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The Effect of Omega-3 Fatty Acids and Vitamin E on the Nephrotoxicity of Cyclosporin A in Hereditary Hypertriglyceridemic Rats

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Summary

It has been suggested that cyclosporin A (CsA) nephrotoxicity can be reduced by the concomitant administration of omega-3 fatty acids or vitamin E. The present study was designed to establish whether the effect of the above substances can also be demonstrated in rats with hereditary hypertriglyceridemia (HTG) whose sensitivity to the nephrotoxic effect is greater than in control AVN rats. CsA administration at a dose of 10 mg/kg/day to HTG rats resulted in a significant rise ($p<0.001$) in serum levels of creatinine (from 66.0±7.6 to 108.4±11.6 μmol/l) and urea (from 8.3±0.7 to 22.3±18 mmol/l) which was not found in AVN rats. The baseline values of systolic blood pressure (SBP) were significantly higher in HTG rats. However, in both strains CsA administration was associated with a similar SBP increase which was not prevented by omega-3 fatty acids (EPAX) or vitamin E administration. Concomitant administration of CsA with EPAX at a dose of 600 mg/kg b.w./day in HTG rats prevented the rise in the serum levels of creatinine (65.4±14.7 μmol/l) and reduced the increase in the serum urea levels (11.9±7.6 mmol/l). Concomitant administration of CsA and vitamin E (at a dose of 25 mg/kg/day) also reduced the increase ($p<0.05$) in the serum levels of creatinine (70.7±14.3 μmol/l) and urea (9.8±3.4 mmol/l) compared to the effects elicited by the administration of CsA alone ($p<0.05$). Administration of CsA alone or in combination with EPAX or vitamin E did not have a marked effect on diuresis, proteinuria, urinary osmolality, urinary excretion of urea, creatinine and potassium. Under all experimental conditions, the rate of urinary excretion of sodium in HTG rats was significantly lower ($p<0.01$) than in AVN rats. The results obtained support the assumption that omega-3 fatty acids and vitamin E at the doses used reduce CsA nephrotoxicity in rats with hereditary hypertriglyceridemia whose sensitivity to the nephrotoxic effect of CsA is significantly higher than in AVN rats.

Key words

Cyclosporin A • Nephrotoxicity • Hereditary hypertriglyceridemic rats • Omega-3 fatty acids • Vitamin E

Introduction

The beneficial immunosuppressive effect of cyclosporin A (CsA) used in organ transplantation is associated with some toxic effects, the most important one being its nephrotoxic action (Thiel *et al.* 1987, Myers *et al.* 1988, Wang and Salahudeen 1995). Acute CsA nephrotoxicity manifests itself primarily by a reversible decrease in renal blood flow and a reduction in the glomerular filtration rate, whereas chronic nephrotoxicity is primarily characterized by irreversible tubulointerstitial fibrosis (Thiel *et al.* 1987). The mechanism of CsA nephrotoxicity is not yet well understood. Some papers have suggested that CsA induces renal microsomal lipid peroxidation and that CsA administration reduces antioxidant glutathione levels (Duruibe *et al.* 1989, Inselmann *et al.* 1990, Wang and Salahudeen 1994). Other studies indicate that CsA nephrotoxicity could be diminished when lipid peroxidation mediated by reactive oxygen species is decreased by the action of vitamin E (Wang and Salahudeen 1995) or fish oil (Elzinga *et al.* 1987, Ventura *et al.* 1993). Some findings, however, suggest that fish oil prevents vascular nephrotoxicity only partially (Berkenboom *et al.* 1996). Our earlier study (Bohdanecká *et al.* 1999) has shown that CsA nephrotoxicity is more pronounced in rats with hereditary hypertriglyceridemia (HTG) than in control AVN rats. A detailed examination of the HTG rats has indicated that the high blood pressure in these rats correlates with plasma triglyceride levels (Kuneš *et al.* 1995). It is also worthwhile noting that ion transport alterations in HTG rats results in an increased Na^+ content in red blood cells (Kuneš *et al.* 1994, Zicha *et al.* 1995, 1997).

