Kocatepe Veteriner Dergisi
Kocatepe Veterinary Journal

Kocatepe Vet J (2015) 8(1): 45-50
DOI: 10.5578/kvj.9054
Submittion: 17.09.2014
Accepted: 09.02.2015

ARAŞTIRMA MAKALESİ

The Investigation of The Effects of Omega 3 Fatty Acids and Sesame Oil on Cyclosporine-A Induced Immunosupressive and Oxidant Effects in Rats

Gülcan AVCI[1*], Aziz BÜLBÜL[2], Mehmet ÖZDEMİR[3], A.Yasemin GÖKSU EROL[4]

[1]*Afyon Kocatepe University Faculty of Veterinary Medicine Department of Biochemistry, Afyonkarahisar, TURKEY*
[2]*Afyon Kocatepe University Faculty of Veterinary Medicine Department of Physiology, Afyonkarahisar, TURKEY*
[3]*Afyon Kocatepe University Faculty of Veterinary Medicine Department of Pharmacology and Toxicology, Afyonkarahisar, TURKEY*
[4]*Akdeniz University Faculty of Medicine Department of Histology and Embryology, Antalya, TURKEY*

S U M M A R Y

The purpose of this study was to investigate the effects of omega-3 (O-3) and sesame oil (SO) on cyclosporine-A (CsA) induced immunosuppressive and oxidant effects in rats. The study was performed on seven groups for 15 days using Wistar Albino rats (n=7),1) Control: physiological saline was given by gastric gavage, 2) CsA: CsA was administered (15 mg/kg/day) subcutaneously (sc),3) CsA+SO: CsA (15 mg/kg/day; sc) and SO (1 ml/kg, gavage) were given, 4) CsA+O-3: CsA, (15 mg/kg/day; sc) and O-3 (100 mg/kg, gavage) were administered, 5) CsA+SO+O-3: CsA (15 mg/kg/day; sc), SO (1 ml/kg, gavage) and O-3 (100 mg/kg, gavage) were given at the same time, 6) SO: only SO (1 ml/kg; gavage) 7) O-3: only O-3 (100 mg/kg, gavage). 24 h after the last treatments animals were sacrificed. Blood, livers and kidneys were collected for biochemical analysis. There was no difference among all groups in terms of nitric oxide (NOx). Changes in the IL-1α, IL-1β levels were insignificant all other groups compared to CsA group. Nonetheless IFN-γ levels were increased in all groups except SO, O-3 groups. Changes in the level of MDA, GSH, SOD and CAT in both kidney and liver tissues were negligible in the CsA group compared to control group. MDA, GSH and SOD levels in the kidney tissue were determined higher in the CsA groups than CsA+O-3 group, nevertheless changes in the liver were insignificant. Given these findings, use of SO and O-3 with CsA may be a new approach for preventing CsA- induced oxidative stress in kidneys.

Key Words: Antioxidants, Cyclosporine-A, Cytokines, Omega-3, Sesame Oil

●●●

Ratlarda Siklosporin A'nın Uyardığı İmmunosupresif ve Oksidan Etkilere Karşı Susam Yağı ve Omega 3 Yağ Asitlerinin Etkisinin Araştırılması

ÖZET

Bu çalışma Wistar Albino rat kullanılarak 15 gün boyunca gerçekleştirildi (n=7). 1)Kontrol: Fizyolojik salin gastrik gavaj ile verildi. 2) CsA: CsA (15 mg/kg/gün) subkutan (sc) olarak uygulandı. 3) CsA+SO grup: CsA (15 mg/kg/day; sc) ve SO (1 ml/kg, gavaj) olarak verildi. 4) CsA+O-3: CsA (15 mg/kg/gün; sc) ve O-3 (100 mg/kg, gavaj) olarak verildi. 5) CsA+SO+O-3: CsA (15 mg/kg/gün; sc), SO (1 ml/kg, gavaj) ve O-3 (100 mg/kg, gavaj) eş zamanlı olarak verildi. 6) SO: sadece SO (1 ml/kg; gavaj) ve 7) O-3: sadece O-3 (100 mg/kg, gavaj). Son uygulamalardan 24 saat sonra ratlar sakrifiye edildi. Biyokimyasal analizler için kan, karaciğer ve böbrek dokuları toplandı. Tüm gruplar arasında NOx bakımında bir fark belirlenmedi. CsA gruba göre diğer tüm gruplarda IL-1α, IL-1β seviyesindeki değişimler önemsizdi. Buna karşın IFN-γ düzeyleri SO ve O-3 hariç diğer tüm gruplarda arttı. Kontrol gruba göre CsA grubunda MDA, GSH, SOD ve CAT düzeylerindeki değişimler hem karaciğer hem de böbrek dokusunda önemsizdi. CsA grubuna göre CsA+O-3 grubunda böbrek dokusu MDA, GSH ve SOD düzeyleri yüksek bulunurken, buna karşın karaciğerdeki değişimler önemsizdi. Bu bulgulara göre, CsA ile birlikte SO ve O-3'ün kullanımı böbreklerde CsA'nın indüklediği oksidatif stresin önlenmesinde yeni bir yaklaşım olabilir.

