Libyan Journal Of Basic Sciences (LJBS)
Vol:7 , No:1 , P: 56 -68 , July. 2017

Antioxidant Activity of Lipoic Acid on Cyclosporine A-Induced Physiological Changes to the Liver in Male Albino Rats

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Received: *May 2017* ***Accepted:*** *July 2017*

Abstract:

This study aimed to assess the protective role of lipoic acid (LA) on liver toxicity of male albino rats induced by cyclosporine (CsA). Forty adult male rats were allocated into four groups: Group (I) served as a control group. Group (II); received orally with CsA (25 mg/kg b.w.), daily for 3 week. Group III: (Recovery CsA group): treated orally with CsA (25 mg/kg b.w.), daily for 3 weeks, then recovered for other 3 weeks. Group IV (LA and CsA group): received LA (100 mg/kg b. w.) orally 1 h before treatment by CsA (25 mg/kg b. w.) daily for 3 weeks. The results indicated that treatment of CsA caused significantly elevated of glucose, serum alanine and aspartate aminotransferases (ALT & AST), gamma glutamate transferase (GGT) activities. While, serum totals protein, albumin and globulin contents showed highly significant decrease resulting liver dysfunction. Hepatic malondialdehyde (MDA) concentration was markedly increased reflecting increased lipid peroxidation, whereas, reduced glutathione (GSH) and superoxide dismutase (SOD) were significantly decreased. On the other hand, LA plus CsA dose-dependently inhibited activities of AST, ALT and GGT. Serum glucose, total protein, albumin and globulin restored. The administration of LA plus CsA exhibited significant reduction of lipid peroxidation while GSH content and SOD activity were enhanced significantly that reflecting improving hepatotoxicity.

In conclusion, the results indicated a negative role of CsA on liver function and oxidative stress in induction toxicity. Thus, lipoic acid plays a positive role on toxicity of liver induced by cyclosporine A.

Keywords: *Lipoic acid, cyclosporine A, oxidative stress, hepatotoxicity.*

Introduction:

As a highly potent immune-suppressive drug, cyclosporine (CsA) remains largely used for the prevention of acute rejection in solid organ transplantation, and for the treatment of various autoimmune diseases. However, CsA can lead to a chronic form of renal damage characterized by a progressive and irreversible deterioration of renal function associated with

interstitial fibrosis, tubular atrophy, arteriolar hyalinosis and glomerulosclerosis [1 and 2].

Alpha-lipoic acid (LA), or 1, 2-dithiolane-3-pentanoic acid, is a naturally occurring dithiol compound synthesized enzymatically in the mitochondrion from octanoic acid. LA is a necessary cofactor for mitochondrial α-ketoacid dehydrogenases, and thus serves a critical role in mitochondrial energy metabolism. In addition to synthesis, LA is also absorbed intact from dietary sources, and it transiently accumulates in many tissues. There is growing evidence that orally supplied LA may not be used as a metabolic cofactor but instead, elicits a unique set of biochemical activities with potential pharmacotherapeutic value against a host of pathophysiologic insults. LA has a potent antioxidant, a detoxification agent and improve age-associated cardiovascular, cognitive, and neuromuscular deficits [3- 5].

This impressive array of cellular and molecular functions has piqued considerable interest among the lay public and the research community for the use of LA both as a nutritive supplement and as a pharmacotherapy. In light of this growing interest, we will attempt to provide an update on the biochemical, toxicological, and pharmacological mechanisms of LA. As many excellent reviews already exist that outline the metabolic role of LA as a covalently bound enzyme cofactor, only a brief summary of this particular aspect of LA function will be presented herein. Instead, a focus mainly on the cellular actions of orally supplied, nonprotein-bound LA will be presented. Pertinent clinical benefits of LA will also be discussed in light of this molecular mechanism [6 and 7].

Therefore, this study investigated the modulating and antioxidant activity of lipoic acid on hepatotoxicity induced by cyclosporine A in male albino rats.

Materials and Methods:

Chemicals

Cyclosporine A (CsA) presents in the form of ampoules under traditional name Sandimmun and provided by Novartis Pharma (Basel, Switzerland). It is present as a clear, yellow liquid supplied in 1 ml ampoules containing 50 mg/ml and was further diluted with olive oil.

