JPP Journal of Pharmacy and Pharmacology

JPP 2005, 57: 1485–1492

Received March 21, 2005
Accepted July 26, 2005
DOI 10.1211/jpp.57.11.0015
ISSN 0022-3573

Attenuation of ciclosporin-induced nephrotoxicity by dietary supplementation of seal oil in Sprague-Dawley rats

Wei Yang, Gene R. Herzberg, Zhili Kang, Lili Wang, Desmond Robb*, Edward Randell, John Smeda, Jieying Xiong, Mohamedtaki Kara and Hu Liu

Abstract

Fish oil, rich in omega-3 (n-3) polyunsaturated fatty acids (PUFAs), has been reported to attenuate nephrotoxicity induced by ciclosporin (cyclosporine A). Harp seal oil is a rich source of n-3 PUFAs. This study investigated the ability of dietary seal oil to reduce nephrotoxicity caused by ciclosporin. Sprague-Dawley rats were maintained on a standard diet (with sunflower oil as lipid, SFO) or a diet enriched with seal oil (with 85% seal oil and 15% sunflower oil as lipid, SO) for four weeks before and four weeks after intravenous administration of ciclosporin (15 mg kg^{-1} daily). Kidney function was assessed by measuring blood urea nitrogen, creatinine clearance, urinary *N*-acetyl-1-β-D-glucosaminidase, 6-keto-prostaglandin $F_{1\alpha}$, thromboxane B_2 and malondialdehyde. Systolic blood pressure (SBP) was monitored. Ciclosporin concentrations in blood were measured using liquid chromatography–tandem mass spectrometry (LC-MS/MS). The fatty acid compositions of the diets and erythrocyte membranes were analysed by gas chromatography (GC). The results showed that nephrotoxicity was induced by ciclosporin in rats maintained on both SO and SFO diets. However, rats fed on SO diet endured less toxicity than those on SFO diet. The n-3 and n-6 PUFAs in the erythrocyte membrane of rats maintained on SO diet were found to be 10.79% and 11.93%, while those in rats maintained on SFO diet were found to be 1.67% and 22.71%, respectively. In conclusion, dietary supplementation of seal oil was found to reduce ciclosporin-induced nephrotoxicity in rats.

School of Pharmacy, Memorial University of Newfoundland, St. John's, NL, Canada A1B 3V6

Wei Yang, Zhili Kang, Lili Wang, Mohamedtaki Kara, Hu Liu

Department of Biochemistry, Memorial University of Newfoundland, St. John's, NL, Canada A1B 3V6

Gene R. Herzberg, Edward Randell, Hu Liu

Faculty of Medicine, Memorial University of Newfoundland, St. John's, NL, Canada A1B 3V6

Desmond Robb, Edward Randell, John Smeda, Jieying Xiong, Hu Liu

Correspondence: H. Liu, School of Pharmacy, Memorial University of Newfoundland, St. John's, NL, Canada A1B 3V6. E-mail: hliu@mun.ca

Funding: The authors wish to express sincere appreciation to the Canadian Institutes of Health Research (CIHR) for the operating grants to HL and LW and from Natural Science and Engineering Research Council of Canada (NSERC) to GRH. Dr Hu Liu was a recipient of the Rx&D-HRF/CIHR Career Award in Health Sciences funded by the Health Research Foundation of Canada's Research Based Pharmaceutical Companies (Rx&D) and CIHR.
*__Note:__ Dr Robb passed away in 2004.

Introduction

Dose-related ciclosporin (cyclosporine)-induced renal dysfunction is the most frequent adverse effect noted with this potent immunosuppressant, despite its unique advantage of lacking myelosuppression compared with other immunosuppressants (Paavonen et al 1981). In both animals and man, ciclosporin was observed to cause nephrotoxicity, characterized by renal function impairment and morphological changes in the kidney. Studies suggested that reduced renal plasma flow (RPF) and glomerular filtration rate (GFR), reflected by decreased creatinine clearance (Clcr) and increased blood urea nitrogen (BUN), may contribute to renal dysfunction. The altered renal haemodynamics is thought to be produced by a ciclosporin-induced imbalance between the production of vasoconstrictors and vasodilators (Perico et al 1986), and by direct toxic effects to vascular endothelia (Antonovych et al 1988), resulting in vasoconstriction of the afferent arterioles. The increased production of endothelin (ET) (Darlametsos et al 2000), altered intracellular calcium regulation (Meyer-Lehnert & Schrier 1998), the activation of sympathetic system (Scherrer et al 1990) and alteration of eicosanoids production (Zoja et al 1990) have all been implicated in the triggering of ciclosporin-induced nephrotoxicity.

Since ciclosporin is used on a long-term basis in organ transplant patients, nephropathy associated with chronic ciclosporin use is of great concern. Different strategies have been used in an attempt to minimize its toxicity. Epidemiological studies have found that fish oil, rich in long chain n-3 polyunsaturated fatty acids (PUFAs), is beneficial in reducing cardiovascular diseases (Dreyeberg et al 1975). Recently, it was

found that ciclosporin-induced nephrotoxicity and thromboxane A_2 (TXA_2) production were reduced in rats when olive oil was replaced with fish oil in the formulation of ciclosporin (Casas et al 1995). Dietary supplementations of fish oil also significantly reduced the incidence of graft rejection, and improved blood pressure and GFR in patients receiving ciclosporin for kidney transplantation (Bennett et al 1995).

