Lipoic Acid Attenuates Cholestasis Induced Cerebral Injury in Rats

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Abstract: Cholestasis is characterized by an abnormal accumulation of bile acids, which is caused by defectiveness in the process of bile acid transport. It is believed that oxidative stress is a likely mediator for cholestatic damage and antioxidant therapy is a recommended therapeutic strategy. The aim of this study was to evaluate protective effect o alpha lipoic acid as an anti oxidant agent on cerebral injury after bile act ligation in rats. forty five adult male wistar rats were randomly assigned to three groups each containing fifteen rats as follows: sham operation (SO) (control), bile duct ligation (BDL), and BDL+LA (25mg/kg). After fourteen day's cerebral tissue sampled for pathologic and biochemical studies. Levels of SOD and GPx antioxidant enzymes were higher in BDL+LA group comparing to BDL group significantly, histologic damage and MDA levels were higher in BDL group comparing to BDL+LA group significantly ($P<0.05$). In our study LA treatment in BDL rats improved cellular SOD and GPx levels and reduced MDA levels in BDL+LA group comparing to BDL group. The findings of our present study showed that LA, with its potent free radical scavenging and antioxidant properties, seems to be a highly promising agent in protecting cerebral tissue against oxidative damage.
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1. Introduction

Cholestasis is characterized by an abnormal accumulation of bile acids, which is caused by defectiveness in the process of bile acid transport. Bile acids are the major products of cholesterol metabolism in the liver, and act as physiological detergents that facilitate absorption, transport, disruption of lipid-soluble fats and vitamins; furthermore it also aids in the excretion of lipids. Retention and accumulation of toxic, hydrophilic bile salts stimulates the production of proinflammatory cytokines and enhances apoptosis which leads to tissue damage (Trauner et al., 1998; Miyoshi et al., 1999), Apoptosis is an integral part of many biological processes, including embryonic development, metamorphosis, hormone-dependent atrophy, and in chemical-induced cell death (Allen et al., 1997; Patel et al., 1994). Hepatic encephalopathy is a well described clinical entity associated with obstructive jaundice and liver failure. The pathophysiological cascade responsible for central nervous system dysfunction under conditions of hepatopathy is not fully elucidated. It is considered to implicate many factors, ranging from ammonia and manganese neurotoxicity (Seyan et al., 2010) to inflammatory cytokines (Seyan et al., 2010) and oxidative stress acting both independently and synergistically. Many cirrhotic patients, up to 50% to 70%, develop hepatic encephalopathy (Quero JC et al., 1996). A neuropsychiatric syndrome characterized by alterations of intellectual function, personality, consciousness and motor coordination (Erceg et al., 2004).

The bile acid concentrations increase in rats after BDL and induce lipid peroxidation, which is probably related to the stimulation of phagocytic activity in polymorphonuclear phagocytes and inflammatory cells (Tomioka et al., 2000; Rivera-Mancía et al., 2009). Therefore, it is believed that oxidative stress is a likely mediator for cholestatic damage and antioxidant therapy is a recommended therapeutic strategy. Alpha-lipoic acid (LA) or thioctic acid (chemical name: 1,2 dithiolane-3-valeric acid or 6,8-dithio-octanoic acid) is a natural dithiol compound which is known as a co-factor in the a-ketoacid dehydrogenase mitochondrial complex and for its complex antioxidant properties (Moini et al., 2002; Biewenga et al., 1997; Bilska et al., 2005). Initially, α-lipoic (LA) was obtained from livers and it has been found naturally in many plants and

animals (Reed et al., 1951). It is absorbed from the diet, biological membranes, and is then taken up by cells and tissues (Packer et al., 1996). LA is easily absorbed and converted into the reduced form of dihydrolipoic acid in a variety of cellular tissues (Packer et al., 1998). Both act as an antioxidant in different environments and mutually form a redox couple.Alpha-lipoic acid, which has been shown to be effective in both the somatic and the autonomic neuropathies in diabetes, normalizes the endoneural bloodflow (Nagamatsu et al., 1995), reduces oxidative stress (Low et al., 1997; Nickander et al., 1996), and improves vascular dysfunction (Morcos et al., 2001; Xie et al., 2012; Vasdev et al., 2011). The aim of this study was to evaluate protective effect o alpha lipoic acid as an anti oxidant agent on cerebral injury after bile act ligation in rats.

