

Lipoic acid prevents hepatic and intestinal damage induced by obstruction of the common bile duct in rats

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Abstract. – INTRODUCTION: Cholestatic liver diseases are characterized by impaired hepatocellular secretion of bile, resulting in intracellular accumulation of bile acids which result in a shift in the oxidant/prooxidant balance in favor of increased free radical activity and injury of different tissues including liver and intestine. The aim of this research was to study protective effect of lipoic acid (LA) as a potent antioxidant in cholestasis induced hepatic and intestinal injury in rats.

MATERIALS AND METHODS: Forty five adult male Wistar rats were randomly assigned to four groups each containing fifteen rats as follows: sham operation (SO) (control), bile duct ligating (BDL), and BDL+LA (25 mg/kg). After fourteen days hepatic and intestinal tissue sampled and blood serum sampled for pathologic and biochemical studies.

RESULTS: Levels of SOD and GPx antioxidant enzymes were higher in BDL+LA group comparing to BDL group, levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ -glutamyltranspeptidase (GGT), and pathologic scores in liver and intestine were lower in BDL+LA group comparing to BDL group significantly, but there is no significant difference in concentrations of total bilirubin between groups.

CONCLUSIONS: Our results showed the protective potential of LA with liver and intestine damage. Despite improvements in operative technique and the development of potent, broad-spectrum antibiotics, biliary tract surgery in patients with obstructive jaundice is still associated with high morbidity and mortality rates. In summary, our results show that BDL induced hepatic and intestinal injury were significantly attenuated by LA administration and the administration of LA could effectively diminish this damage.

Key Words:

Cholestasis, Lipoic acid, Liver, Intestine, Rat.

Introduction

Cholestatic liver diseases are characterized by impaired hepatocellular secretion of bile, resulting in intracellular accumulation of bilirubin, bile acids and cholesterol. Bile duct ligation (BDL) causes complete blockage of cholesterol excretion, and it is a feature of many chronic human liver diseases like primary biliary cirrhosis. Hepatocyte damage by toxic bile acids is assumed to represent a key event for progression of cholestatic liver diseases¹. 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. There is growing evidence suggesting that considerable impairment of oxidative stress regulation may play an important role in cholestatic liver injury³⁻⁵.

It is known that BDL results in a shift in the oxidant/prooxidant balance in favor of increased free radical activity⁶. Enhanced production of reactive oxygen intermediates augments lipid peroxidation by disturbing oxidant-antioxidant balance in hepatic mitochondrial fraction.

Previous studies showed that hepatic mitochondria generate reactive oxygen species (ROS) when isolated hepatocytes are exposed to hy-

drophobic bile acids; mitochondrial free radical production may be an important mechanism in cholestatic liver injury⁷. Accumulation of bile acids, lead to oxidative injury and inflammation in hepatocytes⁸. Over production of hydroxyl radicals in blood and liver from rats with obstructive jaundice has been reported⁹.

Liver damage in extra-hepatic cholestasis is not only influenced by local consequences of biliary obstruction but also by significant systemic alterations¹⁰. Bile duct ligation in rats resulted in significant increases in intestinal permeability and bacterial translocation¹⁰. These mechanisms are probably complementary in the development of systemic endotoxaemia and inflammatory response¹⁰. In attempting to limit the oxidative damage, a number of antioxidants have been tested in experimental bile-duct obstruction models⁴⁻⁶.

Alpha-lipoic acid (LA) or thioctic acid (chemical name: 1,2 dithiolane-3-valeric acid or 6,8-dithio-octanoic acid) is found naturally in mitochondria as the coenzyme for pyruvate dehydrogenase and α -ketoglutarate dehydrogenase¹¹. It is effective in reducing free radicals including lipid peroxides in cellular membranes as well as scavenging free radicals at their mitochondrial source¹². Inside cells and tissues, lipoic acid is reduced to dihydrolipoic acid, which is even more potent as an antioxidant¹¹. For many years LA has been used as a pharmacological agent against diabetic polyneuropathy and is known to be without serious side effects¹³. Furthermore, LA is described as a therapeutic agent in a number of conditions related to liver disease, including alcohol-induced damage, mushroom poisoning, metal intoxicification, CCl₄ poisoning, and hyperdynamic circulation in biliary cirrhosis¹⁴⁻¹⁶. The aim of the present study was to assess the potential protective role of LA in oxidative hepatic and intestinal damage in bile duct ligated rats.