The protective effect of fish oil against ciclosporin-induced nephrotoxicity is ascribed to its high content of n-3 PUFAs, including eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3). These n-3 PUFAs may modify the production of vasoactive eicosanoids, such as the vasodilators prostaglandin E_2 (PGE_2) and prostaglandin I_2 (PGI_2) and the vasoconstrictor TXA_2, by competing with arachidonic acid (AA, C20:4 n-6), which is used as a precursor for the production of these vasoactive agents. This action could reduce ciclosporin-induced arteriolar vasoconstriction, which is thought to be responsible for impaired renal function. There have been conflicting reports (Hansen et al 1995; Kooijmans-Coutinho et al 1996) about the degree of benefit produced by consuming large amounts of n-3 PUFAs with regards to improving renal function. The discrepancies between these studies are most likely due to different experimental protocols, and the quality of fish oil used in different studies. It is known that the source and the degree of oxidization of fish oil can affect its beneficial properties.

Blubber oil from harp seal (seal oil) is a rich source of n-3 PUFAs and has been found to be superior to fish oil in several ways. Firstly, seal oil contains ten times more docosapentaenoic acid (DPA, 22:5 n-3), which is believed to be a more potent anti-atherogenic factor than EPA (Kanayasu-Toyoda et al 1996; Murphy et al 1997). Secondly, like in man, the fatty acids in triacylglycerides (TAGs) in seal oil were found mainly at the *sn*-1 and *sn*-3 positions, whereas the fatty acids in fish oil are mainly at the *sn*-2 position (Puppione et al 1992; Christensen & Hoy 1996). As a result, it is expected that the fatty acids found in seal oil are more easily absorbed by man. Seal oil is also much more stable and less prone to lipid oxidation than fish oil (Nakhla 1997).

This study was conducted to explore the potential of using seal oil to reduce the nephrotoxicity of ciclosporin.

Materials and Methods

Male Sprague-Dawley rats (3-week old, from the Animal Care Services of Memorial University of Newfoundland, St John's, NL, Canada), 56–78 g, were housed under standard conditions, with free access to tap water and SFO diet or SO diet. The SFO diet was prepared according to a formulation published by the American Institute of Nutrition (Reeves et al 1993). The compositions of two diets were identical with the exception of a 70 g sunflower oil per kg diet present in the SFO diet was replaced by 59.5 g of seal oil and 10.5 g of sunflower oil in the SO diet.

All ingredients used in the diets, with the exception of seal oil and sunflower oil, were purchased from ICN Pharmaceuticals Inc. (Montreal, Canada). The ciclosporin injection (Sandimmune IV) was a product of Novartis Pharm Canada Inc. (Dorval, QC, Canada). All other chemicals used were of analytical grade and were purchased from Sigma-Aldrich Canada Ltd (Oakville, ON, Canada), unless otherwise indicated. The seal oil was a gift from the Atlantic Marine Products (Catalina, NL, Canada). The sunflower oil was a product of the Best Foods Inc. (Calgary, AB, Canada).

After three days of acclimation to the new environment, the rats were randomly divided into four groups and were fed either an SFO diet or an SO diet. The rats were studied four weeks before and for four weeks during the daily intravenous administration of ciclosporin ($15\,mg\,kg^{-1}$) or normal saline (NS) via the tail vein. The following is a summary of the groups studied: SFO diet + intravenous injection of NS (control); SFO diet + intravenous injection of ciclosporin; SO diet + intravenous injection of NS (control); SO diet + intravenous injection of ciclosporin.

The procedures used in this study adhered to the Guidelines to the Care and Use of Experimental Animals, as issued by the Canadian Council on Animal Care. Blood and urine samples were taken one week before, and once a week during, the four-week intravenous administration of ciclosporin or NS. Blood samples were taken from the jugular vein. Urine samples were collected using metabolic cages over 24 h following blood sampling.

The blood samples were centrifuged at 3000 *g* for 15 min at 4°C and urine samples were centrifuged at 10 000 *g* for 10 min at 4°C. The urine supernatant and blood serum samples were frozen at −20°C until analysis. Rats were sacrificed by inhaling an overdose of ethyl ether upon completing the study. Before death, blood samples were withdrawn by direct cardiac puncture collected into Vacutainer tubes containing EDTA for the determination of whole blood ciclosporin concentrations. A 2-mL volume of the blood was centrifuged at 840 *g* for 10 min to separate the red blood cells (RBCs). The RBCs were washed with ice-cold NS (×3) and stored at −80°C until analysis of fatty acid compositions by gas chromatography (GC).

Upon sacrifice, the kidneys of rats were perfused in-situ with ice-cold NS until the colour of kidneys changed to pale. Kidneys were then excised; half of the left kidney was fixed in buffered (phosphate-buffered saline, PBS, pH 7.4) 4% paraformaldehyde for histological assessment. The rest of the kidneys were frozen in liquid nitrogen immediately after removal, and stored at −80°C until further biochemical analysis.

BUN, serum and urine creatinine concentrations were determined using Diagnostics Urea Nitrogen (Cat. no. 640) and Diagnostics Creatinine kit (Cat. no. 555; Sigma Diagnostics, St Louis, MO, USA), respectively. Urinary *N*-acetyl-1-β-D-glucosaminidase (NAG) levels were measured by a colorimetric procedure using PPR NAG Test Kit (PPR Diagnostics Ltd, London, UK).