2. Materials and methods

2.1. Animals

Male wistar rats were obtained from laboratory animals care center of Tabriz University of Medical Sciences (Tabriz, Iran). They were allowed free access to a commercial standard diet and water ad libitum. Rats were randomly assigned to three groups, each containing fifteen rats as follows: sham operation, (control), BDL, and BDL+LA. Sham-operated rats served as controls. Except in this group, biliary canals were ligated. Rats were fasted for 12 h before the operation, but were given water.

2.2. Surgery protocol

The animals were anesthetized by intramuscular injection of 50 mg/kg ketamine hydrochloride and 10 mg/kg xylasine. Midline laparotomy was performed under sterile conditions. In sham group, the common bile duct (CBD) was freed from the surrounding soft tissue, and was manipulated without ligation and transaction. In BDL and BDL+LA groups, the CBDs of the rats were identified, double ligated with 5-0 silk, and divided between the ligatures. BDL+LA group was administered by LA 25mg/kg subcutaneously for 14 days (Mythili et al., 2007). The animals were sacrificed on 14th postoperative day with high-dose diethyl ether inhalation. Subsequently, the cerebral tissue was obtained.

2.3. Light Microscopy Analyses

After decapitation, the left hemispheres of the brains were stored in the 10% formaldehyde overnight at 4°C. The samples were then fixed in a 10% buffered formalin solution for 7 days. The left hippocampal regions were obtained from coronal sections of the frontal planes. Formaline-fixed, paraffin-embedded sections (4-µm thickness) were stained with hematoxylin eosin and cresyl violet. Intact hippocampal CA1 pyramidal neurons were semiquantitatively counted in three consecutive 789 μm^2 areas outlined with a counting Gundersen's frame (Gundersen et al., 1988) under 40x magnifications, and in three consecutive hippocampus sections. The histologist was blinded to the animal groups, and the procedure was conducted in a blinded fashion (Onem et al., 2006).

2.4. Assay of antioxidant enzymes

The hippocampus was excised and frozen in liquid nitrogen and stored at -80°C until further preparation. In order to measure anti-oxidant enzyme activity, the samples were homogenized in 1.15% KCL solution. Superoxide dismutase (SOD) activity in tissue was determined by using xanthine and xanthine oxidase to generate superoxide radicals which then react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-henyltetrazolium chloride to form a red formazan dye. The SOD activity was then measured by the degree of inhibition of this reaction (Ransod, Randox Laboratories Ltd. United Kingdom). Results obtained as SOD Unit/mg protein (Paoletti et al., 1986).

Glutathione peroxidase (GPx) activity was measured using the method described by Paglia and Valentine. GPx catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidised glutathione is immediately is converted to reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured (Ransod, Randox Laboratories Ltd. United Kingdom). Results obtained as GPx Unit/mg protein (Paglia et al., 1967).

2.5. Tissue MDA level

Tissue malondialdehyde was determined by the method of Uchiyama and Mihara (Mihara et al., 1983) 3-mL aliquot of 1% phosphoric acid and 1 mL of 0.6% thiobarbituric acid solution were added to 0.5 mL of 10% tissue homogenate. The mixture was heated in boiling water for 45 minutes. After cooling, the color was extracted into 4 mL of n-butanol. The absorbance was measured in a spectrophotometer at 532 nm ($\acute{\varepsilon} = .56 \times 10^5$ mol/L^{-1} cm^{-1}). The amounts of lipid peroxides calculated as thiobarbituric acid reactive substances of lipid peroxidation were expressed as nMol/ml (Kirimlioglu et al., 2008).

2.6. Statistical analysis

Data were expressed as means ± SD. Differences among various groups were tested for statistical significance using the one-way ANOVA test and Tukeys post test. A P value of less than 0.05

denoted the presence of a statistically significant difference.

3. Results

3.1. SOD and GPx level

Levels of SOD and GPx antioxidant enzymes were decreased in hippocampus of the groups subjected to bile duct ligation, but it was less severe in LA treated group. SOD and GPx levels in BDL+LA group were higher than BDL group significantly (P<0.05, Table 1).