With respect to the potentially beneficial effect of omega-3 fatty acids and vitamin E on CsA nephrotoxicity, we designed a study to establish whether this effect can also be demonstrated in HTG rats the kidneys of which seem to be highly sensitive to CsA nephrotoxicity.

Methods

The experiments were performed on male rats with genetically fixed hypertriglyceridemia (Vrána and Kazdová 1990) weighing 320-360 g, kept under standard conditions with water intake *ad libitum* and a normal commercially available diet (protein content 20 %). Wistar rats (AVN strain) weighing 280-310 g, kept under identical experimental conditions, served as normolipemic controls. Both HTG and AVN rats were 110 days old at the start of the experiment.

The experiments were carried out in compliance with the institutes provisions and directive governing the use of experimental animals, and were approved by the local Ethics Committee.

HTG and AVN rats were divided into four groups: Group 1 (CsA) rats received CsA (HTG n=7; AVN n=6), Group 2 (CsA + EPAX) rats were given CsA in combination with EPAX (HTG n=12; AVN n=6), Group 3 (CsA + vitamin E) rats were administered CsA concomitantly with vitamin E (HTG n=13; AVN n=7), and Group 4 (C) rats were controls, receiving no CsA, EPAX or vitamin E (HTG n=6; AVN n=7). The study lasted 21 days.

CsA was administered in the form of Consupren sol. (Galena, Czech Republic) by intragastric tube at a dose of 10 mg/kg/day (0.2 ml/100 g b.w.), diluted with placebo (Consupren vehicle, supplied by the manufacturer). Fish oil (EPAX, 3000 TG Pronova) containing 35% of n-3 fatty acids (eicosapentaenoic and docosahexaenoic acids) was administered at a dose of 600 mg/kg b.w./day and vitamin E (DL-tocopherol) was given in a dose of 25 mg/kg/day. Both products were added into that part of placebo used for diluting CsA. The last dose was invariably administered 24 hours before the end of the experiment.

Blood samples were collected to determine the serum levels of creatinine, urea, sodium and potassium (Hitachi 707, 717 autoanalyzers); triglycerides and cholesterol were determined using commercially available enzymatic kits (Lachema, Brno, Czech Republic).

CsA kinetics were monitored for 24 h at the end of the experiment, i.e. after 21 days of CsA administration. The blood levels were determined 1, 2, 4, 6, 12 and 24 h after CsA administration by the RIA method using a specific antibody (RIA kit Cyclo-Trac SP, USA). The area under the 24-h curves of CsA concentrations in whole blood (AUC_{0-24} mg.h/l) was evaluated using the trapezoidal method.

Urine was collected from rats in metabolic cages over a period of 24 hours. The monitored parameters included urine volume, the amounts of excreted creatinine, urea, sodium and potassium (Hitachi 707, 717 autoanalyzers), proteinuria (Lachema kit) and osmolality (Osmomat 030 osmometer).

Systolic blood pressure was measured non-invasively by tail-cuff plethysmography. Throughout the experiment, the rats' behavior and their body weight were monitored daily. At the end of the experiment, both kidneys were removed under ether anesthesia; the kidneys were immediately weighed and samples for histology were obtained. For evaluation by light microscopy, the kidneys were fixed in 10 % formol and processed by the standard technique. Paraffin slices were stained with HE (hematoxylin-eosin), the PAS method (periodic acid and Schiff's reagent), green trichrome and PASM (basement membrane impregnation by silvering).

Statistical analysis was performed using Friedmann's test (Neményi's modification), Wilcoxon's test and two-way analysis of variance. Data are given as means ± S.D.

Table 1. Plasma levels of triglycerides, total cholesterol, creatinine, urea and the concentrations of cyclosporin A in hypertriglyceridemic (HTG) and control (AVN) rats