The systolic blood pressure (SBP) of rats was measured using the tail plethysmography method. Briefly, rats were

placed in a quiet, temperature-controlled environment (33°C) for 30 min. A tail cuff with a light sensor connected to a pulse amplifier (Model 29; 11TC INC., Woodland Hills, CA, USA) was placed as proximal as possible and 4–6 blood pressure readings were recorded using a 2-channel recorder. The blood pressures reported are the average of 4 or 5 measurements.

Whole-blood ciclosporin concentrations were measured by liquid chromatography–tandem mass spectrometry (LC-MS/MS) following protein precipitation with zinc sulfate. Briefly, 20 μL of anti-coagulated blood was mixed with 80 μL of 0.1 M zinc sulfate followed by addition of 200 μL of 20 $\mu g\,L^{-1}$ ascomycin in acetonitrile as internal standard. This mixture was then centrifuged and 5 μL of the supernatant was injected on a Phenomenex Security Guard Cartridge (Part no: KJ0–4282) to elute in 50–100% methanol gradient containing 2 mM ammonium acetate and 0.1% formic acid at a flow rate of 0.6 mL min^{-1}. The chromatography was performed on a Waters 2795 Alliance HT HPLC system at 55°C. The eluted drugs were detected using a Micromass Quattro LC tandem mass spectrometer operated in electrospray positive-ionization mode.

One part of the freshly excised kidney was mixed with 9 parts of 100 mM ice-cold potassium chloride containing 3 mM EDTA and was homogenized using a glass tissue grinder tube. Lipid peroxidation was then assessed using the OXI-TEK Thiobarbituric Acid Reactive Substances (TBARS) Assay kit (Cat. no: 0801192; ZeptoMetrix Corp., Buffalo, NY, USA) and was expressed as the level of malondialdehyde (MDA) as nmol (mg protein)$^{-1}$.

Thromboxane B_2 (TXB_2) and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) levels in the urine samples were analysed using enzyme immunoassay with Thromboxane B_2 and 6-Keto-Prostaglandin $F_{1\alpha}$ Biotrak EIA System (Cat. no: RPN 220, RPN221; Amersham Biosciences Corp., Piscataway, NJ, USA), respectively. The data were presented as the ratio 6-keto-$PGF_{1\alpha}/TXB_2$.

The fatty acid composition in the SFO and SO diets and phospholipid fatty acid composition in the erythrocyte membrane of rats consuming the respective diets were analysed by GC. Total lipids of the samples were extracted using a modified Folch procedure (Folch et al 1957). The extracted lipid samples were transmethylated with Transmethylation Reagent (94% MeOH, 6% aqueous HCl) and a few crystals of hydroquinone at 60°C for 2 h. Fatty acid methyl esters were separated by GC using an Omegawax 320 capillary column (30 m × 0.32 mm × 0.2 μm thickness) in a Hewlett Packard 5890 Series II gas chromatographer equipped with a flame ionization detector. Separation was performed isothermally at 200°C, with helium as the carrier gas at a flow rate of 1 mL min^{-1}. Identification of the peaks in the chromatograms was made by comparison of retention times with known standards.

Statistical analysis

Results are presented as mean ± s.d. Comparisons between multiple groups were performed by two-way analysis of variance and post-hoc test (Tukey's test) as appropriate. $P < 0.05$ was set as statistical significance.

Results

Kidney function

BUN and Clcr are commonly used clinical parameters of renal function. A reduction in glomerular filtration is associated with increased BUN and decreased Clcr. As shown in Table 1, significantly elevated BUN and decreased Clcr levels

Table 1 The BUN, Clcr and urinary NAG levels in rats fed on the SO diet or SFO diet, measured one week before, and once a week following the commencing of intravenous administration of NS or ciclosporin (Sandimmune IV, 15 mg kg^{-1} daily) for four weeks

	Time	SO + NS	SFO + NS	SO + ciclosporin	SFO + ciclosporin
BUN (mg dL^{-1})	Week 0	17.34 ± 1.68	18.53 ± 2.62	18.37 ± 1.94	16.08 ± 2.83
	Week 1	15.26 ± 1.13	13.47 ± 1.41	17.41 ± 4.47#	17.22 ± 3.78#
	Week 2	16.59 ± 3.13	15.28 ± 1.72	30.62 ± 4.33##	30.77 ± 4.92##
	Week 3	16.43 ± 2.39	15.35 ± 2.26	28.97 ± 7.43##	35.27 ± 8.27##
	Week 4	16.39 ± 2.41	17.15 ± 2.19	33.85 ± 9.51*,##	45.73 ± 7.27##
Clcr (mL min^{-1})	Week 0	1.30 ± 0.25	1.27 ± 0.30	1.32 ± 0.20	1.31 ± 0.29
	Week 1	1.56 ± 0.16	1.60 ± 0.21	1.41 ± 0.22	1.40 ± 0.36
	Week 2	1.50 ± 0.37	1.49 ± 0.28	0.95 ± 0.24##	0.93 ± 0.25##
	Week 3	1.63 ± 0.30	1.51 ± 0.14	0.91 ± 0.18##	0.77 ± 0.19##
	Week 4	1.70 ± 0.53	1.64 ± 0.52	1.19 ± 0.22##	0.88 ± 0.19##
Urinary NAG (mmol h^{-1} L^{-1})	Week 0	8.97 ± 2.16	10.00 ± 1.51	9.66 ± 2.29	8.80 ± 3.39
	Week 1	8.81 ± 2.32	9.50 ± 1.89	10.18 ± 4.68	13.11 ± 4.60
	Week 2	8.67 ± 1.73	9.74 ± 1.70	15.56 ± 1.65##	18.52 ± 5.84##
	Week 3	10.29 ± 2.33	10.78 ± 2.07	20.99 ± 2.59**,##	25.92 ± 3.51##
	Week 4	9.48 ± 1.32*	11.22 ± 3.44	21.57 ± 4.46*,##	26.76 ± 2.82##

Rats were fed seal oil enriched (SO) diet or standard (SFO) diet, respectively, for four weeks before the start of the experiment and were maintained on the respective diets during the four-week of intravenous administration of normal saline (NS) or ciclosporin. Data shown are mean ± s.d. (n = 6–8). *$P < 0.05$, **$P < 0.01$, SO vs SFO; #$P < 0.05$, ##$P < 0.01$, NS injection vs ciclosporin injection.