Anahtar Kelimeler: Antioksidanlar, Siklosiporin-A, Sitokinler, Omega-3, Susam Yağı,

*Corresponding author e-mail: gulcanavci@hotmail.com

INTRODUCTION

Isolated from the fungus *Tolypocladium inflatum*, Cyclosporin A (CsA) is an immunosuppressive agent for the treatment of autoimmune and inflammatory disorders and to prevent rejection of organ transplantation (Tedesko and Haragsim, 2012; Stähelin, 1986). The most important effect of CsA is to reduce the T lymphocyte activity. Cyclosporin binds to cyclophilin of lymphocytes, especially T cells. This complex inhibits calcineurin and prevents the dephosphorylation of nuclear factor of activated T-lymphocytes. Therefore, it was reduced function of effector T-cells activity via inhibited lymphokine and interleukin production (Tedesko and Haragsim, 2012). Whereas, research has recently indicated that longterm use of CsA could lead to its side effects, such as nephrotoxicity, hepatotoxicity, and hypertension (Ponticelli, 2005; De Hornedo et al., 2007), which their side effects are limiting the use of CsA.

Omega-3 fatty acids (O-3) are important in normal growth and health are alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Many studies have shown that these fatty acids play a crucial role in brain function and to reduce risk factors for heart disease, including high cholesterol and high blood pressure, in cardiovascular diseases (Leaf and Weber, 1988; Simopoulos, 2007). In addition, O-3 are also associated with many health benefits, including anti-inflammatory, lowering cholesterol and triglyceride levels, and reducing lipid peroxidation (Shidfaret al., 2008; Kris-Etherton et al, 2001).

Sesame (*Sesamum indicum* L.) oil (SO) contains nearly 85% polyunsaturated fatty acids (85%) such as linoleic acid and oleic acid and avariety of antioxidant vitamins, glycerol esters of different fatty acids, and sesamol, sesamin (Budowski and Markely, 1951; Namiki, 2007). Hsu and Liu, (2004) were reported that sesame oils improving organ damage induced by lipopolisaccaride.

Nevertheless, the mechanisms underlying their side effects of CsA are shown associated with oxidative stress. Thus, use of antioxidant agents associated with CsA treatment may be beneficial to ameliorate CsA-induced side effects. Given the antioxidant effects of O-3 and SO, these fatty acids may benefical effect against CsA-induced oxidative stress in renal and liver of rats. Because of the side effects limit the use of CsA, the present study was designed to investigate the protective effects of O-3 and/or SO against CsA-induced immunosupressive effect and oxidative stress in rats.

MATERIAL AND METHODS

Forty-nine adult male Wistar-Albino rats (weighing 150-200 g) were used in the present study. Rats were allowed to acclimatize for a 1-week period before starting the experiment. The temperature was 25 ± 2°C, and lighting was 12-hour light/dark cycle. Rats in all groups were fed ad libitum with the same standard laboratory chow and tap water *ad libitum*.

The animals were randomly divided into seven groups (with 7 rats per group). Seven groups of 7 rats/group were treated with saline/drugs for 15 days. The groups were as follow: The control group: Rats were given saline by gastric gavage. In group 2, subcutaneous (SC) CsA was administered (15 mg/kg/day). In group 3, CsA (15 mg/kg/day; SC) and SO (1 ml/kg, gavage) were given. In group 4, CsA (15 mg/kg/day; SC) and O-3 (100 mg/kg, gavage) were given orally. In group 5, CsA (15 mg/kg/day; SC) and SO (1 ml/kg,gavage) and O-3 (100 mg/kg, gavage) were given synchronously. In group 6, only SO (1 ml/kg; PO) and in group 7 only O-3 (100 mg/kg, PO) were given. At the end of the study, all rats were anesthetized with an intraperitoneal ketamine (21.2 mg/kg) and xylazine (4.2 mg/kg) combination. Blood samples were obtained, then all rats were sacrificed and livers and kidneys were collected

