Biomedicine & Pharmacotherapy 103 (2018) 75–86

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Betaine treatment protects liver through regulating mitochondrial function and counteracting oxidative stress in acute and chronic animal models of hepatic injury

Reza Heidari[a,*], Hossein Niknahad[a,b], Ala Sadeghi[b], Hamidreza Mohammadi[b], Vahid Ghanbarinejad[b], Mohammad Mehdi Ommati[a], Arghavan Hosseini[b], Negar Azarpira[c], Forouzan Khodaei[b], Omid Farshad[a,b], Elaheh Rashidi[b], Asma Siavashpour[b], Asma Najibi[b], Asrin Ahmadi[b], Akram Jamshidzadeh[a,b,*]

[a] Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[b] Department of Pharmacology and Toxicology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
[c] Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

ARTICLE INFO

Keywords:
Amino acid
ATP
Bile acid
Bioenergetics
Cholestasis
Liver injury
Oxidative stress

ABSTRACT

Betaine is a derivative of the amino acid glycine widely investigated for its hepatoprotective properties against alcoholism. The protective properties of betaine in different other experimental models also have been documented. On the other hand, the exact cellular mechanism of cytoprotection provided by betaine is obscure. The current study was designed to evaluate the hepatoprotective effects of betaine and its potential mechanisms of hepatoprotection in two animal models of acute and chronic liver injury. Bile duct ligation (BDL) was used as a model of chronic liver injury and thioacetamide (TAA)-induced hepatotoxicity was applied as the acute liver injury model. Severe increase in serum markers of liver tissue damage along with significant liver tissue histopathological changes were evident in both acute and chronic models of hepatic injury. It was also found that tissue markers of oxidative stress were significantly increased in BDL and TAA-treated animals. Moreover, liver mitochondrial indices of functionality were deteriorated in both investigated models. Betaine supplementation (10 and 50 mg/kg, i.p) ameliorated hepatic injury as judged by decreased liver tissue histopathological alterations, a significant decrease in tissue markers of oxidative stress, and mitigation of serum biomarkers of hepatotoxicity. On the other hand, betaine (10 and 50 mg/kg, i.p) protected hepatocytes mitochondria in both chronic and acute models of hepatotoxicity. These data indicate that the antioxidative and mitochondria regulating properties of betaine could play a primary role in its mechanisms of hepatoprotection.

1. Introduction

Betaine (Trimethylglycine) enters the human body through different dietary sources [1,2]. Some physiological roles including osmoregulatory properties have been attributed to betaine [1,2]. This chemical also participates in many critical biochemical pathways as a "methyl-donor" [1,2]. The hepatoprotective properties of betaine have repeatedly been mentioned in different experimental models [3–6]. Betaine is also a well-known agent which protect the liver against alcoholism [5,7,8].

Previous investigations have provided compelling evidence that oxidative stress and its associated events play a pivotal role in hepatotoxicity with different etiologies [9–13]. On the other hand, mitochondrial dysfunction has also been reported in several human pathological conditions and xenobiotics-induced cytotoxicity [14]. Mitochondrial injury and inability to maintain sufficient cellular ATP level could lead to cell death [15]. Oxidative stress and mitochondrial dysfunction are involved in the pathogenesis of liver injury in different experimental models [10,16–19]. It has been revealed that TAA model of the acute liver injury is associated with severe oxidative stress and mitochondrial dysfunction [20,21]. On the other hand, accumulation of the cytotoxic bile acid during bile duct obstruction is associated with mitochondrial injury and oxidative stress in the liver [22–25].

It has been found that betaine supplementation might blunt oxidative stress and its consequences in different experimental models [3,4,6,8,26]. Betaine also has been reported to prevent mitochondria-

* Corresponding authors at: Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
E-mail addresses: rheidari@sums.ac.ir (R. Heidari), ajamshid@sums.ac.ir (A. Jamshidzadeh).

https://doi.org/10.1016/j.biopha.2018.04.010
Received 24 February 2018; Received in revised form 29 March 2018; Accepted 2 April 2018

dependent cell death and apoptosis [7,27,28]. Hence, this chemical might provide promising protective properties in animal models of hepatic injury.

