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Effect of acute and chronic treatment of Cyclosporin A on liver and kidney functions in rats

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ABSTRACT

Cyclosporin A is a compound widely used as an immunosuppressive drug, particularly, in case of kidney transplantation to prevent rejection of transplanted organ. This study aimed to investigate the bad side effect of acute and chronic treatment with cyclosporin A on liver and kidneys by measuring liver enzymes and kidney function tests in serum. Male rats were used as experimental model in this study. The results of this study concluded that, chronic treatment with cyclooosprin A leads to increase in serum urea, creatinine, and uric acid significantly compared to control, also, ALT and Alkaline phosphatase activities in serum were increased by chronic administration of cyclosporine A for four weeks. Decrease of serum albumin and total protein were observed significantly compared to control groups.

INTRODUCTION

Kidney transplantation is the preferred method of treatment of end-stage renal disease, which significantly improves the quality of life, but also increases survival when compared to dialysis. Prevention of acute or chronic rejection demands the use of immunosuppressant. However, nephrotoxicity, hepatotoxicity, cardiovascular disease, post-transplantation diabetes mellitus, chronic graft dysfunction and dyslipidemia may all occur as complications of immune-suppressive therapy (Ivandic and Basic-Jukic, 2014). Cyclosporine A (CsA), a cyclic undecapeptide, which is one of the major immunosuppressants, has been used for the prevention of life-threatening transplant rejection responses as well as the treatment of immune diseases.

Although CsA has played an important role in the development of organ transplants, its clinical use has been severely limited due to the nephrotoxicity of CsA, a common and serious side effect (Farh, 1993). CsA is metabolized by the cytochrome p-450, particularly a CYP3A4 isoform, in the liver liver and excreted mainly into the bile. The therapeutic drug monitoring of CsA is essential to optimize the immunosuppressant therapy due to large inter- and intra-individual variability in the pharmacokinetics of this drug (Campana *et al.*, 1996).

In experimental animals, CsA has been shown to cause acute renal vasoconstriction, followed by a decrease in glomerular filtration rate and renal blood flow (English *et al.*, 1987). A down regulation of calbindin D 28 kDa, a vitamin D-dependent calcium binding protein, has been reported to be a critical factor for the renal side-effects of CsA (Steiner *et al.*, 1996).

Cyclosporine A actions

CsA suppresses immune responses mainly by inhibiting production of immune reactive cytokines such as IL2. Intracellular interaction of CsA involves its receptor protein peptidylprolyl cis-trans isomerase (PPIase) cyclophilin and the protein phosphatase 2B calcineurin, inhibiting both the PPIase activity of cyclophilin and calcineurin phosphatase activity. Since calcineurin activity is essential for the dephosphorylation and activation of the nuclear factor of activation of T cells (NFAT), cytokines that are regulated by NFAT are consequently down regulated by CsA (Shaw *et al.*, 1995).

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NFAT is a transcription factor that activates the transcription of cytokines that promote the growth and proliferation of T and B-cells. IL2 that is produced by T lymphocytes in response to antigenic or mitogenic stimulation is also necessary for the proliferation and differentiation of many immune cells including activated T lymphocytes, natural killer cells, lymphokine-activated killer cells, B lymphocytes and macrophages (Suthanthiran *et al.,* 1996). In addition to the inhibitory effect on IL2, CsA inhibits the production of interleukins 1a and 1b, interleukin6, gamma-interferon and other lymphokines (Olyaei *et al.,* 2001). These cytokines together modulate the immune and inflammatory reactions, stimulate the hematopoiesis and also present diverse physiological roles regulating the innate and adaptative immunity (Rezzani, 2004).

MATERIALS AND METHODS

Experimental animals

Eighty male albino rats (*Rattus norvegicus*) weighting 100 – 120 g were purchased from the Egyptian Organization for Serological and Vaccine Production, Egypt, were used as experimental animals throughout the present work. The animals were housed individually in plastic cages and acclimated for 1 week before treatment. Food and water were offered *ad libitum*. Animals were maintained at 22± 2 °C at normal light/dark cycle.

Experimental design

Cyclosporine was purchased as Sandimmune capsules (Novartis International AG, Basel, Switzerland). Cyclosporin A was emulsified in olive oil and administered orally (20mg/Kg/day) in case of chronic dosage and 50mg/Kg as a single dose in acute case. Animals were randomly selected and divided into 4 main groups, each one divided into two subgroups, consisted of 10 animals as follow:

1- Animals of the first group were divided into 2 subgroups, the first serves as a control group, and the second were treated orally with 20mg/Kg/day cyclosporine for one week.

2- Animals of the second group were divided into 2 subgroups, the first serves as a control group, and the second were treated orally with 20mg/Kg/day cyclosporine for two weeks.

3- Animals of the third group were divided into 2 subgroups, the first serves as a control group, and the second were treated orally with 20mg/Kg/day cyclosporine for four weeks.