Biochemical Analysis

Plasma nitric oxide (NOx), interleukin (IL)-1α, IL-1β and IFN-γ levels were measured using a commercial enzyme-linked immunosorbent assays kit (Cayman Chemical, Invitrogen Corporation) according to the manufacturer's instructions. The organs including liver and kidney were collected for clinical biochemistry examination. The tissue samples from each organ were removed immediately and washed in ice-cold saline. Tissues were then homogenized in 1:40 w/v of 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA. After centrifugation at 18 000×g for 15 min at 4◦C, the supernatant was extracted, and kept at -30◦C in advance of assays.

Tissue total protein (Lowry et al., 1951), malondialdehyde (MDA) (Draper and Hadley,1990), reduced glutathione (GSH) (Beutler et al., 1963), catalase (CAT) (Luck, 1965) and superoxide dismutase (SOD) (Sun et al., 1988) were measured spectrophotometrically methods in liver and kidney homogenizate.

Statistical Analysis

Data obtained from experiment animals is expressed with mean ± standard error. The statistical differences between the control and experimental groups were evaluated by one-way ANOVA and Tukey post hoc tests (SPSS for Windows 13.0). A difference in the mean values with $P<0.05$ was considered to be significant.

RESULTS

There was no difference among all groups in terms of NOx. Changes in the IL-1α, IL-1β levels were insignificant all other groups compared to CsA group. Nonetheless IFN-γ levels were increased in all groups except SO and O-3 groups (Table 1). Changes in the level of MDA, GSH, SOD and CAT in both kidney and liver tissues were negligible in the CsA group compared to control group. MDA, GSH and SOD levels in the kidney tissue were determined higher in the CsA groups than CsA+O-3 group, nevertheless changes in the liver were insignificant (Table 2).

DISCUSSION

In addition to its effects on immune system depending on the dose of CsA leads to side effects such as acute and chronic nephrotoxicity, hypertension, hyperlipidemia, gingival hyperplasia, hyperkalemia, hypomagnesaemia, hyperuricemia and thrombotic microangiopathy, neurotoxicity. CsA inhibited-calcineurin in nonlymphatic tissues is thought caused its side effects. Normally, activated calcineurin dephosphorylates regulatory sites on nuclear factor of activated T-lymphocytes (NFATs). However, CsA inhibited-calcineurin prevents the dephosphorylation of NFATs-mediated cyclooxygenase-2 (COX-2) expression and downstream production of arachidonic acid metabolites. Thus, inhibition at this step leads to a reduction of T-cell activation and immune response (Tedesko and Haragsim, 2012). Several studies reported that CsA inhibits endothelial nitric oxide (eNOS) formation, vasodilator factors, prostacyclin and prostaglandin E2 (Oriji, 1999; Hortelano et al., 2000). Many studies indicated that inducible nitric oxide (iNOS)-derived NO level was increased in lipopolysaccharide induced endotoxemia, pathological vasodilatation and tissue damage (Cauwels and Brouckaert, 2007; Yazar et al., 2010). Also CsA, an immunosuppressive agent, is a potent inhibitory of iNOS. Therefore, it can be expected to reduce the blood NO level. However, in this study, there was no changes among all groups in terms of NOx. This result may be associated with the absence of infection induced-iNOS activity in this experimental model.

Moreover, Mariee and Abd-Ellah (2011) indicated that the protective effect of DHA against CsA-induced nephrotoxicity in rats was chiefly associated with the increase of NO bioavailability in the kidney. Similarly , Hsu et al., (2007) suggest that SO potently inhibited cisplatin-induced lipid peroxidation by inhibiting NO generation in mice. Given unchanged NO levels in all groups, this result may due to absence of infection induced-NO activity in this study.