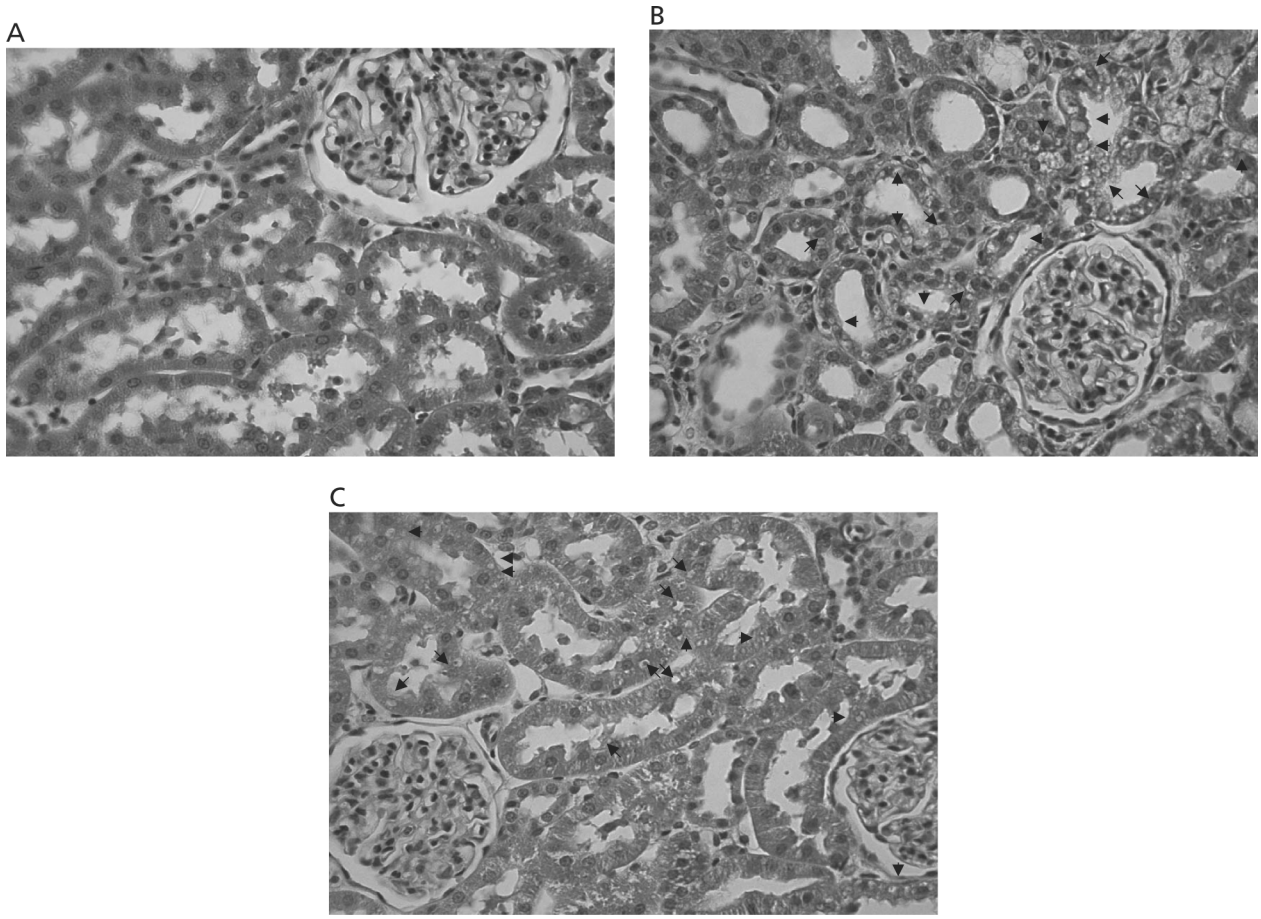

Figure 1 A. Photomicrograph of kidney of rats kept on seal oil enriched (SO) diet following intravenous administration of normal saline (NS) for four weeks, showing a normal tubular interstitial pattern (HE. ×400). B. Photomicrograph of kidney of rats kept on standard (SFO) diet following intravenous administration of ciclosporin (Sandimmune IV, 15 mg kg^{-1} daily) for four weeks, showing a large amount of intracellular vacuoles (arrows) together with brush border loss and atrophy of the tubular cells (HE. ×400). C. Photomicrograph of kidney of rats kept on SO diet following intravenous administration of ciclosporin (Sandimmune IV, 15 mg kg^{-1} daily) for four weeks, showing some intracellular vacuoles (arrows) (HE. ×400).

were observed in rats on both SFO and SO diets, following two weeks of intravenous administration of ciclosporin, compared with those receiving NS, indicating a decreased kidney function. The two parameters continuously deteriorated for the next two weeks. However, rats on the SO diet endured less kidney damage during the last two weeks of ciclosporin administration as shown by the less radical change found in BUN and Clcr in comparison with those on SFO diet. Urinary NAG, a sensitive and reliable indicator of tubular injury, was also found to increase following the administration of ciclosporin with a similar trend to that for BUN.

