). The increased liver oxalate concentration (129%) in hyperoxaluric rats was further increased to 154% upon co-administration of cyclosporin and this was restored to near normal upon pretreatment with vitamin E. Administration of cyclosporin resulted in significant elevation of xanthine oxidase activity and this was not influenced by ammonium oxalate. Pretreatment with vitamin E prevented this increase in xanthine oxidase activity.

The activities of cytosolic enzymes glycolic acid oxidase and lactate dehydrogenase were increased to 130 and 115%, respectively, compared with control values, in rat liver treated with cyclosporin alone and ammonium oxalate alone; this was further enhanced to 150% when cyclosporin was administered to hyperoxaluric rats (Table 2). Pretreatment with vitamin E restored the activities to near normal levels. A positive correlation was found between lipid peroxidation or oxalate and enzymes. Cyclosporin treatment did not change the activity of aspartate transaminase and there was a 10% decrease in alanine transaminase activity compared with the control value. However, significant ($P < 0.001$) decreases in both aspartate transaminase and alanine transaminase activity were observed when cyclosporin was administered to hyperoxaluric rats. Pretreatment with vitamin E restored the activities of these enzymes to normal.

Table 1. Effect of cyclosporin on lipid peroxidation, oxalate and xanthine oxidase in the untreated rat liver and in the liver with and without vitamin E pretreatment.

	Vitamin E untreated						Vitamin E pretreated			
	Control	Cyclosporin	Ammonium oxalate	Cyclosporin +ammonium oxalate	Correlation (r) Lipid peroxidation	Oxalate	Control	Cyclosporin	Ammonium oxalate	Cyclosporin +ammonium oxalate
Lipid peroxidation (nmol malondialdehyde (mg protein)$^{-1}$)	1·40 ±0·16	1·72 ±0·17**	1·91 ±0·16*,†	2·16 ±0·18***,†††,‡‡‡			1·39 ±0·12	1·47 ±0·11	1·43 ±0·13	1·53 ±0·12*
Oxalate (mg (g wet tissue)$^{-1}$)	1·07 ±0·1	1·16 ±0·09*	1·38 ±0·11*,††	1·65 ±0·12†††,‡‡‡	−0·8		0·98 ±0·1	1·03 ±0·09	1·12 ±0·12*	1·21 ±0·12**,††
Xanthine oxidase (units (mg protein)$^{-1}$)[a]	1·00 ±0·09	1·66 ±0·05***	1·15 ±0·07**,†††	1·75 ±0·09*,††,‡‡‡	−0·6	−0·5	1·05 ±0·10	1·12 ±0·09	1·1 ±0·09	1·23 ±0·05***,††,‡‡

Values are means ± s.d. of results from eight animals. [a]One unit = change in optical density of 0·01 min^{-1}. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with control; †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ compared with cyclosporin alone; ‡‡$P < 0.01$, ‡‡‡$P < 0.001$ compared with ammonium oxalate alone.

Table 2. Effect of cyclosporin on the activities of oxalate synthesizing enzymes and transaminases in the untreated rat liver and in the liver with and without vitamin E pretreatment.

	Vitamin E untreated						Vitamin E pretreated			
	Control	Cyclosporin	Ammonium oxalate	Cyclosporin + ammonium oxalate	Correlation (r) Lipid peroxidation	Oxalate	Control	Cyclosporin	Ammonium oxalate	Cyclosporin + ammonium oxalate
Glycolic acid oxidase (μmol pyruvate min^{-1} (mg protein)$^{-1}$)	0·96 ±0·03	1·30 ±0·02***	1·25 ±0·05***,†	1·46 ± 0·10***,††,‡‡‡	+0·80	+0·80	0·93 ±0·07	0·99 ±0·06	1·02 ±0·08*	1·09 ±0·08**,†
Lactate dehydrogenase (μmol glyoxylate min^{-1} (mg protein)$^{-1}$)	0·94 ±0·03	1·12 ±0·06***	1·07 ±0·10**	1·33 ± 0·09***,†††,‡‡‡	+0·74	+0·80	1·01 ±0·07	1·05 ±0·08	1·05 ±0·08	1·10 ±0·08**,†
Aspartate transaminase (μmol $\times 10^{-1}$ pyruvate min^{-1} (mg protein)$^{-1}$)	0·57 ±0·09	0·55 ±0·08	0·47 ±0·07*,†	0·45 ±0·09*,†	−0·51	−0·37	0·6 ±0·12	0·12 ±0·09	0·59 ±0·09	0·55 ±0·1
Alanine transaminase (μmol $\times 10^{-1}$ pyruvate min^{-1} (mg protein)$^{-1}$)	0·72 ±0·05	0·66 ±0·04*	0·60 ±0·04***,††	0·54 ±0·04*,†,‡‡	−0·78	−0·69	0·7 ±0·06	0·69 ±0·09	0·71 ±0·06	0·65 ±0·1

Values are means ● s.d. of results from eight animals. *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$ compared with control; †$P < 0·05$, ††$P < 0·01$, †††$P < 0·001$ compared with cyclosporin alone; ‡‡$P < 0·01$, ‡‡‡$P < 0·001$ compared with ammonium oxalate alone.