Alpha-Lipoic acid (LA) was purchased in the form of a yellow powder from Sigma chemical company (St Louis, Missouri, USA) and was suspended in sterile normal saline, before use.

Experimental animals

Male Wistar albino rats, each weighing 180 ± 20 g, were obtained from animal house in Medical Research Center (MRC), Faculty of Medicine, Ain Shams University. The animals were acclimatized to the laboratory conditions for a period of 14 days. They were maintained at an ambient temperature of 25 ± 3°C, 50 ± 20% relative humidity and 12/12 h of light–dark cycle and were given a standard rat feed and water and libitum. All experimental procedures were conducted according to the ethical standards approved by the Institutional Animal Ethics Committee guidelines for animal care and use, Ain Shams University, Cairo, Egypt.

Experimental protocol

The rats were randomly divided into four groups, each of eight rats as follows:

Group I (Control): received saline (2 ml/kg b. w.) and olive oil (2 ml/kg b. w.) orally for 21 days.

Group II (CsA-treated group): was treated orally by gastric gavage with CsA (25 mg/kg b.w.), daily for 21 days.

Group III (Recovery CsA-treated group): was treated orally by gastric gavage with CsA (25 mg/kg b.w.), daily for 21 days and recover for other 21 days.

Group IV (LA and CsA-treated group): received LA (100 mg/kg b. w.) orally [8], 1 h before treatment by CsA (25 mg/kg b. w.) daily and concurrently for 21 days.

At the end of the experimental period, the animal groups were sacrificed after 24 hr. of the last dose of different administrations and their blood were collected, by carotid bleeding, in centrifuge tubes and serum was obtained from the blood after centrifugation at 3000 rpm for 10 min. The liver tissue was immediately excised, cleared of adhering connective tissue, rinsed in physiological saline, weighed and stored at -20°C until analysis studies.

Methods of analysis

Determination of glucose, total proteins, albumin and globulin were estimated depending on the assays depicted by [9- 11] respectively.

AST and ALT were measured colorimetrically according to [12] and GGT by the method of [13].

Hepatic glutathione (GSH) was spectrophotometrically assayed in the by the method of **[14].** The activity of hepatic SOD was determined by

assessing the inhibition of pyrogallol autooxidation [15]. Malondialdehyde (MDA) was determined in liver by using the method of [16].

Statistical analysis

Statistical analyses of the resulted data were done using InStat version 2.0 (Graph Pad, ISI, Philadelphia, PA, USA, 1993) computer software. The results were expressed as means ±SE). Multiple comparisons were done using one-way ANOVA followed by Tukey-Kramer as a post-ANOVA test. Statistical significance was accepted at $P< 0.001$, $P< 0.01$, $P< 0.05$.

Results

CsA administration significantly increased in serum glucose concentrations ($P<0.001$) in the CsA treated group as compared to the control group I. While, CsA exerts a significant ($P<0.001$) decrease in serum total protein, albumin and globulin contents as compared to the control group I (Table 1). A marked elevation in serum ALT, AST and GGT ($P<0.001$) where, these concentrations tended to increase highly significant compared to the values in the control group I (Table 2). thus, CsA-induced increase in the liver function injury.

The present data showed an elevation in the level of hepatic MDA significantly ($P<0.001$), while a significant ($P<0.001$) reduction in hepatic GSH content and SOD activity was observed in CsA treated group as compared to the control group I (Table 3).

On the other hand, treatment with LA plus CsA a highly significant decrease was observed in glucose concentrations ($P<0.001$) in the LA plus CsA treated groupIV as compared to the CsA group II. A highly significant improvement was achieved in serum total protein, albumin and globulin contents in the LA plus CsA treated group IV as compared to the CsA group II (Table 1).

The present study showed that treatment with LA plus CsA significantly ($P<0.001$) inhibited the liver enzymes ALT, AST and GGT in the LA plus CsA treated group IV as compared to the CsA group II. Hepatic MDA was restored significantly ($P<0.001$), also hepatic GSH content and SOD activity was attenuated in the LA plus CsA treated group IV as compared to the CsA group II (Table 3).

