ELSEVIER

Journal of Nutritional Biochemistry 14 (2003) 480–486

THE JOURNAL OF Nutritional Biochemistry

Beneficial effects of a diet rich in a mixture of n - 6/n - 3 essential fatty acids and of their metabolites on cyclosporine - nephrotoxicity

George Tsipas[a,b,*], Polymnia Morphake[b]

[a]*Laboratory of Zoology, Biochemistry and Molecular Biology. Department of Environmental and Natural Resources. Management School of Natural Resources and Enterprises Management. University of Ioannina, Agrinio 301.00, Greece*
[b]*France-Hellenic Biomedical Research Institute, General Hospital of Agrinio, Agrinio, 301.00, Greece*

Received 30 August 2002; received in revised form 9 May 2003; accepted 10 June 2003

Abstract

In this study we investigated the role of a mixture of n–6/n–3 essential fatty acids, in the cyclosporine model nephrotoxicity.

Administration of cyclosporine in rats decreased creatinine clearance and provoked body weight loss, but it did not induce proteinuria and did not alter the urine volume. These changes were associated with decreased urinary ratios of prostaglandin E/thromboxane B and prostaglandin I/thromboxane B excretions. Light microscopic sections showed that 100% of the animals were affected by histological tubular lesions on their kidneys.

Administration of cyclosporine to animals fed for 3 months on standard chow containing a mixture of n - 6/n - 3 essential fatty acids, restored creatinine clearance, augmented urine volume and prevented body weight loss. The improvement of renal function was accompanied by increased urinary ratios of prostaglandin E/thromboxane B and prostaglandin I/thromboxane B excretions. Light microscopic sections showed that only 40% of the animals demonstrated histological tubular lesions, of minor importance, to their kidneys.

Our results suggest that the metabolites of arachidonic acid can play important role in the development of cyclosporine-nephrotoxicity because they increase the levels of thromboxane A and that the enchanced synthesis of prostaglandins (E) and (I) induced by a mixture of n - 6/n - 3 essential fatty acids, could play a beneficial role in the prevention of this renal dysfunction.

Keywords: n - 6; n - 3 essential fatty acids; Prostaglandins; Thromboxanes; Cyclosporine; Nephrotoxicity

1. Introduction

Cyclosporine (CsA) is an immunosuppressive drug which is successfully used to prevent rejection in organ transplantation and to treat some autoimmune diseases. However, CsA induces renal side effects, as shown by a decrease in glomerular filtration rate and ultrafiltration coefficient regulated by the tone of messagial cells [1,2]. CsA can also affect many other organ systems, such as the liver, the angiovascular system, the central nervous system, bone, muscle and skin [2,3]. A hemolytic uramic syndrome has also been observed [4]. The CsA nephrotoxicity is dose and time dependent with morphologic targets and changes reversible or irreversible [5-7]. Morphological changes to the kidney occur in addition to functional toxicity. Tubular changes consist of vacuolization, megamitochondria and micro-calcification, which are reversible [5]. Arteriolar changes may progress into focal interstitial fibrosis and nephron loss [5,8]. CsA causes intense vasoconstriction in the renal vascular bed. The factors that have been incriminated include increased activity in the renin angiotensin system [9], increased responsiveness to neurohumoral stimuli [10], increased production of thromboxane (TXB_2) [11,12], serotonin (5–HT) [13] and endothelins (ETs) [14,15] and decreased production of prostacyclin (PGI_2) and prostaglandin E_2 (PGE_2) [12,14,15].

In previous studies it has been observed that agents such as glycerol, mercuric chloride, gentamicin, noradrelalin and others, induced acute renal failure associated with highly increased urinary TXB_2 and slightly enhanced urinary PGE_2 and 6-keto-PGF_{1a} excretions [16-20] and that : (A) the use of TXA–synthetase inhibitors, imidazole or OKY–046 or CSS12970 [17-21] or (B) infusion of PGE_1, PGE_2 and PGI_2

* Corresponding author. Tel.: +326410-39503; fax: +326410-33716.
E-mail address: gtsipas@cc.uoi.gr (G. Tsipas).

0955-2863/03/$ – see front matter
doi:10.1016/S0955-2863(03)00102-5

Table 1
Summary of animal treatments

Group	Olive oil 1.8 ml/Kg	CsA 37.4μM(1.8 ml)/Kg	n-6/n-3
1 NR	+	−	−
2 CsA	−	+	−
3 CsA + n-6/n-3	−	+	+

NR: normal rats. CsA: CsA treated rats. CsA + n-6/n-3: animals were fed on standard chow containing a mixture of n-6/ n-3 essential fatty acids in form (10 ml EPO and 100 ml FO) / Kg for 3 months prior to and during the experiment. CsA: dissolved in olive oil. +: one per day, for seven (7) days.

[22-24] and (C) the use of evening primrose oil or fish oil [25], partially prevented the development of this syndrome. Since, it has been observed that CsA–induced nephrotoxicity is also associated with enhanced urinary TXB_2 [11,12,15,26], and even with diminished urinary PGE_2 and 6-keto-PGF_{1a} excretions [12,14,15,27].