4- Animals of the fourth group were divided into 2 subgroups, the first serves as a control group, and the second were treated with one acute dose (50mg/Kg) orally.

The animals of control groups were administered only olive oil orally.

Blood collection

Twenty four hours after stopping treatment with cyclosporine A, animals were anaesthetized by diethyl ether, dissected and blood was collected by heart puncture with syringe (3ml capacity). The required amount of blood was collected in tubes, and the blood allowed to coagulate in water bath at 37 °C for 30 minutes. Serum was separated by centrifugation in cooling centrifuge (Hettich, Germany) at 3000 xg for 15 minutes, transported into another dry and clean Eppendorf tubes and was kept in deep freezer at -20 °C for biochemical analysis.

Biochemical Analysis

Biochemical studies were performed using commercially available kits, and serum levels of creatinine, urea, uric acid, albumin, and total protein (Diamond Diagnostics, Egypt) as well as liver enzymes (Biomeriux, France) were quantified according to the manufacturer's instructions.

Statistical Analysis

Data are expressed as mean±SD. The level of statistical significance was taken at $P < 0.05$, using one way analysis of variance (ANOVA) test followed by Dunnett test to detect the significance of differences between each group and control. All analysis and graphics were performed by using, INSTAT and graphPad Prism software version 4.

RESULTS AND DISCUSSION

The data recorded in table (1) indicate a marked increase in blood urea (18.76%), which was significantly different from control level after two weeks of treatment with CsA , whereas blood urea level underwent a highly significant increase (54.15%) in animal group treated for 4 weeks ($P < 0.01$). In a group treated with an acute dose (50 mg/Kg of CSA) and sacrificed after 24 hours, there is no change in blood urea. Concerning serum creatinine, the data recorded in table (1) indicated a marked increase in serum creatinine level amounted to be 54.6% compared to the control level after 4 weeks of treatment, whereas changes observed after 1 week, 2 weeks and even in case of acute dosage study, were not significant as compared with their respective controls. The data obtained by serum uric acid analysis are recorded in table (1). It is apparent from the results that, the treatment with CSA (20 mg/Kg/day) for 4 weeks, induced marked increase in serum uric acid which amounted to be 28.64% when compared with the control group. Statistical analysis of these data indicated that, this increase was highly significant ($P < 0.01$).

Several mechanisms have been proposed in cyclosporine-induced nephrotoxicity, namely, the activation of the renin-angiotensin system and enhanced sympathetic tone (Dell *et al.,* 2003), increased synthesis of endothelin (Ramirez *et al.,* 2000), inductions of cytochrome P450 enzymes in renal microsomes (Serino *et al.,* 1994), and renal vasoconstriction attributed to an imbalance in releasing of the vasoactive substances, including reduction of vasodilator factors in particular nitric oxide (Yoon *et al.,* 2009). Other studies have clearly demonstrated that cyclosporine-induced oxidative stress plays a pivotal role in producing structural and functional impairment of the kidney (Burdmann *et al.,* 2003). The hypothesis of these nephrotoxicity as

indicated in the present study, has been proposed that CsA alters the balance between vasodilators and vasoconstrictors in kidney with predominance of vasoconstrictors and vascular smooth muscle cells proliferation in the intima and accumulation of cholesterol esters in macrophages that can be transformed in foam cells in vessel wall with narrowing of vessel lumen (Beckman *et al.,* 2002). Several studies indicate that vascular dysfunction induced by CsA results from an increase in vasoconstrictor factors such as endothelin, thromboxane, and angiotensin II and at the same time a reduction of vasodilator factors such as prostacyclin and nitric oxide (NO) (Parra *et al.,* 1998; Markell *et al.,* 1994; Bilchick *et al.,* 2004; Baid *et al.,* 2001; Halliwell and Gutteridge, 1999). Therefore, an imbalance in the release of vasoactive substances is related to renal vasoconstriction. Decreased glomerular filtration rate and renal plasma flow observed in an early stage is known to be related to afferent arteriolar vasoconstriction (Shen *et al.,* 1987). Loss of proximal tubular cells brush border, proximal tubule dilatation, swelling, necrosis, and infiltration of white blood cells in kidney cortex belong to renal tubular toxicities which are considered to be acute (Racusen *et al.,* 1987). Chronic CsA nephropathy is characterized by irreversible renal striped vasculointerstitial fibrosis, inflammatory cell infiltrations and hyalinosis of the afferent glomerular arterioles (Nankivell *et al.,* 2003; Mourad *et al.,* 1998; Myers *et al.,* 1988). The damage in the glomerular and arteriolar vessels produces decreased urea and uric acid urinary excretion, along with reduction of fractional excretion of sodium, lithium, potassium and phosphates, and decreased reabsorption of bicarbonate, hyperchloremia and metabolic acidosis (Young *et al.,* 1995a, 1995b). Chronic ischemia caused by CsA is believed to be associated with reactive oxygen species and lipid peroxidation.