Table1: Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and Malondialdehyde (MDA) levels in hippocampus tissue of rats after bile duct ligation

	MDA **(nMol/ml)**	GPx **(Unit/mg protein)**	SOD **(Unit/mg protein)**
Sham	0.60±0.31	2.86±0.28	2.66±0.19
BDL	1.88±0.27	1.88±0.22	1.63±0.12
BDL+LA	1.07±0.27	2.46±0.32	2.29±0.23

Note. The values are shown as a mean ±SD for rats in each group and difference of (P<0.05) considered significant.

3.2. MDA level

MDA level as an index of lipid peroxidation increased significantly in hippocampus tissue after bile duct ligation. MDA level was lower in BDL+LA group comparing to BDL group significantly (P<0.05) and it was lower in sham group comparing to BDL+LA group significantly (P<0.05, Table 1).

3.3. Histopathology

Studying the histologic samples by light microscopy showed that, the pyramidal neurons in the subfield of the hippocampus were completely normal in appearance in sham group. Number of neurons were higher in the CA1 subfield in the BDL+LA group comparing to BDL group significantly (P <0.05, Table 2).

Table 2: Number of surviving CA1 cells in hippocampus of rats

	Sham	BDL	BDL+LA
CA 1 cells number	198.50±12.44	167.90±10.40	180.70±10.37

Note.Number of CA1 neuron were higher in BDL+LA group than BDL group significantly (P<0.05)

4. Discussion

Cholestasis is encountered in a variety of clinical disorders. It is the main feature of a number of chronic progressive liver diseases, including primary biliary cirrhosis, primary sclerosing cholangitis, allograft rejection, iatrogenic obstruction of bile ducts, and biliary atresia. Cholestasis is now recognized as a disorder characterized by liver oxidants overload (Huang et al., 2003; Portincasa et al., 2007; Sastre et al., 2007). Furthermore, the oxidative stress in cholestatic liver disease is a systemic phenomenon (Ljubuncic et al., 2000; Assimakopoulos et al., 2006), probably encompassing all tissues and organs, even those separated by the blood-brain barrier (Chroni et al., 2006).Similarly, oxidative stress plays an important role in the pathogenesis of toxic tissue injury (Feher et al., 1998).

To reduce the detrimental effects of ROS, besides diminishing its production, organisms have developed their own antioxidant mechanisms including low-molecular-weight antioxidant molecules, i.e., glutathione, melatonin and various antioxidant enzymes, such as SOD and GPx and glutathione reductase. These enzymes activities are higher in the liver than in other tissues (Yuan et al., 2005). Superoxide dismutase (SOD), an oxygen radical scavenger, which converts the superoxide anion radical present in the upper stream of reactive oxygen metabolism cascade, will afford protection from cell damage (Minor et al., 1993). SOD catalyses the dismutation of the superoxide anion (O2-) into H2O2; GSH-Px is a selenoprotein, which reduces lipidic or nonlipidic hydroperoxides as well as H2O2 while oxidizing GSH (Michiels et al., 1994). In our study, we found that cholestasis impaired these enzymes activities, as indicated by the markedly lower activities compared with sham group. LA administration maintaianed the activities of these enzymes significantly comparing to control group (P<0.05). LA is an antioxidant substance that can react at many levels: (1) it neutralizes free radicals formed by direct radical scavenging (hydroxyl radical: HO•), hypochlorous acid and singlet oxygen), (2) it regenerates endogenous antioxidants (GSH, vitamin C, and vitamin E) from their oxidized forms, and (3) it complexes transitional metals (especially iron and copper which are involved in HO• synthesis) (Cakatay et al., 2006). Concerning the clinical ways, dietary antioxidants have attracted attention as preventive and therapeutic agents (Dhalla et al., 2000; Buonocore et al., 2007; Marchioli et al., 1999).