	Group	C	CsA	CsA+EPAX	CsA+vitamin E
Triglycerides	HTG	3.1±0.8 ***	3.2±2.1	2.0±0.6 *	3.0±0.9 **
(mmol/l)	AVN	1.2±0.2	2.3±1.2	1.4±0.3	1.8±0.3
Cholesterol	HTG	2.0±0.7	2.1±0.5	1.3±0.2 ***	1.9±0.2 ***
(mmol/l)	AVN	2.0±0.1	2.0±0.2	1.8±0.1	2.3±0.1
Creatinine	HTG	66.0±7.6	108.4±11.6 ***	65.4±14.7	70.7±14.3 *
(μmol/l)	AVN	63.5±7.1	58.6±3.1	60.1±2.4	58.4±2.8
Urea	HTG	8.3±0.7	22.3±18.0 ***	11.9±7.6 **	9.8±3.4 *
(mmol/l)	AVN	7.6±0.8	7.6±0.9	7.1±1.0	6.9±0.4
CsA AUC_{0-24}	HTG		54.7±7.6	30.5±12.9	40.1±12.1
($mg.h.l^{-1}$)	AVN		47.7±15.8	39.6±2.1	48.0±5.2

*Data are means ± S.D.; C – without treatment; CsA – cyclosporin A; EPAX – fish oil; vitamin E – tocopherol; * $p<0.05$; ** $p<0.01$; *** $p<0.001$ significantly different from corersponding groups of AVN rats.*

Results

It is evident that the mean serum triglycerides levels were higher in all experimental groups of HTG rats than in corresponding AVN rats (Table 1). The differences were significant except for the group receiving CsA only. Table 1 also indicates that, in the groups given CsA + EPAX and CsA + vitamin E, the serum levels of cholesterol in HTG rats were significantly lower than in AVN rats.

CsA administration to HTG rats resulted in a significant rise of serum creatinine and urea levels. By contrast, CsA administration to AVN rats did not cause significant changes in the serum levels of these two substances. Concomitant administration of EPAX prevented the increase in serum creatinine levels in HTG rats. The rise in serum urea levels on concomitant administration of CsA and EPAX was diminished compared to the increase seen on administration of CsA alone. However, urea level remained significantly elevated compared with AVN rats on the same experimental regimen. The concomitant administration of CsA and vitamin E was associated with a smaller yet still significant elevation of the serum levels of creatinine and urea. Finally, the areas under the curves of blood CsA levels (AUC_{0-24}) did not differ significantly between HTG and AVN rats (Table 1).

Table 2 shows that the urinary volume in HTG and AVN rats did not differ significantly. The rate of urinary protein excretion was significantly higher in HTG than in AVN rats under both control and experimental conditions. No significant differences in the rates of creatinine, urea, and potassium excretion were observed between HTG and AVN rats under all experimental conditions. However, a significant difference between HTG and AVN rats was found in urinary sodium

excretion. In HTG rats, the rate of urinary sodium excretion was significantly lower than in AVN rats and this difference was present under all experimental conditions. It is also evident from Table 2 that urinary osmolality in HTG and AVN rats did not differ in any of the experiments.

Table 2. Renal parameters measured in individual experimental groups

	Group	C	CsA	CsA+EPAX	CsA+vitamin E
Urinary volume	HTG	17.2±6.3	15.0±6.5	16.7±8.3	12.6±2.9
(ml/24 h)	AVN	17.2±12.0	20.3±8.0	14.6±6.3	12.4±2.7
Urinary protein	HTG	11.6±4.4 ***	9.4±5.2 ***	10.3±4.5 ***	7.6±2.4 ***
(mg/24 h)	AVN	1.9±0.6	0.9±0.5	1.1±0.2	1.1±0.3
Urinary osmolality	HTG	566±114	633±237	706±288	775±166
(mmol/kg)	AVN	646±283	519±174	636±201	741±195
Urinary sodium	HTG	108±112 ***	96±88 **	112±87 **	125±87 **
(μmol/24 h)	AVN	513±143	302±200	369±225	338±185
Urinary potassium	HTG	1300±280	920±500	1080±280	1050±250
(μmol/24 h)	AVN	1480±280	1120±900	890±290	910±280
Urinary creatinine	HTG	73.8±21.9	62.0±21.9	69.4±18.3	71.1±22.9
(μmol/24 h)	AVN	76.3±14.8	90.2±50.8	68.2±20.5	66.0±7.9
Urinary urea	HTG	5.4±0.8	4.2±2.3	4.6±1.6	4.3±1.5
(mmol/24 h)	AVN	5.4±2.1	5.4±1.9	5.2±1.9	3.7±1.6

For the legend see Table 1.