In this study we investigated whether the use of substances augmenting prostaglandins (PGs) and diminishing thromboxane (TXA) production could also prevent the nephrotoxicity induced by this agent. Our results suggest that a mixture of n - 6/n - 3 essential fatty acids–precursor substances of PGs and TXs of series 1 and 3 respectively-could improve renal dysfunction induced by CsA.

2. Materials and methods

The study was performed on 30 female Wistar rats weighing 180 ± 10 g (± SEM). They were randomly allocated into three groups, each containing ten animals. The temperature of the room in which the animals were housed, was maintained between 22 to 25°C and the humidity at 35 to 40%. For convenience, lighting was controlled allowing 12 h of light. Tap water, standard rat chow or standard chow containing a mixture of n - 6/n - 3 essential fatty acids (EFAs) were available ad libidum until the last day of the experiment.

2.1. Animals and their treatment

The first and second group of animals were fed on standard rat chow (SC). The animals of the third group for 3 months prior to the experiment and throughout the experiment itself were fed on SC containing (10 mL EPO and 100 mL FO)/Kg. EPO (Evening Primrose Oil) contained 72% cis-linoleic acid (cLA) and 9% gamma-linolenic acid (GLA). FO (Fish Oil) contained 5.6% eicosapentaenoic acid (EPA), 2% a–linolenic acid (ALA) and 1.9% linoleic acid (LA). Table 1 summarizes the treatment of the animals of each group.

The animals of the 2nd and 3rd group were injected intraperitoneally with CsA, 37.4 μM (1.8 ml)/Kg per day, for 7 days. The first group received 1.8 ml/Kg olive oil, the solvent of CsA. On the seventh day of the experiment 24h urine was collected using individual metabolic cages. At the end of the experiment the animals were anesthetized with pentothal (sodium thiopental), 3 ml of blood was withdrawn through a femoral artery, and the kidneys were taken for light microscopic sections. Cyclosporine, sandimmun sandoz, concentrated (50 mg/ml) injectable was provided from Sandoz, Basel, Switzerland. Evening primrose oil and Fish oil were kindly supplied by Scotia Pharmaceuticals, Guildord, UK.

2.2. Parameters measured

Urine and plasma creatinine concentration were determined by a method using Fuller's earth in order to eliminate chromogenes [17,18]. Creatinine clearance (Ccr) was calculated by the formula Ccr = (V x Ucr)/Pcr. (V = urine volume ml/Kg/min, Ucr and Pcr = urinary and plasma creatinine concentration). Since we have observed a close correlation between Ccr, inulin clearance, cyanocobalamin clearance and I^{125}–sodium iothalamate clearance, Ccr was utilized to determine the glomerular filtration rate (GFR) [18].

Urinary protein concentration was determined by the method of Goodwin [23]. Proteinuria (PU) was calculated by the formula PU (mg/24h) = UP (mg/ml) x V (ml/24h). (UP = urinary protein concentration, V = 24h urine volume).

Urinary TXB_2, 6-keto-PGF_{1a} (the stable metabolites of TXA_2 and PGI_2, respectively) and PGE_2 were determined by a radioimmunoassay (RIA) method in our laboratory [17, 18]. Tritium–labeled TXB_2 (105 Ci/mmol), 6-keto-PGF_{1a} (157 Ci/mmol) and PGE_2 (169.5 Ci/mmol) were obtained by New England Nuclear, Boston, MA, USA. Standards PGs, by Cayman Chemical, Denver, CO, USA, and monoclonal antibodies against PGE_2, from Institute Pasteur, Paris, France. Polyclonal antibodies against 6-keto-PGF_{1a} and TXB_2 were kindly supplied by Dr A. Hornych, Hopital Broussais, Paris, France. The results obtained by this method were compared against those observed, using a scintillation proximity assay (SPA), an enzyme immunoassay (EIA) method (R = 0.820 and R = 0.959, respectively) [15] and by those observed using two different antibodies to determine PGs and TXB_2 [18]. Since our method using either monoclonal or polyclonal antibodies does not separate the 2 from the 1 and 3 series of PGs and TXs, because of cross reactivity, the results obtained were expressed as TXB_2, PGE_2 and 6-keto(K)-PGF_{1a}.

2.3. Light microscopic (LM) studies

Histological specimens of kidneys were fixed in 10% buffered neutral formalin and embedded in paraffin. The characteristic lesions induced by CsA in the epithelial cells of the proximal tubules were observed in LM sections from

Table 2
Effects of CsA on urine volume (VU), clearance creatinine (Ccr), body weight loss (BWL) and proteinuria (PU), in normal and in n-6/n-3 essential fatty acids treated rats

Group	VU ml/Kg/24h	Ccr ml/Kg/min	BWL %	PU mg/24 h
1 NR	19.9 ± 2.2	2.5 ± 0.1	(+) 0.6 ± 0.4	7.1 ± 2.3
$\mathbf{P}_{(1vs2)}<$	NS	0.0005	0.0005	NS
2 CsA	26.6 ± 3.9	1.3 ± 0.1	(−) 12.2 ± 1.8	11.4 ± 3.8
$\mathbf{P}_{(2vs3)}<$	0.05	0.0005	0.05	NS
3 CsA + n-6/n-3	35.3 ± 2.8	2.9 ± 0.1	(−) 6.6 ± 2.1	14.2 ± 2.9
$\mathbf{P}_{(1vs3)}<$	0.0025	0.01	0.005	0.05