Cytokines, which are cell signalling molecules, are important regulators of both the innate and adaptive immune responses. They are produced by various of the immune cells, such as macrophages, B lymphocytes and T lymphocytes, mast cells. They are described as proinflammatory (TNF-α, IL-1β, IL-6) or anti-inflammatory (IL-4, IL-10, IL-13) cytokines. Cytokine production is stimulated by several different factors, including bacterial or viral infections, inflammation and trauma (Horn et al., 1996, Nororiha et al., 1995). Blood levels of cytokines in physiological conditions are usually low or undetectable concentration. CsA induced immunosuppression leads to the blocking of the transcription of genes encoding several cytokines, especially IL-2. The absence of IL-2 synthesis prevent the activation of T-cell and secondary synthesis of other cytokines such as IL-4, IF-γ (Matsuda and Koyasu, 2000).

In this study, all the cytokines levels in CsA group did not significantly change in comparison with control group. In addition, IL-1α, IL-1β and IF-γ (undetectable) levels were found to be statistically insignificant all in groups compared to CsA group. As expected, the reason for these results may be due to absence of infection induced-cytokines and the short experimental period of this study. Similarly, Elisa Böhmer et al., (2009) demonstrated that CsA treatment (5 or 15 mg/kg, 8 week) decreases blood levels of IL-1α, IL-1β and IL-2, but does not alter IL-6 and IF-γ levels in rats. The present study, IF-γ levels were increased in CsA+SO, CsA+O-3 and CsA+O-3+SO groups compared to CsA group except for SO and O-3 groups. Given the increased IF-γ levels, this result may be related to reduced immunosuppressive effect of CsA by SO.

Reactive free radicals produced in oxidative stress can oxidize of biomolecules within cells, which leads to extensive lipid peroxidation in biological membranes.

Table 1. Results of the analysis of NOx and cytokines in all groups (X ± SEM)

	Control	CsA	CsA+SO	CsA+O-3	CsA+SO+O-3	SO	O-3	P
NOx (µmol/L)	13.06±0.27	13.66±0.63	13.12±0.27	13.16±1.60	12.07±0.54	13.35±0.29	12.97±0.12	0.834
IL-1α (pg/ml)	64.03±15.54	52.36±20.47	42.87±12.12	20.22±4.74	34.49±11.47	85.82±40.97	53.49±15.42	0.391
IL-1β (pg/ml)	#	49.21±28.30	61.84±36.37	38.68±19.47	67.10±41.66	#	13.42±12.49	0.288
IFγ (pg/ml)	#	#	95.71±50.72[a]	11.34±7.74[b]	10.34±8.99[b]	#	#	0.013

[a,b]Different superscripts in the same line indicate a significant difference between groups (P<0.05).
[#]The data obtained were lower than the minimum standard level (undetectable)

Table 2. Results of the analysis of oxidant and antioxidant parameters in liver and kidney homogenizates (X ± SEM)

	Control	CsA	CsA+SO	CsA+O-3	CsA+SO+O-3	SO	O-3	P
Liver- MDA (nmol/mg prot.)	0.029±0.003	0.022±0.004	0.031±0.009	0.021±0.001	0.020±0.001	0.017±0.002	0.017±0.002	0.543
Kidney-MDA (nmol/mg prot.)	0.144±0.029[b]	0.110±0.011[b]	0.200±0.044[b]	0.312±0.030[a]	0.110±0.042[b]	0.162±0.020[b]	0.124±0.039[b]	0.001
Liver-GSH (nmol/mg prot.)	3.40±1.02[ab]	2.03±0.50[bc]	2.50±0.32[abc]	2.19±0.48[bc]	2.48±0.57[abc]	1.09±0.30[c]	3.98±0.41[a]	0.024
Kidney-GSH (nmol/mg prot.)	7.43±1.61[c]	8.96±0.78[bc]	13.94±1.53[ab]	18.12±1.88[a]	16.75±2.91[a]	10.65±1.17[bc]	9.35±2.92[bc]	0.002
Liver-SOD (U/mg prot.)	1.20±0.38[b]	2.39±0.56[ab]	2.66±0.51[a]	1.54±0.23[ab]	1.73±0.38[ab]	1.80±0.21[ab]	2.78±0.21[a]	0.042
Kidney-SOD (U/mg prot.)	5.63±0.71b	5.28±0.95[b]	5.22±0.98[b]	10.58±1.55[a]	8.10±0.75[ab]	5.28±0.99[b]	6.67±1.24[b]	0.006
Liver-CAT (k/mg prot.)	0.013±0.002	0.015±0.003	0.014±0.003	0.014±0.001	0.013±0.002	0.008±0.001	0.012±0.002	0.564
Kidney-CAT (k/mg prot.)	0.007±0.001[cd]	0.005±0.001[d]	0.018±0.002[a]	0.010±0.001[bcd]	0.013±0.002[b]	0.004±0.001[d]	0.011±0.002[bc]	0.000