Morphological change

Light microscopic examination of kidney slides showed no morphological changes with the control where NS was administered (Figure 1A). Numerous intracellular vacuolizations within some of the proximal tubules in rats on SFO diet were noted following the administration of ciclosporin (Figure 1B). The number of proximal tubular vacuolizations in rats on SO diet was less than rats on SFO diet following the administration of ciclosporin (Figure 1C). In addition, brush-border loss and single-cell necrosis were noted in rats on SFO diet.

Haemodynamic alteration

The SBP was found to increase significantly ($P < 0.01$) in rats fed both SO and SFO diets following the administration of ciclosporin when compared with their corresponding controls (Table 2). Although the SBP values in rats fed with SO diet increased less dramatically than those maintained on SFO diet at each week following the administration of ciclosporin, the difference was statistically significant ($P < 0.05$) only at the first week of ciclosporin administration.

MDA content in kidney

Following the administration of ciclosporin for four weeks, renal MDA levels were significantly ($P < 0.01$) elevated in rats on both SFO and SO diets (197.1 ± 37.3 and 208.7 ± 49.2 nmol (mg protein)$^{-1}$, respectively) when

Table 2 SBP in rats measured one week before and once a week during the intravenous administration of NS or ciclosporin (Sandimmune IV, 15 mg kg^{-1} daily) for four weeks

Time	SO + NS	SFO + NS	SO + ciclosporin	SFO + ciclosporin
Week 0	134.7 ± 9.6	133.5 ± 2.7	135.0 ± 8.5	136.6 ± 9.1
Week 1	130.2 ± 3.7	133.2 ± 4.7	138.8 ± 6.6*,##	146.3 ± 4.6##
Week 2	133.7 ± 5.9	131.5 ± 6.2	139.3 ± 4.5##	142.9 ± 3.1##
Week 3	134.7 ± 5.2	135.0 ± 6.1	142.1 ± 11.9##	147.9 ± 7.8##
Week 4	133.0 ± 8.8	127.5 ± 6.0	140.7 ± 7.8##	145.3 ± 8.2##

The rats were fed seal oil enriched diet (SO) or standard diet (SFO) for four weeks before and during the four-week intravenous administration of normal saline (NS) or ciclosporin. Data shown are mean ± s.d. (n = 6–8). $^{*}P < 0.05$, $^{**}P < 0.01$, SO vs SFO; $\#P < 0.05$, $\#\#P < 0.01$, NS injection vs ciclosporin injection.

compared with their corresponding controls (149.1 ± 41.0 and 149.6 ± 36.1 nmol (mg protein)$^{-1}$, respectively) (Figure 2). However, the difference between rats on the SO diet and those on the SFO diet following the administration of ciclosporin was not statistically significant.

Urinary 6-keto-$PGF_{1\alpha}$/TXB_2

The ratios of urinary 6-keto-$PGF_{1\alpha}$/TXB_2 in rats following the administration of ciclosporin for four weeks were significantly ($P < 0.01$) lower (0.53 ± 0.09 and 0.43 ± 0.08 for rats kept on the SO diet and SFO diet, respectively) than those administered with NS (1.23 ± 0.33 and 1.01 ± 0.23, respectively) (Figure 3). A significantly ($P < 0.05$) higher ratio was observed in rats fed with the SO diet than those fed with the SFO diet, following the administration of NS or ciclosporin.

Fatty acid composition in diets and erythrocyte membrane

The fatty acid compositions in the respective diets and erythrocyte membrane were determined. It was found that the SO diet and the SFO diet did not cause the percentage composition of myristic (14:0), palmitic (16:0) and stearic (18:0) acids to differ. However, contents of all other fatty acids were significantly different ($P < 0.0001$). The SFO diet was obviously high in linoleic (18:2 n6) and oleic (18:1 n9) acids with 31.31% of total content of n-6 fatty acids, whereas the total content of n-6 fatty acids in SO diet was only 7.67%. In addition, the SO diet had a considerable amount (17.34%) of n-3 fatty acids, including 20:5 n3, 22:5 n3 and 22:6 n3, whereas there was no detectable level of n-3 fatty acids found in the SFO diet.

The fatty acid composition in the erythrocyte membrane of rats is presented in Table 3. Rats maintained on the SFO diet had significantly higher levels of 20:4 n6 and 22:4 n6 fatty acids in the erythrocyte membrane than those kept on the SO diet. Rats kept on the SO diet had significantly higher levels of palmitoleic (16:1 n7), vaccenic (18:1 n7) and 11-eicosenoic acid (20:1 n9), and the total amount of n-3 fatty acids was approximately six times higher than those kept on the SFO diet. The composition of the fatty acids in the erythrocyte membrane was a reflection of the fatty acids found in the respective diets.