Table 3. Effect of cyclosporin on antioxidant levels in the untreated rat liver and the rat liver with and without vitamin E pretreatment.

	Vitamin E untreated						Vitamin E pretreated			
	Control	Cyclosporin	Ammonium oxalate	Cyclosporin + ammonium oxalate	Correlation (r) Lipid peroxidation	Oxalate	Control	Cyclosporin	Ammonium oxalate	Cyclosporin + ammonium oxalate
Ascorbic acid	1·94 ±0·17	1·60 ±0·16**	1·54 ±0·17***	1·13 ± 0·13***,†††,‡‡‡	−0·8	−0·7	2·12 ±0·20	2·03 ±0·17	1·95 ±0·19	1·73 ±0·13***,††,‡
Vitamin E	3·16 ±0·25	2·73 ±0·21**	2·57 ±0·20***	2·26 ± 0·19***,†††,‡‡	−0·8	−0·7	4·26 ±0·30	3·61 ±0·25***	3·25 ±0·21***,††	3·09 ±0·23***,††
Glutathione	4·51 ±0·18	3·50 ±0·23***	1·29 ±0·17***,†††	1·05 ± 0·16***,†††,‡‡	−0·9	−0·9	4·50 ±0·17	4·48 ±0·18	4·29 ±0·17*,†	4·30 ±0·18*,‡

Values are means ± s.d. of results from eight animals and are expressed as μg (mg protein)$^{-1}$. *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$ compared with control; †$P < 0·05$, ††$P < 0·01$, †††$P < 0·001$ compared with cyclosporin alone; ‡$P < 0·05$, ‡‡$P < 0·01$, ‡‡‡$P < 0·01$ compared with ammonium oxalate alone.

The levels of the antioxidants ascorbic acid and vitamin E were reduced significantly ($P < 0·001$) by both cyclosporin and ammonium oxalate when administered alone or together (Table 3). Vitamin E pretreatment restored the level of ascorbic acid. The glutathione level was reduced significantly in rats treated with cyclosporin but this reduction was highly significant in hyperoxaluric rats with or without cyclosporin co-administration. These reduced glutathione levels were restored to normal in all the experimental groups upon pretreatment with vitamin E. The antioxidants showed negative correlation with liver lipid peroxidation and oxalate.

Compared with control values there was a significant decrease in the activities of glutathione-utilizing enzymes glutathione-*S*-transferase, glutathione peroxidase and glucose-6 phosphate dehydrogenase in the livers both of hyperoxaluric rats and of rats treated with cyclosporin alone. This decrease in activity was highly significant in hyperoxaluric rats receiving cyclosporin (Table 4). The elevated activities of glutathione reductase and γ-glutamyl transpeptidase (140%) in groups treated with cyclosporin alone or with ammonium oxalate alone were further increased to 160% on simultaneous administration of cyclosporin and ammonium oxalate. Interestingly the activity of

Table 4. Effect of cyclosporin on the activities of glutathione metabolizing enzymes in the rat liver with and without vitamin E pretreatment.

	Vitamin E untreated						Vitamin E pretreated			
	Control	Cyclosporin	Ammonium oxalate	Cyclosporin + ammonium oxalate	Correlation (r) Lipid peroxidation	Oxalate	Control	Cyclosporin	Ammonium oxalate	Cyclosporin + ammonium oxalate
Glutathione-*s*-transferase (units $\times 10^{-1}$ (mg protein)$^{-1}$)[a]	4·79 ±0·26	4·1 ±0·23***	4·12 ±0·24***	3·7 ±0·21***,††,‡‡	−0·79	−0·64	4·79 ±0·4	4·7 ±0·60	4·68 ±0·50	4·6 ±0·40
Glucose-6-phosphate dehydrogenase (units (mg protein)$^{-1}$)[a]	3·9 ±0·16	2·97 ±0·14***	3·04 ±0·18	2·67 ±0·12***,†††,‡‡‡	−0·82	−0·68	3·95 ±0·2	3·86 ±0·18	3·49 ±0·16***,††	3·01 ±0·16***,†††,‡‡
Glutathione peroxidase (μ glutathione utilized min^{-1} (mg protein)$^{-1}$)	5·67 ±0·72	5·01 ±0·54*	4·29 ±0·32***,††	3·35 ±0·59***,†††,‡‡	−0·80	−0·70	5·6 ±0·9	5·45 ±0·80	5·52 ±0·90	5·13 ±0·90
Glutathione reductase (units (mg protein)$^{-1}$)[a]	0·72 ±0·07	0·96 ±0·8***	1·04 ±0·07***,†	1·13 ±0·06***,†††,‡	+0·49	+0·61	0·74 ±0·03	0·76 ±0·02	0·77 ±0·03*	0·81 ±0·03***,††,‡
γ-Glutamyl transpeptidase (μmol *p*-nitroaniline min^{-1} (mg protein^{-1}))	2·25 ±0·17	3·13 ±0·18***	3·16 ±0·20***	3·52 ±0·18***,††,‡‡	+0·80	+0·79	2·3 ±0·2	2·6 ±0·23*	2·31 ±0·19	2·75 ±0·20***,‡‡‡