Values are mean ± (s.e.m.), n = 10. Groups 1 and 3 were compared with group 2. Group 1 was compared with group 3. NS = not significant.

the three groups of animals. The development of vacuolization or brush border loss were assessed on a +/2+/3+ scale using the following key. A + mark, a 2+ mark and a 3+ mark was given when 1 to 33%, 33 to 66% and 66 to 100% of the tubules developed vacuolization or rush border loss respectively. Finally, a (–) mark was given when no affected tubule was visible. A lesion induced by CsA was characterized as focal when 3 cells from one tubule section developed vacuolization and diffuse when vacuolization was observed in more than 3 cells. For the assessment of tubular casts we followed the same scale as described above, but we used the following key. A +, 2+, 3+ and a 4+ mark was given when 1 to 25%, 25 to 50%, 50 to 75% and 75 to 100% of the tubules developed tubular casts respectively. The single cell necrosis, interstitial edema and dilatation, were estimated with a plus (+) when observed and with a minus (-) when no necrosis was visible.

2.4. Statistical analysis

Statistical analysis was performed using Student's *t*-test and $P < 0.05$ was considered to be significant.

3. Results

3.1. Effect of CsA on renal function in normal rats

The administration of CsA (for 7 days) to the animals decreased Ccr and provoked body weight loss (BWL) while, in the dose given, it did not alter urine volume (VU) and did not induce proteinuria (PU) (Table 2). These functional changes were associated with significantly increased urinary TXB_2 and significantly decreased PGE_2 and 6-keto-PGF_{1a} excretions. These alterations highly diminished the ratios of PGE_2/TXB_2 and 6-keto-PGF_{1a}/TXB_2 (Table 3). LM sections showed that all kidneys were affected but the lesions, such as mainly tubular diffuse vacuolization (VCL) (10 rats), brush border loss (BBL) (6 rats), single cell necrosis (SCN) (4 rats), tubular casts (TC) (2 rats) and interstitial edema (ITODE) (2 rats), were reversible (Table 4).

3.2. Effect of CsA on renal function in n - 6/n-3 essential fatty acids treated rats

The administration of CsA (for 7 days) to the animals fed for 3 months on SC containing (10 ml EPO and 100 ml FO)/Kg almost completely protected the animals against the nephrotoxicity induced by CsA. Thus the mixture of n - 6 / n - 3 EFAs completely restored Ccr, significantly prevented BWL and increased VU (Table 2). These functional changes were associated with significant increases in urinary PGE_2 and 6-keto-PGF_{1a} and a significant fall in TXB_2 excretions. These alterations highly increased the ratios of PGE_2/TXB_2 and 6-keto-PGF_{1a}/TXB_2 (Table 3). LM sections showed that only 4 of 10 animals were affected, but the lesions were of less importance such as VCL (4 rats) (Table 4).

Table 3
Effects of CsA on urinary TXB_2, PGE_2, and 6-K- PGF_{1a} excretions, on the ratios of PGE_2/TXB_2 and 6-K-PGF_{1a}/TXB_2 in normal and in n-6/n-3 essential fatty acids treated rats

Group	TXB_2	PGE_2	6-K-PGF_{1a}	PGE_2/TXB_2	6-K-PGF_{1a}/TXB_2
	(pmol / Kg / 24 h)				
1 NR	296 ± 14	1068 ± 66	403 ± 27	3.6 ± 0.3	1.4 ± 0.1
$\mathbf{P}_{(1vs2)}<$	0.0005	0.0005	0.025	0.0005	0.0005
2 CsA	776 ± 82	487 ± 68	293 ± 34	0.7 ± 0.1	0.4 ± 0.1
$\mathbf{P}_{(2vs3)}<$	0.0005	0.0025	0.0005	0.0025	0.005
3 CsA + n-6/n-3	278 ± 32	1263 ± 163	710 ± 59	5.5 ± 1.3	3.3 ± 0.9
$\mathbf{P}_{(1vs3)}<$	NS	NS	0.0005	NS	0.05

Values are means ± (s.e.m.), n = 10. Groups 1 and 3 were compared with group 2. Group 1 was compared with group 3. NS = not significant.