[a,b,c,d]Different superscripts in the same line indicate a significant difference between groups (P<0.05)

Lipid peroxidation has been implicated in disease states such as atherosclerosis, kidney damage and others (Mylonas and Kouretas, 1999). Several studies support the hypothesis that CsA-induced toxicity may be the consequence of oxidative stress, oxidation and cross-linking of cellular thiols and membrane lipid peroxidation (Ichikawa et al., 1994). Zhong et al., (1998) reported that CsA (25 mg/kg) caused hypoxia and increased production of a new free radical species and exclusively in the kidney (Zhong et al., 1998). Similarly, Haberland et al., (1997) stated that CsA (30 mg/kg) treatment is associated with stimulation of oxygen radical formation in liver and kidney. However, in this study, the level of MDA, GSH, SOD and CAT in both kidney and liver tissues were not found to be significantly in the CsA group compared to control group. The reason for these results may be related to the short experimental period or insufficient dose of CsA, MDA, GSH and SOD levels in the kidney tissue were determined higher in the CsA+O-3 group than CsA group, nevertheless changes in the liver were insignificant (Table 2). In CsA+SO group CAT and GSH levels in kidney and SOD activity in liver were found to be increased compared to CsA group. In kidney, GSH and CAT activity were higher in the CsA+O-3+SO group than CsA group but not liver tissue. These results show that the administration of SO and O-3 alone or combined with CsA may be alleviated CsA-induced oxidative stress. In fact, it is known that SO and O-3 are potent antioxidants (Hsu and Liu, 2006; Yamashita et al., 2000). In previuos study, we reported that the use of O-3 or SO has showed similar protective effects against CsA-induced nephrotoxicity, because of revealed by a remarkable decrease in histopathological changes and apoptotic cell count (Goksu Erol et al., 2013), but they do not have any pro-apoptotic effects on the liver (Göksu Erol et al., 2011). Hsu et al., (2004) stated that SO alleviates oxidative stress-associated renal injury by inhibiting NO formation and proinflammatory cytokine generation in endotoxemic rats.

As a result based on these findings, we stated that administration of SO or O-3 combined with CsA may be ameliorated CsA-induced immunosuppresive and oxidant effects. But, their implementation alone is not sufficient to show these benefical effects. Given their free radical scavenging properties use of SO and O-3 with CsA may be a new approach for preventing CsA- induced oxidative stress in kidneys.

Acknowledgement: This research was supported by funds from The Scientific Research Council of Afyon Kocatepe University, Afyonkarahisar, TURKEY (Project Number: 10 REK 04) and this paper was a part of this project.

REFERENCES

Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione.Journal of Laboratory and Clinical Medicine. 1963; 61: 882-888.

Böhmer AE, Ribeiro Corrêa A.M, de Souza DG, Knorr L, Hansel G, Corbellini LG, Driemeier D, Portela LV, Souza DO. Long-term cyclosporine treatment: Evaluation of serum biochemical parameters and histopathological alterations in Wistar rats. Experimental and Toxicologic Pathology. 2011; 63: 119-123.

Budowski P, Markley KS. The chemical and physiological properties of sesame oil. Chemical Reviews. 1951; 48: 125-151.

Cauwels A, Brouckaert P. Survival of TNF toxicity: dependence on cascades and NO. Archives of Biochemistry and Biophysics. 2007; 462: 132-139.

De Hornedo JP, De Arriba G, Fernandez MC, Benito S, Cid TP. Cyclosporin A causes oxidative stress and mitochondrial dysfunction in renal tubular cells. Nefrologia. 2007; 27: 565-573.

Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods in Enzymology. 1990; 186: 421-431.

Goksu Erol AY, Avcı G, Sevimli A, Ulutas E, Ozdemir M. The protective effects of omega 3 fatty acids and sesame oil against cyclosporine A-induced nephrotoxicity. Drug and Chemical Toxicology. 2013; 36: 241-248.

Göksu Erol AY, Bülbül A, Avcı G, Özdemir M, Akkaya Ö. The Protective Effects of Omega-3 Fatty Acids and Sesame Oil on Cyclosporine-A Induced Liver Apoptosis. Journal of Academic Research in Medicine. 2011; 1: 8-11.