Materials and Methods

Animals

Forty five adult male Wistar rats weighing 220-280 g were used. Animals were housed under continuous observation in appropriate cages in a room in which a 12-12 h light-dark cycle was maintained. 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.

Surgery Protocol

The animals were anesthetized by intramuscular injection of 50 mg/kg ketamine hydrochloride and 10 mg/kg xylazine. 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 25 mg/kg subcutaneously for 14 days¹⁷. The animals were sacrificed on 14th postoperative day with high-dose diethyl ether inhalation. Subsequently, their hepatic and intestinal tissues were removed and blood samples were obtained. The blood samples were put in tubes, and then centrifuged at 2000 g for 5 min. Upper clear supernatant (serum) was taken and the activities of alanine aminotransferase (ALT) (units/l), aspartate aminotransferase (AST) (units/l), γ -glutamyltranspeptidase (GGT) (units/l), and the concentrations of total bilirubin (TB) (mg/dl) in plasma were determined by commercial kits (Pars Azmoon, Tehran, Iran) and standard auto-analyser methods (Alcyon 300, Abbott Laboratories, North Chicago, IL, USA).

Morphologic Analysis

The specimens fixed in 10% formalin were embedded in paraffin. Sections of 4 μ m were prepared, stained with hematoxylin and eosin, and then examined by a blinded pathologist under a light microscope. The histopathologic scoring analysis was performed according to previously described methods with modifications. For liver, the assessment was expressed as the sum of the individual score grades from 0 (no findings), 1 (mild), 2 (moderate), to 3 (severe) for each of the following 6 parameters from liver sections: cytoplasmatic color fading, vacuolization, nuclear condensation, nuclear fragmentation, nuclear fading, and erythrocyte stasis. For the intestine, the histology was scored by using the following grading scale: grade 0, normal mucosa; grade 1, development of subepithelial space at the apex of the villous capillary congestion; grade 2, extension of subepithelial space with moderate lifting of the epithelial layer from the lamina propria;

grade 3, massive epithelial lifting down the side of the villi and a few tips may be denuded; grade 4, denuded villi with lamina propria and dilated capillaries exposed; and grade 5, digestion and disintegration of lamina propria, hemorrhage and ulceration^{18,19}.

Assay of Antioxidant Enzymes

The left lobe of liver and intestinal tissue were frozen in liquid nitrogen and stored at -80°C until further preparation. In order to measure antioxidant enzyme activity, the samples were homogenized in 1.15% KCl solution. Superoxide dismutase (SOD) activities 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., Crumlin, UK). Results obtained as SOD Unit/mg protein²⁰.

Glutathione peroxidase (GPx) activity in liver and intestinal tissues 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., Crumlin, UK). Results obtained as GPx Unit/mg protein²¹.

Tissue MDA Level

Tissues malondialdehyde was determined by the method of Uchiyama and Mihara²². 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 (Amersham Pharmacia Biotech UK Ltd., Little Chalfont, Buckinghamshire, UK) at 532 nm ($\epsilon = .56 \times 10^5 \text{ mol/L}^{-1} \text{ cm}^{-1}$). The amounts of lipid peroxides calculated as thiobarbituric acid reactive substances of lipid peroxidation were expressed as nMol/ml²³.

Statistical Analysis

Data were expressed as means $\pm$ 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.

Results

SOD and GPx Level

Levels of SOD and GPx antioxidant enzymes were decreased in hepatic and intestinal tissue in both 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 I).

MDA Level

MDA level as an index of lipid peroxidation increased significantly in hepatic and intestinal tissue in both of the groups subjected to 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 I).