Table 4
Parameters appreciated in light microscopic renal sections in CsA treated rats before and after the animals have been fed on n-6/n-3 essential fatty acids

Group		Vacuolization		B.B.L.	S.C.N.	T.C.	IT.OED.	DTL.
	A.R.	Diffuse	Focal					
NR	1	±	−	−	−	−	−	−
	2	+	−	−	−	−	−	−
CsA	1	+	−	+	−	−	−	−
	2	2+	−	2+	2+	+	−	−
	3	3+	−	−	+	−	−	−
	4	3+	−	+	−	+	−	−
	5	+	−	+	−	−	−	−
	6	2+	−	−	−	−	+	−
	7	2+	−	+	−	−	−	−
	8	−	2+	−	+	−	−	−
	9	+	−	−	+	−	−	−
	10	3+	−	+	−	−	+	−
CsA + n-6/n-3	1	+	−	−	−	−	−	−
	2	2+	−	−	−	−	−	−
	3	−	2+	−	−	−	−	−
	4	−	+	−	−	−	−	−

A.R.: affected rats, Diffuse – Focal vacuolization, B.B.L.: brush border loss, S.C.N.: single cell necrosis, T.C.: tubular casts IT.OED.: interstitial oedema, DTL: dilation −: no change, ±: no remarkable, +: moderate, 2+: severe, 3+: extra severe.

4. Discussion

In previous studies it has been observed that renal failure induced by glycerol, mercury chloride, gentamycin and other substances, could be partially prevented when the animals were : (A) injected with agents inhibiting TXA synthesis (imidasole, OKY–046, CSS12970) [17-21] (B) infused with PGs (PGE_1, PGE_2, PGI_2) [22-24] and (C) fed on substances decreasing TXA_2 and enhancing PG–production [19]. However, these agents only partially prevented the development of acute renal failure, a fact indicating that other vasoconstrictive substances can also play a role in the development of this syndrome. The catecholamines, the renin - angiotensin system and vasopressin, as far as we know, do not play a major role [18,20]. The metabolites of arachidonic acid (AA) are involved in the nephrotoxicity induced by CsA, as in the renal dysfunction induced by glycerol [17,18], mercuric chloride [19], gentamicin [20] and others. Since CsA increased TXA_2, involved in the development of acute renal failure [16-20] and contrary to glycerol, mercury chloride, gentamicin, it even diminished PGE_2 and PGI_2, agents that have been observed to prevent acute renal failure [22,23], thus the nephrotoxicity induced by CsA should have been more serious. However, although CsA diminished PG production the renal failure observed was less important compared to that induced by glycerol, mercuric chloride and gentamicin which was associated with increased TXA_2 as well as PG production [17-20]. Furthermore, CsA has also been observed to stimulate the release of other potent vasoconstrictor factors, such as the 5–HT [13], the endothelins [14,15]. Also a partial protection against the nephrotoxicity induced by CsA was obtained using ketanserine (KTS), an antagonist of S_2 -serotoninergic and a_2 -adrenergic receptors [13] or nifidepine (NFD), an inhibitor of endothelins (ETs) [14,15]. In order to elucidate why nephrotoxicity induced by CsA is of less importance compared to that provoked by glycerol, mercuric chloride, gentamicin and others, it is necessary to investigate whether the above mentioned agents also induce the release (A) of endothelins and 5–HT, (B) of other potent vasoconstrictor factor(s) still unknown, (C) whether additional mechanisms are involved in the development of this syndrome or (D) whether the release of other potent vasodilator factor(s) still unknown is induced by CsA.

Our results suggest that animals fed for 3 months on SC containing n - 6 and n - 3 EFAs such as evening primrose oil and fish oil significantly improved renal function in the cyclosporine model nephrotoxicity. The mechanism by which the mixture of n - 6 / n - 3 EFAs prevents the CsA–nephrotoxicity is probably the following:

The n - 3 EFAs successfully inhibit the metabolisms of n - 6 EFAs at the desaturation steps, because the ability of these to compete with the n - 6 EFAs is considerably greater than the reverse [28-30]. The elongation steps appear to be so rapid that no effective competition takes place. This competition has important practical consequences (Fig. 1). When large doses of n - 3 EFAs are administered such as fish oils, there is a drop in the concentration of AA in various lipid fractions. This occurs because of a direct displacement of AA from these fractions and partly because of reduced formation of AA from LA [29-32]. Furthermore, if high doses of fish oil are administered in the presence of GLA there is no drop in DGLA such as the one that occurs with fish oil supplementation alone, because n - 3 EFAs inhibit conversion of LA to GLA and of DGLA to AA but have little effect on the conversion of GLA to DGLA [29,32-36]. Instead, DGLA rises in parallel with EPA and docosahexaenoic acid (DHA). This has the important effect