Haberland A, Henke W, Grune T, Siems W, Jung K, Schimke I. Differential response of oxygen radical metabolism in rat heart, liver and kidney to cyclosporine A treatment. Inflammation Research. 1997; 46: 452-454.

Horn DL, Opal SM, Lomastro E. Antibiotics, cytokines, and endotoxin: A complex and evolving relationship in gram-negative sepsis. Scandinavian Journal of Infectious Diseases. Supplementum. 1996; 101: 9-13.

Hortelano S, Castilla M, Torres AM, Tejedor A, Bosc'a L. Potentiation by nitric oxide of cyclosporin A and FK506-induced apoptosis in renal proximal tubule cells. Journal of the American Society of Nephrology. 2000; 11: 2315–2323.

Hsu DZ, Chen KT, Lin TH, Li YH, Liu MY. Sesame oil attenuates cisplatin induced hepatic and renal injuries by inhibiting nitric oxide-associated lipid peroxidation in mice. Shock. 2007; 27: 199-204.

Hsu DZ, Liu MY. Sesame oil protects against lipopolysaccharide-stimulated oxidative stress in rats. Critical Care Medicine. 2004; 32: 227-231.

Kris-Etherton P, Eckel RH, Howard BV, Jeor S St, Bazzare TL. AHA Science Advisory: Lyon Diet Heart Study. Benefits of a Mediterranean-style, National Cholesterol Education Program/American Heart Association Step I Dietary Pattern on Cardiovascular Disease. Circulation. 2001; 103: 1823 -1825.

Leaf A, Weber PC.. Cardiovascular effects of n-3 fatty acids. The New England Journal of Medicine. 1988; 318: 549-557.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry. 1951; 193: 265-275.

Luck H. Catalase, In: Methods in Analysis, Bergmeyer H.U. (ed.), Academy Press, London. 1965; pp. 855-884.

Mariee AD, Abd-Ellah MF. Protective effect of docosahexaenoic acid against cyclosporine A-induced nephrotoxicity in rats: a possible mechanism of action. Renal Failure. 2011; 33: 66-71.

Matsuda S, Koyasu S. Mechanisms of action of cyclosporine. Immunopharmacology. 2000; 47: 119-125.

Mylonas C, Kouretas D. Lipid peroxidation and tissue damage. In Vivo (Athens, Greece). 1999; 13: 295-309.

Namiki M. Nutraceutical functions of sesame: a review. Critical Reviews in Food Science and Nutrition. 2007; 47: 651-673.

Noronha IL, Niemir Z, Stein H, Waldher R. Cytokines and growth factors in renal disease. Nephrology Dialysis Transplantation. 1995; 10: 775-786.

Oriji GK. Nitric oxide in cyclosporine A-induced hypertension: endothelin receptors gene expression. Prostaglandins, Leukotriens and Essential Fatty Acids. 1999; 61: 41-44.

Ponticelli C. Cyclosporine: from renal transplantation to autoimmune diseases. Annals of the New York Academy of Science. 2005; 1051: 551-558.

Shidfar F, Keshavarz A, Hosseyni S, Ameri A, Yarahmadi S. Effects of omega-3 fatty acid supplements on serum lipids, apolipoproteins and malondialdehyde in type 2 diabetes patients. Eastern Mediterranean Health Journal. 2008; 14: 305-313.

Simopoulos AP. Omega-3 fatty acids and athletics. Current Sports Medicine Reports. 2007; 6: 230-236.

Stähelin H. Cyclosporin A:Historical background. Progressin Allergy. 1986; 38: 19-27.

Sun Y, Oberley LW, Li YA. Simple method for clinical assay of superoxide dismutase. Clinical Chemistry. 1988; 34: 497-500.

Tedesco D, Haragsim L. Cyclosporine: A Review. Journal of Transplantation, Article ID 230386, 7 pages, doi:10.1155/2012/230386

Yazar E, Er A, Uney K, Bulbul A, Avci GE, Elmas M, Tras B. Effects of drugs used in endotoxic shock on oxidative stress and organ damage markers. Free Radical Research. 2010; 44: 397-402.

Zhong Z, Arteel GE, Connor HD, Yin M, Frankenberg MV, Stachlewitz RF, Raleigh JA, Mason RP, Thurman RG. Cyclosporin A increases hypoxia and free radical production in rat kidneys: prevention by dietary glycine. American Journal of Physiology-Renal Physiology. 1998; 275: F595-F604.