

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

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SUMMARY

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 (NO_x). Changes in the IL-1 α , IL-1 β 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

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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 kurban edildi. Biyokimyasal analizler için kan, karaciğer ve böbrek dokuları toplandı. Tüm gruplar arasında NO_x bakımında bir fark belirlenmedi. CsA gruba göre diğer tüm gruplarda IL-1 α , IL-1 β 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, Siklosporin-A, Sitokinler, Omega-3, Susam Yağı,

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INTRODUCTION

Isolated from the fungus *Tolyposcladium 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 (Shidfare et 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 a variety 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 beneficial 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 immunosuppressive 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 $25 \pm 2^\circ\text{C}$, 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 (NO_x), interleukin (IL)-1 α , IL-1 β 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 \times g for 15 min at 4 $^\circ\text{C}$, the supernatant was extracted, and kept at -30 $^\circ\text{C}$ 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 \pm 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-1 α , IL-1 β 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 (Orij, 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-1 β , 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-1 α , IL-1 β 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-1 α , IL-1 β 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±0.71 ^b	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 previous 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 (Göksu 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 immunosuppressive and oxidant effects. But, their implementation alone is not sufficient to show these beneficial 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.

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