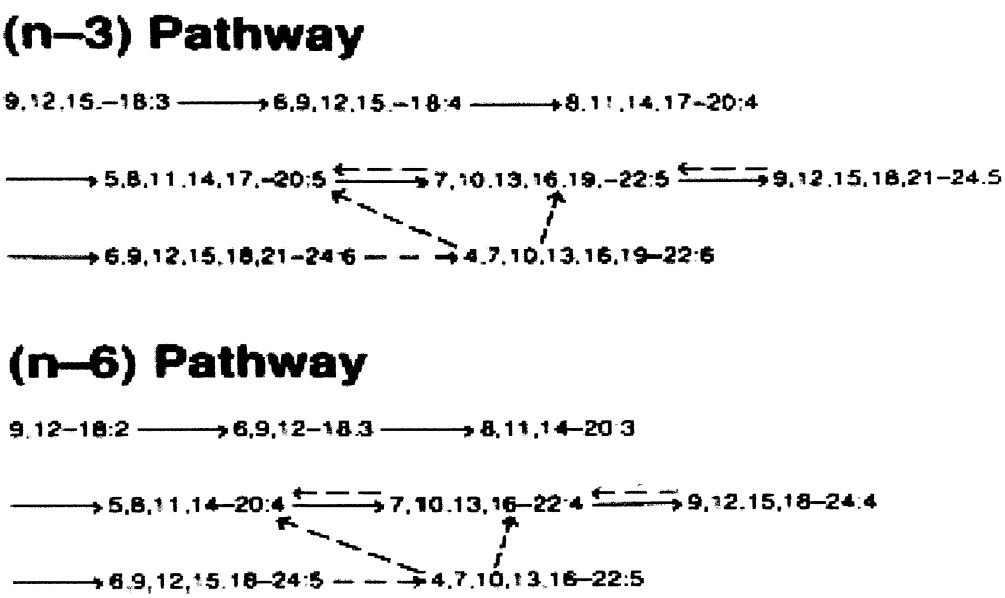

Fig. 1. Revised pathways for the biosynthesis of n - 3 and n - 6 PUFA. The solid arrows denote reactions localized in the endoplasmatic reticulum while the dashed arrows show partial degradative reactions taking place in peroxisomes (H. Sprecher 2000). The 8,11,14-20:3 (DGLA), 5,8,11,14-20:4 (AA) and 5,8,11,14,17- 20:5 (EPA) are precursor substances of PGs and TXs of series 1,2 and 3 respectively.

of raising the levels of metabolites of (A) DGLA, notably PGE_1 and 15–OH-DGLA and (B) EPA precursor of the series of PGs and TXs (PGE_3, PGI_3, PGD_3 and TXA_3).

DGLA may exerts its effects by its metabolite PGE_1 (potent vasodilator, antiinfla-mmatory, anti-aggregatory, etc agent) [19], which raises levels of cyclic–AMP, thus inhibiting phospolipase and limiting the release of AA into the free form, a necessary step in its conversion to pro–inflammatory (TXA_2) [17-20] metabolites [29,37,38]. Its other metabolite 15–OH–DGLA, inhibits the conversion of free AA to leukotrienes and other metabolites of 5-and 12–lipoxygenases [29,37]. Furthermore, it has been observed that DGLA increased relative to AA and also enchanced the ex vivo capacity of platelets to produce PGE_1 and PGE_2, probably by inhibiting conversion of PGH_2 to TXA_2 [29,37, 38] and therefore redirecting the conversion of PGH_2 to its prostanoid metabolites PGE_2, PGI_2 and PGD_2 (vasodilator, anti-aggregatory and natriuretic substances). These metabolites, such as PGE_1 have been observed to protect against acute renal failure in different nephrotoxicity models and in the CsA-nephrotoxicity [19,22-24,39,40].

The nephroprotection effect is also reinforced by the metabolites of EPA, which have the same anti-aggregatory effect as the 2 series, while TXB_3 (TXA_3) is inactive [41-43]. One could postulate that EPA exerts its beneficial action against the development of CsA–nephrotoxicity by an action of PGE_3 and PGI_3 [35,44] similar to that of PGE_1, PGE_2 and PGI_2 [45-47]. In addition EPA and DHA (the delta–6–desaturase product of EPA), compete with AA for cyclooxygenase and lipo-oxygenase systems [29,35]. Furthermore, it has been observed that (A) diet of n - 3 EFAs alone, diminishes the synthesis of the AA-derived prostaglandins PGE_2, PGF_{2a} and PGI_2 [34,35,48-50], while the production of TXA_2 does not diminish [36] and that (B) diet of both n - 6 / n - 3 EFAs reduces the concentration of TXA_2 [36].

In another study, the administration of EPA in fish oil has been shown to reduce renal damage due to cyclosporine [51]. However while fish oil may reduce thromboxane production, it also inhibits the formation of PGE_1 and PGE_2 which would be expected to have adverse effects. On the other hand, the daily administration of fish oil has a favorable effect on renal hemodynamics and blood pressure in renal-transplant recipients treated with cyclosporine. However, fish oil supplementation did not have any beneficial effect on lipid profile, renal function, incidence of acute rejection episodes, or 1–year graft survival [52,53].

It is important to note that in our experiment we observed that the mixture of the n - 6 / n- 3 EFAs, as well as in previous studies with n - 6 EFAs alone and KTS prevented BWL [13,25] but OKY-046 or NFD or n - 3 EFAs singly administered [12,15,25] did not. Morever, only the mixture of n - 6 / n - 3 EFAs minimized the morphological and histological changes induced by CsA and this improvement could be attributed to the great augmentation of the ratios PGE (E_1, E_2, E_3)/TXB_2 and PGI (I_1, I_2, I_3)/TXB_2 than in the other cases of n - 6 EFAs or KTS administration [13,25]. Thus, the beneficial effect of the mixture of n - 6 / n - 3 EFAs elevate this considerably as a nutrient and a prescription pharmaceutical against other therapeutic treatments because the risk of adverse events and side effects is exceedingly low. We also wish to note that the level of the active TXB_2 obtained is much lower since the antibodies, even the monoclonal, used for the determination of TXBs and PGs by the radioimmunoassay (RIA) method, do not separate the 2 from the 1 and 3 series of PGs and TXs, because of cross

reactivity. Thus, the TXB observed in CsA + n - 6 / n - 3 treated animals consisted of a mixture of active TXB_2 and inactive TXB_1, TXB_3 [41-43], while the PGE and PGI consisted of a mixture of E_1, E_2, E_3 and I_1, I_2, I_3 respectively.