Values are means ± s.d. of results from eight animals. [a]One unit = change in optical density of 0·01 min^{-1}. *$P < 0·05$, ***$P < 0·001$ compared with control; †$P < 0·05$, ††$P < 0·01$, †††$P < 0·001$ compared with cyclosporin alone; ‡$P < 0·05$, ‡‡$P < 0·01$, ‡‡‡$P < 0·001$ compared with ammonium oxalate alone.

glutathione reductase, but not that of γ-glutamyl transpeptidase, was restored to near normal when animals were pretreated with vitamin E. There was positive correlation between liver lipid peroxidation and oxalate whereas there was negative correlation for glutathione-*S*-transferase, glutathione reductase and glucose-6 phosphate dehydrogenase.

Discussion

Lipid peroxidation is regarded as one of the basic mechanisms of tissue damage by free radicals (Esterbauer et al 1991). Increased liver levels of malondialdehyde, lactate dehydrogenase and glycolic acid oxidase were measured in the current study of cyclosporin-treated hyperoxaluric rats. It is interesting to note that the levels of glutathione and the other antioxidants vitamin E and ascorbic acid in hyperoxaluric rat liver were significantly lower than those of control rats, whereas cyclosporin had less effect on these antioxidant levels. Liver is the major source of extracellular antioxidant glutathione (Lauterburg et al 1984). Administration of cyclosporin selectively reduces glutathione in the brain (Henricsson et al 1990). Duruibe et al (1989) have shown that reduced levels of both hepatic and renal glutathione contributes to the toxicity observed during treatment with cyclosporin. A significant reduction in glutathione levels has also been shown in hyperoxaluric rat liver (Selvam & Ravichandran 1991). The increased activity of glutathione-synthesizing enzymes such as glutathione reductase and γ-glutamyl transpeptidase and reduced activity of the glutathione-utilizing enzymes glutathione-*S*-transferase and glutathione peroxidase suggest disturbances in glutathione metabolism. NADPH is required for glutathione generation through the activity of glucose-6-phosphate dehydrogenase (Gaetani et al 1989). The reduced activity of glucose-6-phosphate dehydrogenase observed in cyclosporin-treated hyperoxaluric rat liver might reduce the level of reduced glutathione and thereby increase the susceptibility of cells to lipid peroxidative damage.

The reduced activity of alanine transaminase might lead to an increase in the glyoxylate pool and thereby increase glycolic acid oxidase activity, resulting in hyperoxaluria (Selvam & Ravichandran 1991). Oxalate alone can induce lipid peroxidation (Ernster & Nordenbrand 1967). Glycolic acid oxidase and xanthine oxidase are known to synthesize oxalate and to produce hydrogen peroxide and superoxide anions (Gutteridge et al 1985). The increased activity of glycolic acid oxidase and xanthine oxidase on administration of cyclosporin to hyperoxaluric rats might result in an increase in free radicals and thence lipid peroxidation.

The impact of hyperoxaluria on cellular enzymes and antioxidants seems to aggravate lipid peroxidative damage caused by cyclosporin. The highly

significant correlation (r) between levels of these enzymes and of antioxidants and liver lipid peroxidation and oxalate confirms that the hepatotoxicity arising from lipid peroxidation and depletion of antioxidants was aggravated by hyperoxaluria.

The antioxidants α-tocopherol (vitamin E), ascorbic acid and glutathione are interrelated in recycling processes. Recycling of tocopheroxyl radicals to tocopherol is achieved by reaction with ascorbic acid and the dehydroascorbic acid formed in this reaction is reduced to ascorbic acid by non-enzymatic reaction with glutathione. Because we observed a significant reduction in the level of ascorbic acid, recycling of tocopheroxyl radicals to tocopherol must have been hindered, resulting in elevated levels of lipid peroxidation. A similar observation has been reported in vitamin B_6-deficient rat kidney (Selvam & Ravichandran 1991). The increased levels of the antioxidants ascorbic acid and glutathione resulting from pretreatment with vitamin E might thus normalize the lipid peroxidation reaction and related biochemical changes which in turn protects the cells from the increased risk of peroxidative damage as a result of administration of cyclosporin to hyperoxaluric rats.

Cyclosporin promotes the lipid peroxidation-mediated biochemical changes which are aggravated in hyperoxaluria. Pretreatment with vitamin E quenches the cyclosporin-induced changes and thereby prevents cellular damage.

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