Although the beneficial properties of betaine in different experimental models have been well-studied, the precise mechanism(s) of cytoprotection behind remained obscure [3,4,8]. The current study was designed to evaluate the effect of betaine supplementation on liver mitochondrial function and oxidative stress in two experimental models of acute (Thioacetamide; TAA treatment) and chronic (Bile duct ligation; BDL) liver injury. The data could help to clear the mechanism of hepatoprotection provided by betaine as well as developing new therapeutic strategies against liver injury with different etiologies.

2. Materials and methods

2.1. Chemicals

4,2-Hydroxyethyl,1-piperazineethanesulfonic acid (HEPES), 3-(*N*-morpholino)propanesulfonic acid (MOPS), Dimethyl sulfoxide (DMSO), D-mannitol, Bovine serum albumin (BSA), Thiobarbituric acid (TBA), 3-[4,5dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), dithiobis-2nitrobenzoic acid (DTNB), Glutathione (GSH), 2′,7′-Dichlorofluorescein diacetate (DCFH-DA), Betaine (Tri-methyl glycine), Malondialdehyde (MDA), Sucrose, *n*-Propanol, *n*-Butanol, Sodium citrate, Potassium chloride, di-Sodium hydrogen phosphate (Na_2HPO_4), Sodium succinate, Glacial acetic acid, Magnesium chloride, Dithiothreitol, Rhodamine123 (Rh 123), Coomassie brilliant blue, Ethyleneglycol-bis (2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), and Ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium bicarbonate, Trichloroacetic acid (TCA), Sodium acetate, and Hydroxymethyl amino methane-hydrochloride (Tris-HCl) were purchased from Merck (Darmstadt, Germany). All salts for preparing buffer solutions were of analytical grade and prepared from Merck (Darmstadt, Germany).

2.2. Animals

Male Sprague Dawley rats (200–250 g) were obtained from Animal Breeding Center of Shiraz University of Medical Sciences, Shiraz Iran. Rats were housed in cages on wood-chip bedding at a temperature of 23 ± 2 °C and relative humidity ≈40%. Animals had free access to food (Behparvar®, Tehran, Iran) and tap water. Animals were handled according to the guidelines approved by a local ethics committee at Shiraz University of Medical Sciences, Shiraz, Iran (95-01-36-12046).

2.3. Animal surgery and bile duct ligation (BDL) as the model of chronic liver injury

Animals were anesthetized (A mixture of Ketamine 80 mg/kg and Xylazine 10 mg/kg, i.p), a midline incision was made, and the common bile duct was localized, doubly ligated, and cut between these ligatures [29]. The sham operation comprised laparotomy and bile duct identification and manipulation without ligation. The treatments in chronic liver injury model were as follow: 1) Sham-operated; 2) BDL; 3) BDL + Betaine (10 mg/kg/day, i.p); 4) BDL + Betaine (50 mg/kg/day, i.p).

2.4. Animal model of acute liver injury

Thioacetamide-induced hepatotoxicity is extensively used as an animal model of acute liver injury [30]. In the current investigation, thioacetamide-induced liver injury was achieved by i.p injection of thioacetamide (200 mg/kg) to rats [31]. Betaine (10 and 50 mg/kg, i.p) was administered for three consecutive days before thioacetamide challenge. The treatments were as follow: 1) Control (Vehicle-treated); 2) Thioacetamide; 3) Betaine (10 mg/kg, i.p) + Thioacetamide; 4) Betaine (50 mg/kg, i.p) + Thioacetamide.