In conclusion, the alterations observed in CsA-nephrotoxicity by inhibition of TXB_2 production and stimulation of PGE and PGI, suggest that diet enriched with a mixture of n - 6 / n - 3 EFAs plays an important role in the development (TXA_2) and prevention (PGE and PGI) of this syndrome. However, other vasoactive factors such as serotonin [13], endothelins (ET-1, ET-2, ET-3, ET-4 and endothelin - b), nitric oxide [1,5,54], neuropeptide Y [55], vasoactive intestinal polypeptide [56], calcium, reactive oxygen intermediates [1,27] and perhaps others still unknown, as well as additional mechanisms concerning the antagonism between T–helper 1/T-helper 2 cells, must also be considered to be involved in the CsA–nephrotoxicity.

Acknowledgments

This paper is part of a continuing series of studies on the cyclosporine–nephrotoxicity. The paper is dedicated to the memory of the late N. Papanikolaou (1923-1996) who was a leader in the investigation of the nephrotoxicity mechanisms and participated in the initial steps of the program. We would like to thank Scotia Pharmaceuticals Ltd, England, for their donation of evening primrose oil and fish oil and Dr. A. Hornych for polyclonal antibodies against 6Keto–PGF_{1a} and TXB_2 that were kindly supplied by Hopital Broussais, Paris, France. We also thank Dr. Kostas Bourtzis for critical comments on a draft of the manuscript, Anestis Agapiadis for his help with the English editing and E. L. Gkika for technical assistance.