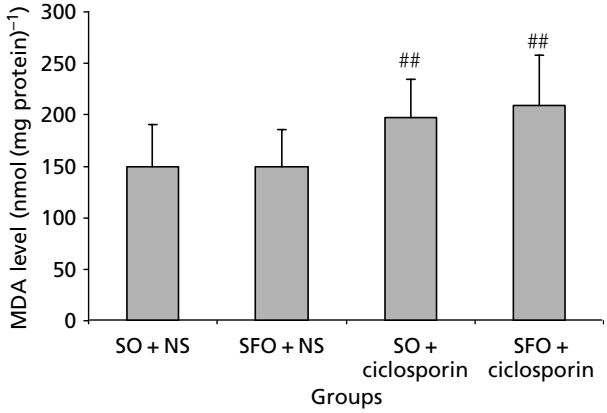

Figure 2 MDA content in kidney tissue in rats kept on seal oil enriched (SO) diet or standard (SFO) diet and administered NS or ciclosporin (CsA; Sandimmune IV, 15 mg kg^{-1} daily) intravenously for four weeks. Rats were fed SO or SFO diet for four weeks before and during the four-week intravenous administration of normal saline (NS) or ciclosporin. Data shown are mean ± s.d. (n = 6–8). $^{*}P < 0.05$, $^{**}P < 0.01$, SO vs SFO; $\#P < 0.05$, $\#\#P < 0.01$, NS injection vs ciclosporin injection.

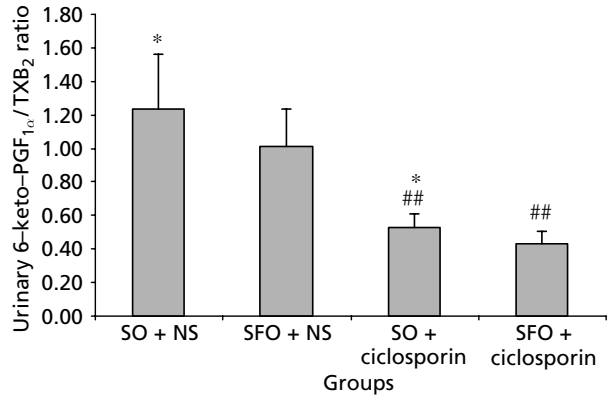

Figure 3 Urinary 6-keto-$PGF_{1\alpha}$/TXB_2 values in rats kept on seal oil enriched (SO) diet or standard (SFO) diet and administered normal saline (NS) or ciclosporin (CsA; Sandimmune IV, 15 mg kg^{-1} daily) intravenously for four weeks. Rats were fed SO diet or SFO diet for four weeks before and during the four-week intravenous administration of NS or ciclosporin. Data shown are mean ± s.d. (n = 6–8). $^{*}P < 0.05$, $^{**}P < 0.01$, SO vs SFO; $\#P < 0.05$, $\#\#P < 0.01$, NS injection vs ciclosporin injection.

Table 3 Percent phospholipid fatty acid composition of rat erythrocyte membrane

Phospholipid fatty acid	Erythrocyte membrane			
	SO + NS (n = 6)	SFO + NS (n = 6)	SO + ciclosporin (n = 7)	SFO + ciclosporin (n = 8)
14:0	0.82 ± 0.25	0.84 ± 0.57	0.74 ± 0.20	0.42 ± 0.06**
16:0	31.11 ± 8.55	23.95 ± 3.33	32.56 ± 2.16	31.13 ± 5.26
16:1 n7	1.38 ± 0.40	0.72 ± 0.40*	1.92 ± 0.46	0.49 ± 0.08**
18:0	11.90 ± 4.40	11.71 ± 3.96	11.01 ± 0.71	13.19 ± 5.60
18:1 n9	7.22 ± 1.77	6.89 ± 1.17	11.20 ± 1.07	10.68 ± 1.58
18:1 n7	3.37 ± 0.73	2.82 ± 0.36	4.37 ± 0.21	2.47 ± 0.41**
18:2 n6	3.99 ± 1.28	5.42 ± 0.95	5.20 ± 0.31	6.29 ± 1.21
20:1 n9	0.56 ± 0.11	0.29 ± 0.21	0.69 ± 0.10	0.32 ± 0.04*
20:4 n6	6.60 ± 3.45	18.62 ± 1.77*	6.56 ± 1.46	14.84 ± 5.15*
20:5 n3	6.90 ± 4.65	0.71 ± 0.59**	5.07 ± 1.20	0.32 ± 0.06**
22:4 n6	ND	1.59 ± 0.31**	0.17 ± 0.01	1.58 ± 0.53**
22:5 n3	2.05 ± 0.98	0.40 ± 0.11**	2.24 ± 0.58	0.38 ± 0.20**
22:6 n3	3.02 ± 1.38	1.31 ± 0.23*	3.48 ± 1.10	0.97 ± 0.18**
Σ saturates	43.83	36.50	44.31	46.63
Σ mono	12.53	10.72	18.18	11.49
Σ n6	10.59	25.63	11.93	22.71
Σ n3	12.28	2.42	10.79	1.67

The rats were fed seal oil enriched (SO) diet or standard (SFO) diet for four weeks before and during the four-week intravenous administration of normal saline (NS) or ciclosporin. Data shown are mean ± s.d. $^{*}P < 0.05$, $^{**}P < 0.01$, SO vs SFO; $\#P < 0.05$, $\#\#P < 0.01$, NS injection vs ciclosporin injection. ND, not detectable.

Blood ciclosporin concentration

The ciclosporin concentration in the blood samples taken 24 h after the four-week intravenous administration of ciclosporin was found to be 4417 ± 1003 and $4231 \pm 664\ \mu g\,L^{-1}$ in rats maintained on the SO diet and SFO diet, respectively. There was no statistical difference between the two groups.