Serum Biochemical Parameters

At fourteen days post BDL, serum ALT, AST and GGT levels were significantly lower in the BDL+LA group, comparing to BDL group ($p < 0.05$). Serum ALT, AST and GGT levels were lower in sham group comparing to BDL+LA

Table I. Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and Malondialdehyde (MDA) levels in hepatic and intestinal tissue of rats after bile duct ligation.

I. MDA	I. SOD	I. GPX	HMDA	H. SOD	H. GPX	
1.08 $\pm$ 0.71	3.14 $\pm$ 0.42	4.10 $\pm$ 0.35	0.88 $\pm$ 0.23	3.33 $\pm$ 0.48	4.24 $\pm$ 0.46	Sham
2.55 $\pm$ 0.48	1.65 $\pm$ 0.29	2.62 $\pm$ 0.34	2.50 $\pm$ 0.43	1.68 $\pm$ 0.27	2.67 $\pm$ 0.39	BDL
1.85 $\pm$ 0.25	2.37 $\pm$ 0.40	3.27 $\pm$ 0.43	1.28 $\pm$ 0.19	2.46 $\pm$ 0.30	3.32 $\pm$ 0.41	BDL+LA

Levels of SOD and GPx antioxidant enzymes were decreased in hepatic and intestinal tissue 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 ($p < 0.05$). H: Hepatic; I: Intestinal.

group significantly ($p < 0.05$, Table II). But there was no significant difference in bilirubin levels between BDL group and BDL+LA group.

Histological Changes

In histopathological evaluation, there were no pathological changes in liver tissue of the sham group. Liver specimens from rats after BDL exhibited focal necrosis and infiltration of leukocyte. LA treatment significantly decreased these pathological changes. Histological tissue damage was milder in the BDL+LA group than that in the BDL group ($p < 0.05$, Table III).

Animals from control group presented no histological alteration in intestinal tissue samples. The intestine specimens of the BDL group showed histopathological alterations. Histopathological intestinal damage scores of the groups are summarized in the Table. Intestinal damage score was significantly higher in BDL group than those of BDL+LA group significantly ($p < 0.05$, Table III).

Discussion

Cholestasis, an impairment in bile formation, occurs in many of human liver diseases. Retention and accumulation of toxic hydrophobic bile salts within hepatocyte may cause hepatocyte toxicity

There are many physiological processes in the human body, which generate reactive oxygen species. ROS are unstable and highly reactive chemical molecules. The univalent reduction of oxygen in mitochondria leads to superoxide radical (O_2^-). Afterward hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^\cdot$) are produced. Other types of free radicals are nitric oxide radical ($NO^\cdot$), peroxynitrite ($ONOO^-$), and lipid peroxy radical ($LOO^\cdot$). When production of oxidants overwhelms the cellular antioxidant capacity, the state of oxidative stress occurs, resulting in molecular and cellular tissue damage and severe meta-

bolic malfunctions caused by oxygen radical-mediated toxicity. Specific enzymes and low molecular weight substances eliminate ROS and contribute to the redox balance in the cell²⁵.

Glutathione peroxidase (GPx) plays an important role in the metabolism of hydrogen and lipid peroxides by using reduced glutathione (GSH) as a hydrogen donor resulting in the formation of oxidized glutathione (GSSG)²³. In BDL rats, glutathione peroxidase activity is reported to be decreased in many tissues¹³. In this study, GPx activity was decreased in hepatic and intestinal tissue after BDL significantly.

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. There were reports describing the efficacy of SOD on BDL injury of the liver^{26,27}. SOD catalyses the dismutation of the superoxide anion (O_2^-) into H_2O_2 . GSH-Px is a selenoprotein, which reduces lipidic or nonlipidic hydroperoxides as well as H_2O_2 while oxidizing GSH²⁷. In our study, we found that BDL impaired SOD activity, and LA administration suppressed SOD decrease after BDL in hepatic and intestinal tissues of rats.