References

[1] Azou BL´, Azou J, Medina W, Frieauff W, Cordier A, Cambar J, Wolf A. In vitro models to study mechanisms involved in cyclosporine A–mediated glomerular contraction. Arch Tox 1999;73(8–9): 337–45.
[2] Myers BD. Cyclosporine nephrotoxicity. Kidney Intl 1986;30:964–7.
[3] Rush DN. Cyclosporine toxicity to organs other than the kidney. Clin Biochem 1991;14:101–5.
[4] McCauley J, Bronsther O, Fung J, Starzl TE. Treatment of cyclosporin - induced haemolytic uraemic syndrome with FK 506. Lancet 1989;2:1516.
[5] Mihatsch MJ, Thirl G, Ryffel B. Morphology of cyclosporin nephropathy. Prog Allergy 1986;38:447–50.
[6] Bennett WM, Elzinga L, Kelley V. Pathophysiology of cyclosporine nephrotoxicity. Role of eicosanoids. Transplant Proc 1988;XX(supp 3):628–33.
[7] Bennett WM. Mechanisms of acute and chronic nephrotoxicity from immunosuppressive drugs. Renal Failure 1996;18(3):453–60.
[8] Ryffel B, Foxwell MB, Gee A, Greiner B, Woerly G, Mihatsch JM. Cyclosporine–relationship of side effects to mode of action. Transplantation 1988;46:90S–96S.
[9] Lusting S, Stem N, Eggena P. Effect of cyclosporin on blood pressure and renin - angiotensin axis in rats. Am J Physiol 1987;253:H1596–H1600.
[10] Colub MS, Berger ME. Direct augmentation by cyclosporin A of the vascular contractile response to nerve stimulation. Hypertension 1987;9(supp III):96–100.
[11] Coffman TM, Carr DR, Yarger WE, Klotman PE. Evidence that renal prostaglandin and thromboxane production by chronic cyclosporine nephro-toxicity. Transpl 1987;43:282–5.
[12] Tsipas G., Morphake P., Darlametsos I., Bariety J., Gkika E.L., Hornych A., Manos G., Papanikolaou N.A selective TXA - synthetase inhibitor, OKY-046, partially protected rats against the nephrotoxicity induced by cyclosporine. Proceedings 3rd International Satelite Symposium on acute renal failure. University Studio Press, Halkidiki, Greece, 1993. pp 277–89.
[13] Darlametsos I, Morphake P, Bariety J, Hornych A, Tsipas G, Gkikas G, Papanikolaou N. Effect of Ketanserine in cyclosporine–induced renal dysfunction in rats. Nephron 1995;70:249–54.
[14] Copeland KR, Yatscoff RW. Comparison of the effects of cyclosporine and its metabolites on the release of prostacyclin and endothelin from mesangial cells. Transplantation 1992;53(3):640–5.
[15] Papanikolaou N, Darlametsos I, Tsipas G, Morphake P, Bokas S, Gkikas G, Hornych A, Bariety J, Gkika EL, Karageorgou I, Patsialos K. Effects of OKY–046 and nifedipine in CsA-induced renal dysfunction in rats. Prostagl Leuk and Essential Fatty Acids 1996;55: 249–56.
[16] Benabe JE, Klehr S, Hoffman MK, Morrison AR. Production of TXA_2 by the kidney in the glycerol–induced acute renal failure in rabbit. Prostaglandins 1980;19:333–7.
[17] Hatziantoniou C, Papanikolaou N. Renal effects of inhibitor of thromboxane A_2–synthetase OKY–046. Experientia 1986;42:613–5.
[18] Papanikolaou N, Chatziantoniou C, Dontas A, Gkika EL, Paris M, Gkikas G, Bariety J. Is thromboxane a potent antinatriuretic factor and is it involved in the development of acute renal failure? Nephron 1987;45:277–82.
[19] Papanikolaou N, Chatziantoniou C, Darlametsos I, Gkika EL, Irvine R. Alterations of $HgCl_2$-induced autoimmune glomerulonephritis and A.R.F. in Brown Norway rats. Omega-6 essential fatty acids. Liss A., New York, 1990. pp. 391–412.
[20] Papanikolaou N, Peros G, Morphake P, Gkikas G, Maraghianne D, Tsipas G, Kostopoulos K, Arambatze C, Gkika EL, Bariety J. Does gentamicin induce acute renal failure by increasing renal TXA_2? Prostagl Leuk and Essential Fatty Acids 1992;45:131–6.
[21] Grauer GF, Greco DS, Behrend EN, Fettman MJ, Mani I, Getzy DM, Reinhart GA. Effects of dietary n - 3 fatty acid supplementation versus thromboxane synthetase inhibition on gentamicin-induced nephrotoxicosis in healthy male dogs. Am J Vet Res 1996;57(6):948–56.
[22] Mendal A, Miller J. Protection against ischemic acute renal failure by prostaglandin infusion. Prostaglandins Leuk Med 1982;8:361–73.
[23] Lifschitz MD, Barnes JL. Prostaglandin I_2 attenuates ischemic acute renal failure in the rat. Am J Physiology 1884;247:F714–F717.
[24] Crafa F, Gugenheim J, Saintpaul MC, Cavanel C, Lapalus F, Ouzan D, Militerno G, Mouiel J. Protective effects of prostaglandin E (1) on normo-thermic liver inchemia. Eur Surg Res 1991;23(5–6):278–84.
[25] Morphake P, Bariety J, Darlametsos I, Tsipas G, Gkikas G, Hornych A, Papanikolaou N. Alteration of cyclosporine–induced nephrotoxicity by gamma linolenic (GLA) acid and eicosapentaenoic acid (EPA) in Wistar rats. Prostag Leuk and Essential Fatty Acids 1994;50:29–35.
[26] Hardy G, Stanke–Labesque F, Deveaux G, Devillier P, Sessa C, Bessard G. Cyclosporine A and Cremophor EL induce contractions of humman saphenous vein: involvement of thromboxane A (2) receptor-dependent pathway. J Cardiov Pharm 2000;36(6):693–8.
[27] Parra T, de Arriba G, de Lema GP, Rodriguez–Puyol D, Rodriguez–Puyol M. Cyclosporine a nephrotoxicity: Role of thromboxane and reactive oxygen species. J Lab Clin Med 1998;131:63–70.