Table 3. Systolic blood pressure (SBP) and body weight (BW) at the beginning and at the end of experiment in hypertriglyceridemic (HTG) and control (AVN) rats

	Group	C	CsA	CsA+EPAX	CsA+vitamin E
SBP	HTG	138.0±13.3**	153.0±17.6*	163.8±13.9***	166.4±10.5***
(mm Hg)	AVN	121.5±10.6	136.6±6.6	141.8±9.9	138.4±6.2
BW (g)					
Beginning	HTG	317±13	344±38	314±38	364±32
End		359±13^{++}	282±41^{+++}	268±34^{++}	329±34^{++}
Beginning	AVN	187±19	309±11	280±14	296±11
End		224±16^{+++}	299±14	268±15	295±19

*Data are means±S.D.; * $p<0.05$; ** $p<0.01$; *** $p<0.001$, significant difference of SBP between HTG and AVN rats. ++ $p<0.01$; +++ $p<0.001$, significant difference of BW between beginning and end of the experiment. For other legend see Table 1.*

Table 3 clearly shows that CsA administration to HTG rats was associated with a significant rise in systolic blood pressure which could not be prevented by the concomitant administration of EPAX or vitamin E. However, systolic blood pressure was always significantly higher in HTG rats, i.e. even under control conditions (without CsA administration).

Table 3 also gives the body weight of HTG and AVN rats at the start and end of the experiment. HTG rats exhibited a small significant decrease ($p<0.001$) in body weight after CsA administration as compared with the baseline. Concomitant administration of EPAX or vitamin E reduced but did not prevent this decrease; the differences compared with the baseline remained significant ($p<0.01$). The body weight of AVN rats did not change significantly under any of the experimental conditions.

CsA administration was associated with more severe histological lesions in HTG than in AVN rats. The kidneys of HTG rats receiving CsA alone exhibited vacuolization of tubular epithelial cells, hypergranulation of the juxtaglomerular apparatus and smooth muscle cell hypertrophy in the arterioles (Fig. 1), and occasionally even fibrin insudate deposition in accordance with the findings of Ryffel (1986).

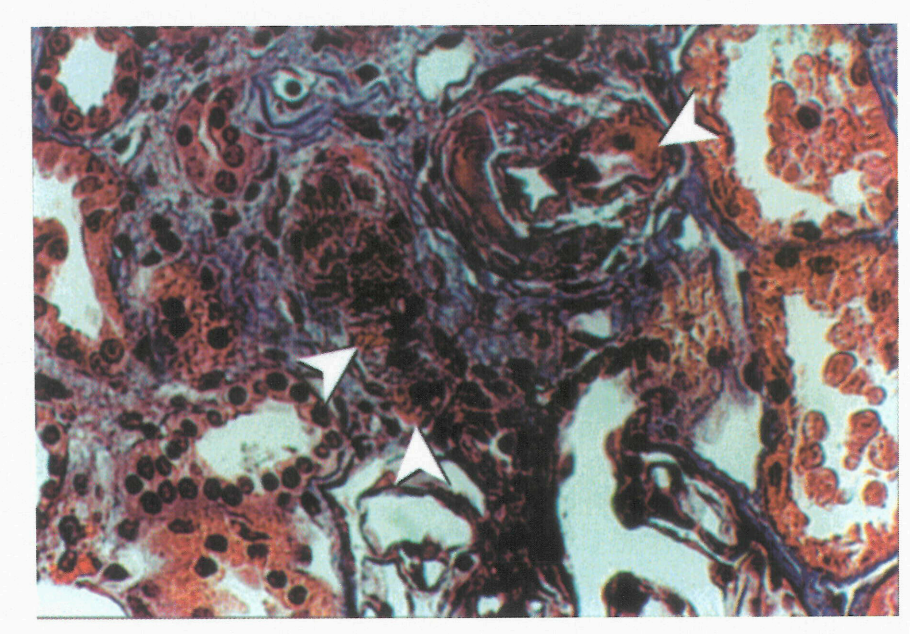

Fig. 1. *Typical histological picture of HTG kidney receiving cyclosporin A. Fibrinoid insudates of arterioles are indicated by arrows. Original magnification 504x, staining ASOg.*

Following the concomitant administration of CsA and EPAX, discrete microvacuolization in a small number of tubular epithelial cells and minor hyalinization was present in the kidney of one rat only. All other kidneys in this group showed only occasional fine hypergranulation of the juxtaglomerular apparatus. Similar changes were observed after concomitant administration of CsA and vitamin E. AVN rats showed only minimal changes (occasionally mild vacuolization of the tubular epithelium) under all experimental conditions.