Animals were anesthetized (Thiopental, 80 mg/kg, i.p) and their blood and liver samples were collected. Supportive therapy by administering 5% dextrose-containing 0.45% sodium chloride and 0.2% potassium chloride (2.5 ml/kg body weight, S.C), was given to avoid hypoglycemia and renal failure [32]. Control animals (Vehicle-treated) received normal saline as the thioacetamide solvent.

2.5. Serum biochemistry

Animals were anesthetized (Thiopental, 50 mg/kg, i.p) and their blood, liver, and kidney samples were collected. Blood was collected from the abdominal aorta, transferred to standard tubes (Improvacuter®; gel and clot activator-coated tubes; Guangzhou, China) and centrifuged (3000 *g*, 10 min, 4 °C) to prepare serum. Mindray BS-200® autoanalyzer (Mindray chemistry analyzers for low-volume laboratories, Guangzhou, China) was used to analyze serum biochemistry. Standard kits (Pars Azmun®, Tehran, Iran) were employed to assess serum gamma-glutamyl transpeptidase (γ-GT), alkaline phosphatase (ALP), Creatinine (Cr), glucose, phosphate, calcium, uric acid and blood urea nitrogen (BUN) [33].

2.6. Reactive oxygen species formation

Reactive oxygen species (ROS) in the liver was estimated as previously described [20,34,35]. Briefly, liver tissue (500 mg) was homogenized in 5 ml of ice-cooled Tris-HCl buffer (40 mM, pH = 7.4, 4 °C). Samples of the resulted tissue homogenate (100 µL) were mixed with Tris-HCl buffer (pH = 7.4, 1 mL) and 2′, 7′dichlorofluorescein diacetate (DCF-DA; Final concentration 10 µM). The mixture was incubated in the dark (37 °C; 15 min). Finally, the fluorescence intensity of the samples was assessed using a FLUOstar Omega® multi-functional microplate reader with $\lambda_{excitation} = 485$ nm and $\lambda_{emission} = 525$ nm [34,36].

2.7. Lipid peroxidation

The thiobarbituric acid reactive substances (TBARS) were measured as an index of lipid peroxidation in the liver tissue [20]. Briefly, the reaction mixture was consisted of 500 µL of liver tissue homogenate (10% w/v in KCl, 1.15% w/v), 1 mL of thiobarbituric acid (0.375%, w/v), and 3 mL of phosphoric acid (1% w/v, pH = 2) [37]. Samples were mixed well and heated (100 °C). After the incubation period (45 min), the mixture was cooled, and then 2 mL of *n*-butanol was added. Samples were vigorously mixed and centrifuged (10,000 *g* for 10 min) [38]. Finally, the absorbance of developed color in n-butanol phase was measured at $\lambda = 532$ nm using an Ultrospec 2000®UV spectrophotometer (Pharmacia Biotech, Uppsala, Sweden) [20].

2.8. Hepatic glutathione content

Liver samples (500 mg) were homogenized in 8 ml of ice-cooled (4 °C) EDTA solution (0.04 M). Then, 5 mL of the prepared homogenate were added to 4 mL of distilled water (4 °C) and 1 mL of trichloroacetic acid (50% w/v; 4 °C). The mixture was vortexed and centrifuged (10,000 *g*, 4 °C, 15 min) [37]. Then, 2 mL of the supernatant was mixed with 4 mL of Tris-HCl buffer (40 mM, pH = 8.9), and 100 µl of DTNB (10 mM in methanol) [38,39]. The absorbance of the developed color was measured at $\lambda = 412$ nm using an Ultrospec 2000®UV spectrophotometer (Pharmacia Biotech, Uppsala, Sweden) [20].

2.9. Ferric reducing antioxidant power (FRAP) of liver tissue

The FRAP assay measures the formation of a blue colored Fe^{2+}-tripyridyl-triazine compound from the colorless oxidized Fe^{3+} form by the action of electron-donating antioxidants [40]. In the current study, the working FRAP reagent was prepared by mixing 10 volumes of