MDA is a secondary product of oxidative stress formed during lipid peroxidation and it is released as a result of the toxic effect of ROS in rats after bile duct ligation¹⁹. Increased concentrations of MDA reflect the level of lipid peroxidation in tissues and it is considered as a marker of tissue injury²⁹. There are several reports indicating that levels of MDA increases after bile duct ligation in rats^{30,31}. Our findings are in agreement with previous works reporting high levels of MDA after BDL. In the present investigation, 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

Table II. Serum alanine aminotransferase (ALT) (units/l), aspartate aminotransferase (AST) (units/l), γ -glutamyltranspeptidase (GGT) (units/l), and the concentrations of total bilirubin (TB) (mg/dl).

AST	ALT	GGT	Bilirubin	
36.20 $\pm$ 9.43	33.10 $\pm$ 6.00	6.42 $\pm$ 1.61	0.10 $\pm$ 0.02	Sham
110.00 $\pm$ 3.05	120.43 $\pm$ 13.83	16.14 $\pm$ 1.57	0.30 $\pm$ 0.15	BDL
73.12 $\pm$ 9.29	79.87 $\pm$ 6.19	12.87 $\pm$ 2.03	0.32 $\pm$ 0.07	BDL+LA

Serum ALT, AST and GGT levels were significantly lower in the BDL+LA group, comparing to BDL group ($p < 0.05$). But there was no significant difference in bilirubin levels between BDL group and BDL+LA group.

Table III. Histologic score of hepatic and intestinal tissue in sham, BDL and BDL+LA group.

Intestinal histologic score	Hepatic histologic score	
0.30 ± 0.48	0.20 ± 0.42	Sham
2.28 ± 0.75	9.85 ± 2.34	BDL
1.25 ± 0.70	4.75 ± 1.38	BDL+LA

LA treatment significantly decreased the pathological changes in hepatic and intestinal tissue. Histological tissue damage was milder in the BDL+LA group than that in the B.

known. Reductions in MDA levels in the LA-treated rats may be due to its antioxidant and free-radical scavenging effect³².

By protecting cell membranes, LA probably reduces the deleterious effects of oxidative stress in living cells. Hagen et al³³ demonstrated that lipoic acid supplementation of old rats markedly improves the average mitochondrial membrane potential and restores the cellular oxygen consumption in hepatocytes to that of young rats. Rats on this feeding regimen were significantly more active, which further shows that lipoic acid acts physiologically to increase general metabolic activity. They also showed that feeding lipoic acid significantly attenuates the age-related increase in hepatocellular oxidant production as well as lipid peroxidation. This reduction in oxidative stress may be directly attributable to increased unbound dihydrolipoic acid or indirectly due to higher levels of other antioxidants. Shaafi et al³⁴ reported that treatment with LA significantly inhibited spinal cord ischemia/reperfusion lipid peroxidation, and maintained cellular GPx and SOD enzymes. In addition, LA treatment also improved neurologic deterioration seen following spinal cord ischemia/reperfusion.

Ozturk et al²⁶ reported that dexamethasone increased villus height, total mucosal thickness, and villus density in the ileum and decreased tubulo-interstitial lesions in the kidney. These effects of dexamethasone may be related to dexamethasone reducing the effects of small bowel and kidney oxidative stress and histological alterations in bile duct-ligated rats.

In the BDL group in our study, elevated serum ALT, AST, GGT and bilirubin activities indicated liver damage. Additionally, reduced serum ALT, AST, GGT activities in the BDL plus LA group showed significant improvement in liver tissues, although there was no significant decrease in bilirubin levels in BDL+LA group comparing to BDL group. Histopathological examination also showed damage in the BDL group hepatic and

intestinal tissue with improvement in BDL+LA group. Our results showed the protective potential of LA with liver and intestine damage.

Despite improvements in operative technique and the development of potent, broad-spectrum antibiotics, biliary tract surgery in patients with obstructive jaundice is still associated with high morbidity and mortality rates^{35,36}.

Conclusions

Our findings show that BDL induced hepatic and intestinal injury were significantly attenuated by LA administration and the administration of LA could effectively diminish this damage.

Conflict of Interest

None to declare.

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