Table (1): The effect of LA on CsA - induced changes on serum glucose, total proteins, albumin and globulin contents.

Groups	Parameters			
	Glucose mg/dl	**TP g/dl**	**Alb g/dl**	**Glob g/dl**
G I (Control)	112.07 ± 0.570	5.44 ± 0.009	1.91 ± 0.034	3.75 ± 0.141
G II: (CsA)	292.22 ± 0.388 a**	3.37± 0.094 a**	1.15± 0.046 a**	1.60± 0.099 a**
G III: (Recov.)	156.16 ± 1.214 ab**	4.63 ± 0..161 ab**	1.63 ± 0.046 a*b**	2.77 ± 0.057 ab**
GIV (LA and CsA)	120.53 ± 0.551 a*b**	5.09 ± 0..063 b*	1.66 ± 0.038 b**	3.11 ± 0.079 a*b**

Data are expressed as mean ± S.E. (n = 6 in each group).
a: Significant change at p< 0.05 with respect to control group I.
b: Significant change at p< 0.05 with respect to group II.
*Highly significant change at p < 0.01.
**Very highly significant change at p < 0.001.
N.S: Non significant change.

Table (2): The effect of LA on CsA - induced changes on serum liver enzymes.

Groups	Parameters		
	ALT U/ml	**AST U/ml**	**GGT U/ml**
G I (Control)	12.38 ± 0.092	19.52 ± 0.130	10.46 ± 0.095
G II: (CsA)	26.97 ± 0.097 a**	67.79 ± 0.166 a**	21.33 ± 0.108 a**
G III: (Recov.)	24.73 ± 0.243 ab**	48.79 ± 0.217 ab**	16.54 ± 0.103 ab**
GIV (LA and CsA)	15.61 ± 0.131 ab**	27.33 ± 0.149 ab**	14.89 ± 0.065 ab**

Data are expressed as mean ± S.E. (n = 6 in each group).
a: Significant change at p< 0.05 with respect to control group I.
b: Significant change at p< 0.05 with respect to group II.
*Highly significant change at p < 0.01.
**Very highly significant change at p < 0.001.
N.S: Non significant change.

Table (3): The effect of LA on CsA - induced changes on hepatic GSH, SOD and MDA levels.

Groups	Parameters		
	Hepatic		
	MDA U/g wet tissue	**GSH U/g wet tissue**	**SOD U/g wet tissue**
G I (Control)	30.34 ± 0.071	28.45 ± 0.115	81.31 ± 0.083
G II: (CsA)	56.20 ± 0.049 a**	56.14 ± 0.044 a**	63.28 ± 0.057 a**
G III: (Recov.)	30.32 ± 0.062	28.41± 0.093	81.38 ± 0.0859
GIV (LA and CsA)	38.28 ± 0.058 ab**	76.39 ± 0.076 ab**	40.20 ± 0.049 ab**

Data are expressed as mean ± S.E. (n = 6 in each group).
a: Significant change at p< 0.05 with respect to control group I.
b: Significant change at p< 0.05 with respect to group II.
*Highly significant change at p < 0.01.
**Very highly significant change at p < 0.001.
N.S: Non significant change.

Discussion:

Deciphering new biological pathways that contribute to CsA hepatotoxicity is of great importance because they may lead to the development of early biomarkers of liver injury.

CsA treatment to control rats resulted in significant increase in serum glucose concentration compared to control (group I). These results are in agreement with those recorded by [17 and 18] who reported that CsA is believed to have a direct toxic effect on pancreatic beta cells, whereas a reversible suppression of insulin release has also been documented. Other studies have also demonstrated that greater cyclosporine dosages and trough levels were associated with higher insulin values and indices of Insulin Resistance (IR). CsA induced an endoplasmic reticulum (ER) stress in human tubular cells lead to its dysfunction which may be induced by calcium disturbances, hypoxia, ATP and glucose deprivation or chemicals caused kidney injuries such as ischemia-reperfusion, chronic ischemia or calcineurin-inhibitors nephrotoxicity [19].