The liver is responsible for detoxification and elimination of potentially harmful substances. It is an important target organ for xenobiotic compounds. For this reason, hepatotoxicity is the most prominent adverse drug reaction leading to the failure of candidate drugs in preclinical or clinical trials. As clearly presented in table (2) there is no significant change in serum alkaline phosphatase activity after 1 week of treatment, but a highly significant increase occurred after 4 weeks of treatment which amounted to be 35.53% (($P < 0.01$). The data recorded in table (2) indicated that in all groups there are no significant changes in serum AST activity as compared with their respective controls in case of both chronic or acute studies. On the other hand, Serum ALT showed significant increase in both 2 weeks and 4 weeks treatment with CsA ($P < 0.05$).

Highly significant decrease of serum albumin were noticed after 2 weeks and 4 weeks of treatment (Table 2). The percentage of decrease were found to be -16.6% and -26.23%, respectively. However no significant change was recorded in case of acute treatment with the test article, also there is a significant decrease in total protein concentration in 2 weeks treated group with -11.2% compared to control (Table 2).

Cyclosporine A (CsA)-induced hepatotoxicity could be due to a reduction in α2β1 integrin expression that may either be from the direct effect of CsA itself or from reactive oxygen species (ROS) overproduction (Mostafavi-Pour *et al.,* 2013).

Adverse effects caused by CsA include hepatotoxicity that can lead to the development of cholestasis (Dandel *et al.,* 2010), fatty liver (Pagadala *et al.,* 2009), and cardiovascular complications due to hyperlipidemia (Hulzebos *et al.,* 2004). The primary mechanism of action underlying the hepatotoxicity of CsA is prevention of the mitochondrial permeability transition pore from opening leading to oxidative stress and impairment of mitochondrial functions (Wolf *et al.,* 1997).

This is most likely followed by induction of NFκB signaling driving expression of pro-inflammatory cytokines (e.g. TNFα, Il1α, and Il1β) and endoplasmatic reticulum (ER) stress, causing a disturbed vesicles formation necessary for protein, lipid, and bile acid trafficking (Szalowska *et al.,*2013). In addition, it was reported that expression of Fxr and its target genes was de-regulated upon treatment with CsA in different human and rodent in vitro liver models as well as rodents in vivo (Kienhuis *et al.,*2103).

Table 1: Kidney function tests as affected by chronic or acute treatment of albino rats with cyclosporin A.

Treatment Periods	One week		Two weeks		Four weeks		Acute dose	
	Control Mean±SD	Treated Mean±SD	Control Mean±SD	Treated Mean±SD	Control Mean±SD	Treated Mean±SD	Control Mean±SD	Treated Mean±SD
Urea (mg/dl)	47.56±5.23	48±4.22	45.98±3.35	54.61*±3.11	48.47±2.72	74.72**±7.52	47.95±2.55	50.02±4.12
Creatinine(mg/dl)	1.58±0.22	1.6±0.21	1.53±0.24	1.82±0.23	1.61±0.2	2.49**±0.21	1.59±0.25	1.66±0.24
Uric acid(mg/dl)	5.62±0.29	5.75±0.32	5.63±0.29	5.99±0.24	6.11±0.34	7.86**±0.21	5.92±0.24	6.01±0.18

(*) significant difference compared to control group ($P < 0.05$).
(**) highly significant difference compared to control group ($P < 0.01$).

Table 2: Liver function tests as affected by chronic or acute treatment of albino rats with cyclosporin A.

Treatment Periods	One week		Two weeks		Four weeks		Acute dose	
	Control Mean±SD	Treated Mean±SD	Control Mean±SD	Treated Mean±SD	Control Mean±SD	Treated Mean±SD	Control Mean±SD	Treated Mean±SD
ALT (U/L)	82±8.33	79±7.7	80±7.9	95*±6.33	83±7.5	97*±8.55	79±8.88	85±8.5
AST (U/L)	260±7.5	265±8.05	266±8.05	268±2.88	220±5.06	223±8.88	232±6.05	235±6.9
Alkaline phosphatase(U/L)	140.64±17.94	142.35±10.22	140±16.9	129.19±10.14	138.64±10.14	190.62**±16.61	144±11.23	143.52±11.23
Albumin (gm/dl)	5±0.15	4.9±0.22	5.3±0.14	4.42**±0.25	5.05±0.17	3.71**±0.43	4.9±0.19	4.88±0.33
Total protein (gm/dl)	7.49±0.25	7.42±0.21	7.78±0.3	6.91**±0.19	7.6±0.33	7.06±0.39	7.49±0.29	7.48±0.22
Globulins(gm/dl)	2.49±0.25	2.5±0.22	2.48±0.2	2.49±0.19	2.55±0.18	3.35**±0.4	2.59±0.33	2.6±0.31
A/G ratio	2.18±0.1	2±0.13	2.12±0.15	1.84*±0.23	2.05±0.22	1.1**±0.14	2.14±0.21	2.11±0.26

(*) significant difference compared to control group ($P < 0.05$).
(**) highly significant difference compared to control group ($P < 0.01$).

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