Discussion

It is known that ciclosporin alters renal function and causes nephrotoxicity. This study clearly showed that intravenous administration of ciclosporin at a dose of $15\ mg\,kg^{-1}$ daily for four weeks resulted in nephrotoxicity in rats, characterized by elevated BUN and urinary NAG, and decreased Clcr. It was also shown that rats maintained on the SO diet exhibited a reduced level of renal functional impairment, less severe morphological changes in the kidney and less increase of SBP following the administration of ciclosporin in comparison with those maintained on a regular diet.

The decreased renal toxicity and change in SBP in rats maintained on the SO diet following the administration of ciclosporin ($15\ mg\,kg^{-1}$ daily) did not appear to be associated with the concentration of ciclosporin in blood, as the concentration of ciclosporin in blood was comparable between SO and SFO diet groups. As analysed by GC, the SO diet contains about 17% n-3 and 7.7% n-6 PUFAs, while the SFO diet contains 31% n-6 with no detectable level of n-3 PUFAs. The difference in dietary fatty acid composition was reflected in the fatty acid composition of phospholipids found in the erythrocyte membrane of rats maintained on the respective diets. The n-3 PUFA content in erythrocyte membrane was six times higher in rats fed on the SO diet than those on the SFO diet, while n-6 PUFA was two times lower. It was reported that the phospholipid fatty acid composition of erythrocyte membrane could be used to predict the fatty acid compositions in other membranes, including kidney (Gibson et al 1984). Therefore, it is expected that the n-3 PUFA levels in other tissues would be higher in rats on the SO diet than those on the SFO diet. The differences in response to ciclosporin administration between rats maintained on the SO and SFO diets is likely ascribed to the difference in their n-3 PUFA levels in cell membranes.

Among the postulated mediators involved in the ciclosporin-induced vascular pathology, accumulating evidence has revealed the involvement of eicosanoids, including PGs and TXs. The production of PGs and TXs may be influenced by dietary fatty acid intake. Ciclosporin administration was reported to have to led to an increased production of vasoconstrictor TXA_2 by platelets and suppressed production of favourable vasodilator PGs (Zoja et al 1990). Supplementation of seal oil, rich in n-3 PUFAs (EPA, DHA, DPA), resulted in increased levels of n-3 PUFAs incorporated into the phospholipid of membranes. Accordingly, it could lead to the reduced production of 2-series and increased production of 3-series of PGs and TXs. Members of the 3-series of PGs (PGI_3,

PGE_3) generated from n-3 PUFAs are equally effective as vasodilatory and anti-aggregatory agents when compared with their 2-series of analogues (PGI_2, PGE_2) generated from n-6 PUFAs. However, TXA_3 is inactive and does not share the vasoconstrictive property of TXA_2 (Needleman et al 1979). Therefore the shift to 3-series PGs would maintain vasodilatation and antithrombotic effects whereas the shift to TXA_3 would reduce vasoconstriction. Furthermore, it was found that EPA in marine oils could also increase PGI_2 and PGE_2, but not TXA_2, production (Abeywardena et al 1989). Thus, supplementation of marine oils rich in n-3 PUFAs would lead the eicosanoids production favouring a net vasodilatory effect. Evidence of this shift is provided by the significantly higher ratio of urinary 6-keto-$PGF_{1\alpha}$/TXB_2 (stable metabolites of PGI_2 and TXA_2, respectively) in the SO-diet-fed rats, compared with the SFO-diet-fed rats.

Besides the vasoconstrictive effect, a number of other mechanisms and mediators have been proposed to account for the nephrotoxicity of ciclosporin. It was observed that ciclosporin induced renal lipid peroxidation under both in-vitro and in-vivo conditions (Inselmann et al 1990). Lipid peroxidation caused by ciclosporin could be reduced by lazaroid, an antioxidant (Wang & Salahudeen 1994), suggesting a possible role of free radical oxygen species (ROS) in ciclosporin-associated toxicity. Our observation of significantly increased MDA levels in kidney following the administration of ciclosporin agrees with the reports. We also found that following the administration of ciclosporin for four weeks, rats maintained on the SO diet had a lower level of MDA in the kidney when compared with rats maintained on the SFO diet. This may be due to the potential antioxidative properties of the SO diet (Nakhla 1997).

Conclusion

This study suggested that dietary supplementation of seal oil reduced ciclosporin-induced nephrotoxicity in-vivo. The beneficial effects of a seal oil supplemented diet may be due to the modulation of prostanoids biosynthesis leading to vasodilatation to offset the renal vasoconstriction induced by ciclosporin.

While our results showed some promise, further studies are required to confirm the full potential of sea oil intake in reducing ciclosporin-induced nephrotoxicity.