Also, a significantly decreased serum total protein, albumin and globulin contents with CsA treated rats (group II) when compared with control (group I). These results are in agreement with [20] who found that CsA

is hepatotoxic including inhibition of hepatic protein synthesis. The protein depression may be due to loss of protein by reduced protein synthesis and increased proteolytic activity or degradation. In this connection [21] reported that the CsA mechanism may be associated with hyperlipidemia in humans in vivo. Human hepatoma cells indicated that CsA increases hepatic lipoprotein production and reduces lipoprotein clearance, generation of reactive oxygen species, nitric oxide, lipid peroxidation, mitochondrial dysfunction and induction of apoptosis [22]. Also, [23] reported that significant proteinuria was seen in the cisplatin-treated rats. This is further supported by the lower serum albumin levels in the treated rats. On the other hand, CsA is metabolized to a number of first and second generation metabolites by the P-450 mixed function oxidase group of enzymes. These products have long been thought responsible for its toxic effects. The similarity between the location of toxicity and the cytochrome P-450 system supports the link between toxicity and metabolism, the ability of CsA to produce free radicals has also been linked to its metabolism [24]. Cytochrome P-450 reductase transfers electrons to molecular oxygen, which in turn is cleaved into oxygen radicals. The main radical produced by P-450 activity is superoxide with smaller amounts of hydrogen peroxide and hydroxyl. Other speculative sources of CsA-driven ODFR production in the kidney are NADPH oxidase in mesangial cells and uncoupling of the mitochondrial electron transport chain [25].

In this study, CsA induced a marked elevation in serum ALT, AST and GGT. These results agree with [26]. who observed impaired liver function in a number of renal, cardiac, or bone marrow transplant recipients receiving CsA, some instances there was a transient increase in hepatic aminotransferase and bilirubin levels with CsA. It was also observed an increase in different enzymatic activities (alkaline phosphatase, lactate dehydrogenase, NADPH diaphorase) and provided good evidence that the oxidative stress was part of the mechanism of CsA-mediated toxicity in liver cells [27]. The elevated serum enzymatic activity generally reflects cell damage, because these enzymes are released into the circulatory fluid when cell membrane integrity is damaged as a result of toxaemia [28]. It is known that hepatotoxicity usually occurs post-transplantation. In addition, a part of patients had hepatotoxicity in the immediate post-transplant period during intravenous CsA administration [29].

In this study CsA rats (group II) showed a significant increase in the levels of hepatic MDA with excess production of hydrogen peroxide in living cells, accompanied with a significant decrease in GSH and SOD that leads to decline in the activity of the antioxidant enzymes depletion of both the GSH and protein thiols. Similar biochemical changes were previously reported in other studies [28 and 29] which then give rise to increased hydroxyl radical formation. The effects of reactive oxygen species (ROS) on cellular and

extracellular components of organisms have been investigated extensively in recent years. CsA promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxides. These ROS enhance the peroxides and reactive hydroxyl radicals. These lipid peroxides and hydroxyl radical may cause cell membrane damage and thus destroy the cell. It also inhibits the activities of free radical quenching enzymes such as catalase, superoxide dismutase and glutathione peroxidase. CsA to produce ROS was observed in the present study by increased amount of renal and hepatic lipid peroxides (LPO). The intracellular generation of hydrogen peroxides (H_2O_2) could be involved in the initiation of CsA toxicity in rat, caused cell membrane damage like lipid peroxidation which leads to the imbalance between synthesis and degradation of enzyme protein. The excess production of ROS may be due to its ability to produce alteration in mitochondria by blocking the permeability transition pore. Reactive oxygen metabolites are generated by a specialized phagocytic cells (neutrophils) as cytotoxic agents to fight invading micro-organism, a process known as the respiratory or oxidative burst. Therefore, phagocytes use the membrane bound NADPH oxidase complex which catalyzes one electron, reduction of O_2 into O^{-2}. The ROMs are generated in biological system via several enzymatic and non-enzymatic pathways [30 and 31].