Discussion

Our results support the assumption that enhanced CsA nephrotoxicity in rats with hereditary hypertriglyceridemia, demonstrated in our previous paper (Bohdanecká *et al.* 1999), may be modulated in some parameters by the concomitant administration of EPAX or vitamin E. This assumption is supported by the findings that the administration of the above substance to HTG rats receiving CsA was capable of preventing the increase in serum creatinine levels and attenuating the rise in serum urea levels.

It is also clear from our results that CsA administration was associated with an increase in systolic blood pressure not only in HTG rats but also in AVN rats. However, the concomitant administration of EPAX or vitamin E did not prevent the rise in systolic blood pressure. These findings suggest that the changes in renal function caused by CsA administration in HTG rats cannot be explained by the changes in blood pressure, since concomitant administration of EPAX or vitamin E

was associated with normalization of serum creatinine level, although arterial hypertension persisted in these rats. The observed changes in blood pressure are consistent with the histological findings on the juxtaglomerular apparatus.

Cyclosporin A availability, assessed by AUC_{0-24}, suggests that this parameter in HTG rats was significantly lower whenever the rats were concomitantly treated with EPAX or vitamin E. In AVN rats, the differences were not significant. How far EPAX and vitamin E may affect intestinal CsA absorption in HTG rats cannot be established from these data. For these reasons, the relationships between AUC_{0-24} and serum creatinine levels were analyzed in selected groups of HTG rats (Table 4).

Table 4. Correlation analysis of the relationship between AUC_{0-24} of CsA and serum creatinine levels in individual experimental groups of HTG rats

Group	CsA	CsA + EPAX	CsA + vitamin E
r coefficient	0.396	0.161	0.460
Significance	n.s.	n.s.	n.s.

n.s. – not significant

There was no significant relationship between AUC_{0-24} and serum creatinine levels in any of the groups studied. These results do not support the assumption that the relatively lower CsA nephrotoxicity in HTG rats could be due to reduced CsA bioavailability.

Furthermore, Table 1 shows that the respective values of AUC_{0-24} in HTG rats, receiving CsA concomitantly with EPAX or vitamin E, did not differ significantly from those found in AVN rats. As regards the urinary findings, HTG rats had significantly higher proteinuria than AVN rats, even under control conditions. CsA administration (either alone or in combination with EPAX or vitamin E) did not enhance proteinuria. These interesting findings remain to be elucidated.

HTG and AVN rats differ in renal sodium excretion, which was lower in HTG rats under all experimental conditions. The assumption that tubular Na^+ transport in HTG rats differs from that in AVN rats is in agreement with the findings of Kuneš *et al.* (1994) showing altered Na^+ transport in erythrocytes of HTG rats.

The results obtained are consistent with the data reported by Elzinga *et al.* (1987), Jevnikar *et al.* (1988), Torras *et al.* (1992, 1994), Berthoux *et al.* (1992) and Ventura *et al.* (1993), supporting the assumption that omega-3 fatty acids reduce CsA nephrotoxicity. Our findings are also in accordance with the data reported by Anderson *et al.* (1994) and Adhirai and Selvam (1997) indicating that concomitant administration of vitamin E lowers CsA nephrotoxicity.

The present data also support the assumption about the beneficial effect of omega-3 fatty acids and vitamin E, which can be demonstrated in rats with hereditary hypertriglyceridemic rats in which Cyclosporin A nephrotoxicity was shown to be significantly higher than in control AVN rats. The potential clinical applicability of these findings was suggested by Berthoux *et al.* (1996) demonstrating a favorable effect of omega-3 fatty acids in a one-year randomized study in renal transplant recipients. However, more clinical trials are needed to verify these findings.

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