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Taurine treatment preserves brain and liver mitochondrial function in a rat model of fulminant hepatic failure and hyperammonemia

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ABSTRACT

Ammonia-induced mitochondrial dysfunction and energy crisis is known as a critical consequence of hepatic encephalopathy (HE). Hence, mitochondria are potential targets of therapy in HE. The current investigation was designed to evaluate the role of taurine treatment on the brain and liver mitochondrial function in a rat model of hepatic encephalopathy and hyperammonemia. The animals received thioacetamide (400 mg/kg, i.p, for three consecutive days at 24-h intervals) as a model of acute liver failure and hyperammonemia. Several biochemical parameters were investigated in the serum, while the animals' cognitive function and locomotor activity were monitored. Mitochondria was isolated from the rats' brain and liver and several indices were assessed in isolated mitochondria. Liver failure led to cognitive dysfunction and impairment in locomotor activity in the rats. Plasma and brain ammonia was high and serum markers of liver injury were drastically elevated in the thioacetamide-treated group. An assessment of brain and liver mitochondrial function in the thioacetamide-treated animals revealed an inhibition of succinate dehydrogenase activity (SDA), collapsed mitochondrial membrane potential, mitochondrial swelling, and increased reactive oxygen species (ROS). Furthermore, a significant decrease in mitochondrial ATP was detected in the brain and liver mitochondria isolated from thioacetamide-treated animals. Taurine treatment (250, 500, and 1000 mg/kg) decreased mitochondrial swelling, ROS, and LPO. Moreover, the administration of this amino acid restored brain and liver mitochondrial ATP. These data suggest taurine to be a potential protective agent with therapeutic capability against hepatic encephalopathy and hyperammonemia-induced mitochondrial dysfunction and energy crisis.

1. Introduction

Hepatic encephalopathy (HE) is a deleterious clinical complication accompanied by acute and chronic liver injury [1]. Although the exact cause of HE is not known, there is agreement on the predominant role of ammonia in HE etiology [2]. Ammonia is metabolized by the liver to the urea in healthy subjects. Damaged livers are unable to metabolize ammonia. Hence, this toxic chemical is elevated in the systemic circulation and, eventually, damages the brain. Ammonia is a neurotoxin that mostly influences astrocytes in the central nervous system (CNS) [3,4]. It also has several direct toxic effects on neurons [4]. Ammonia causes brain edema, oxidative stress, and inflammation when its level rises in HE [5]. Consequently, a decline in brain function occur in patients with HE [5]. Hyperammonemia also affects hepatocytes and liver function [6].

Disturbed mitochondrial function and oxidative stress are implicated in ammonia-induced cytotoxicity [3,7]. It has been reported that brain energy metabolism is interrupted in chronic

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and acute HE [8–10]. Ammonia negatively affects several key enzymes that are responsible for energy metabolism in mitochondria [11]. Hence, targeting mitochondria and bioenergetics failure represents a potentially useful approach for the treatment and reduction of the side effects of HE and hyperammonemia disorders.

Taurine (2-aminoethanesulfonic acid, TAU) is a non-protein amino acid. The concentrations of TAU in the brain, heart, and muscle are high [12]. Several physiological and pharmacological roles are attributed to TAU [13]. This amino acid is an antioxidant, membrane stabilizer, osmoregulator and, probably, a neurotransmitter [14–16]. Bile acid conjugation is a well-known process mediated by TAU in the liver [17]. On the other hand, TAU exhibits hepatoprotective properties against the deleterious effects of a wide range of xenobiotics [18–22]. TAU also has been shown to have a profound effect on CNS [23,24]. It has been demonstrated that TAU acts as an osmoregulator, protects neurons, prevents astrocytes swelling and encounters oxidative stress in CNS [25–28].

The effect of TAU on cell mitochondria is reported in several investigations [14,29,30]. TAU regulates mitochondrial pH, affects mitochondrial GSH and antioxidants, and preserve the mitochondrial membrane potential [14,31–33].

As mentioned, a mitochondrial injury and energy crisis serve as key mechanisms in the liver and brain injury. Hence, targeting cellular mitochondria in HE might serve as a therapeutic option. Previously, we found that taurine administration effectively lowered blood and brain ammonia and its associated complications in different models of acute and chronic liver injury [34]. The current investigation was designed to evaluate the effect of TAU treatment on brain and liver mitochondrial function in a rat model of acute liver failure and hyperammonemia.