MDA is a secondary product of oxidative stress formed during lipid peroxidation and it is released as a result of the toxic effect of reactive oxygen species in rats after bile duct ligation (Orellana et al., 2000). Increased concentrations of MDA reflect the level of lipid peroxidation in tissues and it is considered as a marker of tissue injury (Draper et al., 1990). There

are several reports indicating that levels of MDA increases after bile duct ligation in rats (Canturk et al., 1998; Karaman et al., 2003). Our results are in agreement with previous works reporting high levels of MDA. In the present study, levels of MDA in the LA-treated rats were significantly lower than in the BDL group. Although tissue MDA levels were clearly decreased by LA, its exact mechanism is not known. Reductions in MDA levels in the LA-treated rats may be due to its antioxidant and free-radical scavenging effect. By protecting cell membranes, LA probably reduces the deleterious effects of oxidative stress in living cells (Shaafi et al., 2011; Ying et al., 2010).

Huang et al., (2009) reported that, melatonin treatment decreased liver and systemic oxidative stress, increased liver antioxidant activity, and improved spatial memory in developing rat with BDL-induced cholestasis. They showed that BDL-induced cholestasis in developing rats had worse spatial memory and increased liver and systemic oxidative stress as compared with jaundice-free rats; 2) melatonin treatment, in a dose-dependent manner, decreased liver and systemic oxidative stress, increased liver antioxidant activity, and improved spatial memory in developing rat with BDL-induced cholestasis. The underlying mechanisms of increased systemic oxidative stress during cholestasis may be due to the retention of toxic bile acids, which stimulate the generation of reactive oxygen species (ROS) in hepatocytes and live mitochondria (Sastre et al., 2007), and consequently hepatocellular necrosis and apoptosis. The increased ROS may cause target organs damage (e.g., brain, heart, and kidney) via systemic circulation (Ljubuncic et al., 2000; Tokaç et al., 2013; Liu et al., 2012). Huang et al., (2010) reported that cholestatic rat had a poorer performance in acquisition memory when compared with jaundiced-free rat. Ammonia exerts a deleterious effect on cerebral function and is considered to play an important role in the pathogenesis of hepatic encephalopathy (Lockwood et al., 2004). In this regard, Jover et al., (2006) added hyperammonia diet in BDL rat to simulate hepatic encephalopathy that occurs in humans. They suggest that, systemic oxidative stress, instead of ammonia, plays a role in the cognitive deficit in young rat with BDL-induced cholestasis. ROS are involved in several diseases, including ischemic injury, Alzheimer's disease, Parkinson's disease, and Down's syndrome all of which affect cognitive processes (Sayre et al., 2001; Perry et al., 2002; Butterfield et al., 2007). ROS can cause disruption of calcium homeostasis, membrane damage, and cell death (Keller et al., 1998), and has a detrimental effect on several key enzymes involved in glutamate and glucose transport (Keller et al., 1998; Lauderback et al., 2001); all of the above-mentioned biologic effects can result in cognitive deficit. Although, we could not detect either MDA or GSH/GSSG differences in brain cortex or hippocampus, we postulate that other players in the oxidants/antioxidants system might play a role. Alternatively, other brain regions that are involved in spatial memory might account for the cholestasis induced spatial dysfunction in our rats. Generation of ROS contributes to endothelial and cellular dysfunction, resulting in increased BBB permeability and cerebral edema. Besides the breakdown of BBB barrier, the failure of the Na/K pumps, and the altered electrolyte balance of the cell, may also contribute to brain edema and pathological changes in the cellular function (De Vries et al., 1997). With respect to brain pathology, in experimental animals, histologic changes consisting of atrophy, pyknosis, and neuronophagia were observed after 7 days of obstructive jaundice at the basal ganglia, putamen, and red nucleus, whereas after 13 days these changes were spread to the thalamus substantia nigra and cortex of the canine brain (Furukawa et al., 1991). The current study revealed that markers of oxidative stress, which could eventually lead to structural damage, were present as early as 5 days after the bile duct ligation. In our study 14 days after BDL, number of neurons in hippocampus was reduced significantly, while treatment of LA attenuated the reduction of neurons in hippocampus comparing to control group significantly. It has been proposed that antioxidant therapy may be useful for preventing the deleterious effect of oxidative stress on the nervous system during BDL (Chroni et al., 2006).

5. Conclusion

In our study LA treatment in BDL rats improved cellular SOD and GPx levels and reduced MDA levels in BDL+LA group comparing to BDL group. The findings of our present study demonstrate that LA, with its potent free radical scavenging and antioxidant properties, seems to be a highly promising agent in protecting cerebral tissue against oxidative damage.

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