On the other hand, the present results illustrated that the antioxidant LA administration had an ameliorating effect on the changes of the biochemical parameters associated with CsA challenge. This effect was indicated by improvement of serum glucose concentration. This protection was manifested as significant reduction in serum levels of urea and amelioration of apoptotic markers [32]. Voltage-dependent anion channels (VDAC), known as mitochondrial porins, are membrane proteins encoded by nuclear gene and synthesized in ribosome. VDAC plays crucial roles in the physiological and pathologic process, including energy metabolism and cell apoptosis. VDAC was actually more sensitive to oxidative stress-induced cell death [33]. In addition, free radical scavengers may also be helpful in prolonging survival time of dopaminergic neurons [34]. In this respect, LA could attenuate neuronal damage and loss through counteracting oxidative stress, possibly via regulating antioxidant defense system as well as inhibition of free radical generation [35]. LA and its reduced form dihydrolipoic acid are present in all prokaryotic and eukaryotic cells and considered a vitamin, can be synthesized in human cells. LA is involved in the regulation of carbohydrate and lipid metabolism by converting blood glucose into energy [36], improves glycemic control.

Also, a significant enhanced in total proteins and albumin levels and that may be attributed to CsA antioxidant property. Also, LA plus CsA administration helps to normalize the activities of ALT, AST and GGT.

Coincident with these findings [37 and 38] showed that administration of LA produced a good protective capability against hepatic injury. LA altered expression of hepatic clock genes and level of enzymes associated with lipid metabolism in old rats [39]. LA may function to entrain circadian cycles and therefore limit down-stream pathophysiologies associated with hepatic clock dysfunction. LA has beneficial effects on age-related dyslipidemia via the hepatic clock in old rats, examine the rhythmic nature of the circadian clock with age and how LA supplementation may affect hepatic clock function and when clock-associated lipid metabolism proteins should be increasing [41]. Moreover, [40] reported that pretreatment of α-LA markedly reduced the serum ALT and AST activity, decreased the tissue myeloperoxidase (MPO) activity and lipid peroxidation, but increased SOD and GSH levels. LA alone can inhibit tumor cell proliferation and clone formation with time dependence [28], and inhibited radiation-induced fibrosis and the expression of pro-fibrotic genes via inhibition of p65 acetylation. In particular, p65 acetylation correlates with the expression of pro-inflammatory and pro-fibrotic genes during fibrosis, inflammation [42].

In the present study, administration of LA prior to CsA treatment markedly ameliorated LPO in the rat liver as manifested by decreased MDA level accompanied by increased GSH content and SOD activity. In agreement with the present findings [43] mentioned that LA is a naturally occurring cofactor within pyruvate dehydrogenase and α-keto-glutarate dehydrogenase. It also, Free LA has the ability to scavenge superoxide, hydrogen peroxide, hydroxyl radicals, and peroxynitrite, and can also recycle glutathione (GSH), α-tocopherol and ascorbic acid. *In vitro*, α-lipoic acid decreased plasma susceptibility to oxidation [44] was protective against haemolysis of human erythrocytes induced by peroxyl radicals [45] and increased GSH synthesis in isolated human erythrocytes [46]. It also, α-lipoic acid attenuated superoxide generation and kidney expression of NADPH oxidase in diabetic rats, and it was concluded that α-lipoic acid improves pathology in diabetes by reducing oxidative stress [47]. Thus, LA has been shown to reduce oxidative stress both in vivo and vitro studies. Reduction of hepatic GSH, SOD activity in CsA-treated rats was observed in this study, which was similar to the previous studies [48]. LA has proved to possess lipid lowering, anti-lipoperoxidative and antioxidant properties [49]. It has been demonstrated to play an important role in regulating antioxidative capacity by increasing SOD, GSH and catalase activities by upregulating the gene expression of SOD, GSH and catalase [50].

Conclusion:

In conclusion, LA is main active components for immuno-modulating and antioxidant activities, differ greatly in the chemical composition and physical properties, show the same basic multivitamins and protect the

immune cells from oxidative damage. Thus, LA have a potential protective effect against CsA toxicity, improved the hepatic function with decrease the liver tissue damage of CsA-induced hepatotoxicity in rats.

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