Anti-oxidative effect of Lipoic Acid on Cholestasis Induced Crebellum Toxicity

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Abstract: Cholestasis is a common pathophysiological process in many human diseases leading to the accumulation of toxic bile salts within the liver. Accumulation of bile acids may cause injury in different organs through oxidative pathway. Lipoic acid could be a potential therapeutic agent in the controlling different disorders that an imbalance of the cellular oxidoreductive status. The aim of this study was to evaluate anti oxidative effect of LA on cerebellar tissue after bile duct 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 cerebellar 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 ($P<0.05$). 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 cerebellar tissue against oxidative damage.
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1. Introduction

Cholestasis, extrahepatic or intrahepatic, is a common pathophysiological process in many human diseases leading to the accumulation of toxic bile salts within the liver (Prado et al., 2003). It seems likely that the detergent action of bile salts is responsible for solubilization of plasma membranes and cell death, which in turn may lead to oxidative stress, oxidation of reduced glutathione (GSH), and lipid peroxidation (Baron et al., 1999). There is growing evidence suggesting that considerable impairment of oxidative stress regulation may play an important role in cholestatic tissue injury (Montilla et al., 2001; Ohta et al., 2003). Oxidative stress occurred when the physiological balance between radical-generating and radical-scavenging is disrupted in favor of the former. As a result, the production of certain oxidation products is increased. The attack of oxygen free radicals on cellular lipids results in the formation of aldehydic lipid hydroperoxide decomposition products like malondialdehyde (MDA), which is traditionally used as a reliable marker of lipid peroxidation (Ljubuncic et al., 2006). Tissue's antioxidant capacity can also be assessed by measurement of thiol redox state (TRS), which is affected by the levels of many other thiol or disulfide components and an estimation of them would give a more representative profile of the thiol antioxidant reserves. Specifically, the quantification of the low oxidative stress markers, i.e., reduced glutathione (GSH), cysteine (CSH), and protein thiols (PSH) reflects the effective sum of all cooperative chain-breaking thiol antioxidants (Chroni et al., 2006). Therefore, it is believed that oxidative stress is a likely mediator for cholestatic damage and antioxidant therapy is a recommended therapeutic strategy. Lipoic acid (LA) has been known as a potent antioxidant, of which antioxidant effects are attributed to direct radical scavenging and metal chelation. Humans obtain LA from their diet and via de novo mitochondrial synthesis (Biewenga et al., 1997; Bilska et al., 2005). Exogenous LA is quickly absorbed, transported to the intracellular compartment, and reduced to dihydrolipoic acid (DHLA) under the action of certain enzymes: mitochondrial dihydrolipoyl dehydrogenase, cytosolic glutathione reductase, and thioredoxin reductase (Jones et al., 2002). The reduction process results in two free thiol groups, which are responsible for the superior antioxidant effect of the reduced form (DHLA) as compared to the oxidized form (LA). Besides acting as a potent antioxidant, LA

increases and maintains levels of antioxidants such as ubiquinone, glutathione, and ascorbic acid (Han et al., 1995; Roy et al., 1998). Furthermore, LA could be a potential therapeutic agent in the controlling different disorders that an imbalance of the cellular oxidoreductive status takes role (Ashour et al., 2011; Moreira et al., 2007). The aim of this study was to evaluate anti oxidative effect of LA on cerebellar tissue after bile duct 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 cerebellar tissue was obtained.

2.3. Assay of antioxidant enzymes

The cerebellar tissue was 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 liver tissue was determined by using xanthine and xanthine oxidase to generate superoxide radicals which then react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium 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 in cerebellar tissue 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).

2.4. Tissue MDA level

Tissues 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).

2.5. 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 cerebellar tissue 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).

3.2. MDA level

MDA level as an index of lipid peroxidation increased significantly in cerebellar 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).

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

	GPx (U/mg protein)	SOD(U/mg protein)	MDA(nMol/ml)
Sham	2.79±0.15	2.65±0.19	0.55±0.27
BDL	1.92±0.28	1.80±0.17	1.73±0.32
BDL+LA	2.49±0.19	2.06±0.26	1.07±0.26

Levels of SOD and GPx antioxidant enzymes were decreased in cerebellum in both of the groups subjected to bile duct ligation, but it was less severe in LA treated group. MDA level was lower in BDL+LA group comparing to BDL group significantly ($P<0.05$).

4. Discussion

It is known that increased concentration of bile acids induce lipid peroxides, probably related to the stimulation of phagocytic activity in the polymorphonuclear leukocytes and inflammatory cells, which are present after biliary tract obstruction and enhance the tissue injury (Montilla et al., 2001; Toklu et al., 2007). Several clinical and experimental studies have shown that oxygen free radicals have a role in the pathogenesis of tissue injury in obstructive jaundice (Peres et al., 2000). Free radicals have also been related to the underlying mechanism involved in hepatic encephalopathy (Norenberg et al., 2004) as well as in the neuropsychiatry syndrome in cirrhosis and acute liver failure (Cruz et al., 2001). It has been suggested that ammonium enhances free-radical generation and reduces the concentration of GSH and antioxidant enzymatic activity (Norenberg et al., 2004). The induction of oxidative stress in BDL rats is considered a systemic derangement that affects different tissues including liver, kidney, heart, and brain (Ljubuncic et al., 2000). Reactive oxygen species (ROS), namely superoxide and hydroxyl free radicals, together with hydrogen peroxide, are believed to be directly toxic, and ROS can initiate free radical- mediated chain reactions. ROS damage the building structures of the cell membrane, nucleus, and genetic material by causing scission, carbonylation, fragmentation, cross-linking, and oxidation. These structural changes lead to the decrease or loss of protein biological function (Ghoneim et al., 2002; Cakatay et al., 2008). Highly reactive ROS directly attacks lipids, proteins in the biological membranes and cause their dysfunction. Degradation of polyunsaturated fatty acids in cell membranes by ROS results in the destruction of membranes and formation of MDA, which is an indicator of ROS generation (Nita et al., 2001). The compound 8-hydroxy-2′-deoxyguanosine (8-OHdG) is an oxidant of deoxyguanosine and a marker for oxidative DNA damage.9 Oxidant and antioxidant statuses are vital for regulation of homeostasis. Glutathione (GSH) and superoxide dismutase (SOD) are involved in the antioxidant system and are important for the protection of tissue from oxidative damage. GSH is a tripeptide. Its oxidized form, the dimer GSSG, which is involved in the transport of certain amino acids, is a coenzyme for various enzymes and protects against oxygen radicals and toxic compounds. GSH removes toxic substances from the environment and protects tissue from harmful substances after biotransformation. SOD, which catalyzes the dismutation of superoxide to hydrogen peroxide, catalyzes the conversion of two O2 molecules into H2O2 and O2. SOD exists in mitochondrial (Mn-SOD) and cytoplasmic (Cu/Zn-SOD) forms (Nita et al., 2001). Oxidative stress is also involved in the regulation of almost all cellular processes, including proliferation, differentiation, stress responses, and cell death (Barber et al., 1994). In our study pretreatment with LA attenuated cerebellum toxicity through maintaining cellular GPx and SOD content and decreased MDA levels.

5. Conclusion

The results of the present study show that after 2 week BDL is associated with intense oxidative stress in cerebellum. BDL increased MDA content and reduced GPX and SOD content in the cerebellum.

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