References

Abeywardena, M. Y., Fischer, S., Schweer, H., Charnock, J. S. (1989) In vivo formation of metabolites of prostaglandins I2 and I3 in the marmoset monkey (Callithrix jacchus) following dietary supplementation with tuna fish oil. *Biochim. Biophys. Acta* **1003**: 161–166

Antonovych, T. T., Sabnis, S. G., Austin, H. A., Palestine, A. G., Balow, J. E., Nussenblatt, R. B., Helfrich, G. B., Foegh, M. L., Alijani, M. R. (1988) Cyclosporine A-induced arteriolopathy. *Transplant Proc.* **20** (Suppl. 3): 951–958

Bennett, W. M., Carpenter, C. B., Shapiro, M. E., Strom, T. B., Hefty, D., Tillman, M., Abrams, J., Ryan, D., Kelley, V. R. (1995) Delayed omega-3 fatty acid supplements in renal transplantation. A double-blind, placebo-controlled study. *Transplantation* **59**: 352–356

Casas, A., Hotter, G., Rosello-Catafau, J., Fernandez-Cruz, L., Gelpi, E. (1995) Prostanoids and cyclosporine-mediated nephrotoxicity in rats: a critical appraisal. *Prostaglandins Leukot. Essent. Fatty Acids* **52**: 49–53

Christensen, M. S., Hoy, C. E. (1996) Effects of dietary triacylglycerol structure on triacylglycerols of resultant chylomicrons from fish oil- and seal oil-fed rats. *Lipids* **31**: 341–344

Darlametsos, I. E., Papanikolaou, E. N., Varonos, D. D. (2000) Effect of nifedipine in Cyclosporine-induced nephrotoxicity in rats: roles of the thromboxane systems. *Pros. Leuk. EFAS* **63**: 263–269

Dreyeberg, J., Bang, H. O., Hjorne, N. (1975) Fatty acids composition of the plasma lipids in Greenland Eskimos. *Am. J. Clin. Nutr.* **28**: 958–966

Folch, J., Lees, M., Sloane Stanley, G. H. (1957) Simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* **226**: 497–509

Gibson, R. A., McMurchie, E. J., Charnock, J. S., Kneebone, G.M. (1984) Homeostatic control of membrane fatty acid composition in the rat after dietary lipid treatment. *Lipids* **19**: 942–951

Hansen, J. M., Lokkegaard, H., Hoy, C. E., Fogh-Andersen, N., Olsen, N. V., Strandgaard, S. (1995) No effect of dietary fish oil on renal hemodynamics, tubular function, and renal functional reserve in long-term renal transplant recipients. *J. Am. Soc. Nephrol.* **5**: 1434–1440

Inselmann, G., Hannemann, J., Baumann, K. (1990) Cyclosporine A induced lipid peroxidation and influence on glucose-6-phosphatase in rat hepatic and renal microsomes. *Res. Commun. Chem. Pathol. Pharmacol.* **68**: 189–203

Kanayasu-Toyoda, T., Morita, I., Murota, S. (1996) Docosapentaenoic acid (22:5, n-3), an elongation metabolite of eicosapentaenoic acid (20:5, n-3), is a potent stimulator of endothelial cell migration on pretreatment in vitro. *Prostaglandins Leukot. Essent. Fatty Acids* **54**: 319–325

Kooijmans-Coutinho, M. F., Rischen-Vos, J., Hermans, J., Arndt, J. W., van der Woude, F. J. (1996) Dietary fish oil in renal transplant recipients treated with cyclosporin-A: no beneficial effects shown. *J. Am. Soc. Nephrol.* **7**: 513–518

Meyer-Lehnert, H., Schrier, R. W. (1988) Cyclosporine A enhances vasopressin-induced Ca^{2+} mobilization and contraction in mesangial cells. *Kidney Int.* **34**: 89–97

Murphy, M. G., Wright, V., Ackman, R. G., Horackova, M. (1997) Diets enriched in menhaden fish oil, seal oil, or shark liver oil have distinct effects on the lipid and fatty-acid composition of guinea pig heart. *Mol. Cell. Biochem.* **177**: 257–269

Nakhla A. (1997) *The potential of seal oil as w-3 polyunsaturated fatty acid supplements*. Ph.D. thesis, Memorial University of Newfoundland. September 1997

Needleman, P., Raz, A., Minkes, M. S., Ferrendelli, J. A., Sprecher, H. (1979) Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. *Proc. Natl Acad. Sci. USA* **76**: 944–948

Paavonen, T., Jarvelainen, H., Kontiainen, S., Hayry, P. (1981) Effects of cyclosporine A on in vitro proliferative activity and synthesis of isolated human lymphoid cell subpopulations. *Clin. Exp. Immunol.* **43**: 342–350

Perico, N., Benigni, A., Bosco, E., Rossini, M., Orisio, S., Ghilardi, F., Piccinelli, A., Remuzzi, G. (1986) Acute cyclos-

porine A nephrotoxicity in rats: which role for renin-angiotensin system and glomerular prostaglandins? *Clin. Nephrol.* **25** (Suppl. 1): S83–S88

Puppione, D. L., Kuehlthau, C. M., Jandacek, R. J., Costa, D. P. (1992) Positional analyses of triacylglycerol fatty acids in the milk fat of the antarctic fur seal (Arctocephalus gazella). *Lipids* **27**: 637–639

Reeves, P. G., Nielsen, F. H., Fahey, G. C. (1993) AIN-93 diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **123**: 1939–1951

Scherrer, U., Vissing, S. F., Morgan, B. J., Rollins, J. A., Tindall, R. S., Ring, S., Hanson, P., Mohanty, P. K., Victor, R. G. (1990) Cyclosporine-induced sympathetic activation and hypertension after heart transplantation. *N. Engl. J. Med.* **323**: 693–699

Wang, C., Salahudeen, A. K. (1994) Cyclosporine nephrotoxicity: attenuation by an antioxidant-inhibitor of lipid peroxidation in vitro and in vivo. *Transplantation* **58**: 940–946

Zoja, C., Benigni, A., Renzi, D., Piccinelli, A., Perico, N., Remuzzi, G. (1990) Endothelin and eicosanoid synthesis in cultured mesangial cells. *Kidney Int.* **37**: 927–933