[28] Sprecher H. Metabolism of highly unsaturated n-3 and n-6 fatty acids. Biochim Biophys Acta 2000;1486(2-3):219–31.
[29] Horrobin FD. GLA: an interediate in essential fatty acid metabolism with potential as an ethical pharmaceutical and as a food. Rev Contemp Pharmacoth 1990;1:1–45.
[30] Huang YS, Smith RS, Redden PR, Cantrill RC, Horrobin DF. Modification of liver fatty acid metabilism in mice by n - 3 and n - 6 delta-6–desaturase substrates and products. Biochimica et Biophysica Acta 1991;1082(3):319–27.
[31] Nassar BA, Huang YS. The influence of dietary manipulation with n - 3 and n - 6 fatty acids on liver and plasma phospolipid fatty in rats. Lipids 1986;21:652–6.
[32] Cleland GL, Gibson AR, Neumann M, French KJ. The effect of dietary fish oil supplement upon the content of DGLA in Human plasma phosoholipids. Prost Leuk and Essential Fatty Acids 1990;40: 9–12.
[33] Manku MS, Morse–Fisher N, Horrobin DF. Changes in human plasma essential fatty acid levels as a result of administration of linoleic acid and gamma-linolenic acid. Eur J Nutr 1988;42:55–60.
[34] Arntzen KJ, Brekke OL, Vatten L, Austgulen R. Reduced production of PGE (2) and PGF (2 alpha) from decidual cell cultures supplemented with n-3 Polyunsaturated fatty acids. Prostagl & Other Lipid Med 1998;56(2–3):183–95.
[35] Yeo YK, Lim AY, Lee JY, Kim HJ, Farkas T, Kim D. Eicosapentaenoic and docosahexaenoic acids reduce arachidonic acid release by rat kidney microsomes. J Biochem Mol Biol 1999;32(1):33–8.
[36] Bell JG, Tocher DR, Macdonald FM, Sargent JR. Effects of diets rich in linoleic (18/2 n-6) and alpha linolenic (18/3 n - 3) acids on the growth, lipid class and fatty-acids compositions and eicosanoid production in juvenile turbot (S*cophathalmus–maximus L*). Fish Physiol and Bioch 1994;13(2):105–18.
[37] Stone KJ, Willis AL, Hart M, Kirtland SJ, Kernoff PBA, McNicol GP. The metabolism of dihomo–g–linolenic acid in man. Lipids 1979;14(2):174–80.
[38] Fan YY, Chapkin RS. Phospholipid sources of metabolically elongated gamma- linolenic acid-conversion to prostaglandin - E (1) in stimulated mouse macrophages. J Nutr Biochem 1993;4:602–7.
[39] Ryffel B, Donatsch P, Hiestand P, Mihatsch MJ. PGE_2 reduces nephrotoxicity and immunosuppression of cyclosporine in rats. Clin Nephrol 1986;25:S95–S99.
[40] Mansi MK, Alkhudair WK, Huraib S. Treatment of erectile dysfunction after kidney transplantation with intracavernosal self–injection of prostaglandin E_1. J Urol 1998;159(6):1927–30.
[41] Needleman P, Raz A, Minkes MS, Ferrendelli A, Sprecher H. Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. Proc Natl Acad Sci (USA) 1979;76: 944–7.
[42] Abeywardena MY, Fisher S, Schweer H, Charnock JS. In vivo formation of metabolites of PGI_2 and PGI_3 in the marmoset monkey (*Callithrix jacchus*) following dietary supplementation with tuna fish oil. Biochem Biophys Acta 1989;1003:161–6.
[43] Know JS, Snook JR, Wardlaw GM, Hwang DH. Effects of diet high in saturated fatty acids, canola oil, or safflower oil on platelet function, thromboxane B_2 formation and fatty acid composition of platelet phospholipids. Am J Nutrition 1991;14:351–9.
[44] Hishinma T, Yamazaki T, Mizugaki M. Effects of long-term supplementation of eicosapentanoic and docosahexanoic acid on the 2-, 3-series prostacyclin production by endothelial cells. Prostagl & Other Lipid Med 1999;57(5–6):333–40.
[45] Niwa T, Asada H, Yamada K. Prostaglandin E_1, infusion therapy in chronic glomerulonephritis–a double–blind, crossover trial. Prostagl Leukot Med 1985;19:227.
[46] Hinglais N, Palletier L, Vial C. Effect of PGE_1 in Brown Norway rats with mercury induced autoimmune disease. Clin Immun Immunop 1986;40:401–9.
[47] Hamazaki T, Fischer S, Urakaze M, Sawazaki S, Yano S. Comparison of the urinary metabolites of prostaglandin and thromboxane of the 2– and 3–series in a Japanese fishing and a Japanese farming village. Prostaglandins 1986;32:655–64.
[48] Achard F, Gilbert M, Benistant C, Ben Slama S, DeWitt DL, Smith WL, Lagarde M. Eicosapentanoic and docosahexaenoic acids reduce PGH Synthase 1 expression in bovine aortic endothelial cells. Bioch and Bioph Res Commun 1997;241(2):513–18.
[49] Benistant C, Achard F, Slama SB, Lagarde M. Docosapentaenoic acid (22:5, n - 3): Metabolism and effect on prostacyclin production in the endothelial cells. Prostagl Leuk and Essential Fatty Acids 1996;55(4): 287–92.
[50] Nieuwenhuys CMA, Feijge MAH, Offermans RFG, Kester ADM, Hornstra G, JWM. Modulation of rat platelet activation by vessel wall-derived prostaglandin and platelet-derived thromboxane: effect of dietary fish oil on thromboxane–prostaglandin balance. Atherosclerosis 2001;154(2):355–66.
[51] Elzinga L, Kelley VE, Houghton DC, Bennett MW. Modification of experimental nephrotoxicity with fish oil as a vehicle for cyclosporine. Transplantation 1987;43(2):271–4.
[52] Homan van der Heide JJ, Hilo H, Donker JM, Wilmink JM, Tegzess AM. Effect of dietary fish oil on renal function and rejection in cyclosporine–treated recipients of renal transplants. New Engl J Med 1993;329(11):769–73.
[53] Santos J, Queiros J, Silva F, Cabrita A, Rodrigues A, Henriques AC, Sarmento AM, Pereira MC, Guimaraes S. Effects of fish oil in cyclosporine–treated renal transplant recipients. Transplantation Proc 2000;32(8):2605–8.
[54] Rubanui GM, Parker Botelho LH. Endothelins. FASEB J 1991;5: 2713.
[55] Fried G, Samuelsson U. Endothelin and neuropeptide Y are vasoconstrictors in human uterine blood vessels. Am J Obstet Gynecol 1991; 164:1330.
[56] Blank MA, Fuortes M, Nyren O, Jaffe BM. Effect of endothelin - 1 and vasoactive intestinal polypeptide in the feline colon. Life Sci 1991;48:1937.