2. Materials and methods

2.1. Chemicals

Dimethyl sulfoxide (DMSO), D-mannitol, Fatty acid-free bovine serum albumin (BSA) fraction V, Thiobarbituric acid (TBA), 4,2-hydroxyethyl,1-piperazineethanesulfonic acid (HEPES), 3-(*N*-morpholino)-propane sulfonic acid (MOPS), *n*-butanol, 3-[4,5dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), dithiobis-2nitrobenzoic acid (DTNB), Glutathione (GSH), 2′,7′-dichlorofluorescein diacetate (DCFH-DA), 2-aminoethanesulfonic acid (Taurine), Malondialdehyde (MDA), Sucrose, KCl, Na_2HPO_4, $MgCl_2$, Rhodamine123 (Rh 123), Coomassie brilliant blue, Hydrochloric acid (HCl), Sodium succinate, ethylene glycol-bis (2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid (TCA), Ammonium chloride, Hydroxymethyl aminomethane hydrochloride (Tris-HCl) were purchased from Merck (Dardamstd, Germany). All salts for preparing buffer solutions were of analytical grade and prepared from Merck (Dardamstd, Germany).

2.2. Animals

Sprague-Dawley rats (200–250 g) were obtained from Experimental and comparative Medicine Research Center of Shiraz University of medical Sciences, Shiraz Iran. Rats were housed in cages on wood bedding at a temperature of 23 ± 1 °C. Animals had free access to tap water and a standard chow diet. Animals received humane care and use and were handled according to the animal handling protocol approved by a local ethics committee at Shiraz University of Medical Sciences (94-01-36-9823).

2.3. Animal model of acute liver failure

Thioacetamide is extensively used as a model of acute hepatic failure [35]. Thioacetamide-induced fulminant hepatic failure was achieved by three consecutive i.p injections of thioacetamide (400 mg/kg) to rats (n = 30; 6 rats/group) at 24-h intervals [36]. TA (500 and 1000 mg/kg, i.p) was administered for three consecutive days, two hours after each dose of thioacetamide. The treatments were as follow: 1) Control (Vehicle-treated); 2) Thioacetamide; 3) Thioacetamide + Taurine 500 mg/kg; 4) Thioacetamide + Taurine 1000 mg/kg; 5) Taurine 1000 mg/kg.

On the fourth day (24 h after the last dose of thioacetamide), animals were anesthetized (thiopental, 80 mg/kg, i.p) and their blood, brain, and liver were collected. Supportive therapy by administering 5% dextrose (25 ml/kg body weight, S.C) containing 0.45% sodium chloride and 0.2% potassium chloride, was given to avoid weight loss, hypoglycemia and renal failure [36]. Control animals (Vehicle-treated) received normal saline as the thioacetamide solvent. The sole TA (1000 mg/kg, i.p) was administered to ensure its safety [34].

2.4. Motor coordination and activity tests

All motor coordination and activity tests were conducted for each group five hours before animal anesthesia and blood and tissue sample collection.

2.4.1. Rotarod test

Following a reported procedure [37], each rat underwent five sessions of rotarod performance on a rotarod apparatus. The speed of the rotarod was 5 and 15 rpm. Each session had three trial for each rat with 10 min interval. The time, up to which the rats stayed on the rotating rod, was recorded automatically [37,38].

2.4.2. Open field test

Open field behavior is used as an index of animals' locomotor activity in the animal models of hyperammonemia and hepatic encephalopathy [39,40]. The apparatus was made of a white wood box (100 cm L × 100 cm W × 30 cm H, box floor was divided into 25 squares of 20 × 20) [41–43]. The open field arena was equipped with a webcam (2.0-Megapixel, Gigaware, UK) and all activities were monitored and recorded from a separate room. Animals behavior was recorded for fifteen minutes and the total number of crossed squares were counted (Total locomotion) [34].

2.4.3. Gait test

Rats' hind paws were wetted with ink. Afterward, using a runaway procedure, the animals were allowed to walk down on a paper strip (60 cm long, 10 cm wide) from the brightly lit corridor toward a dark side. The distance between the points of the left and right paws was measured and recorded [37].

2.5. Blood biochemistry and tissue histopathology

A MindrayBS-200® auto analyzer and standard kits were used to measure plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) [44]. Plasma ammonia was measured with standard commercial kits [45]. To determine brain ammonia content, samples (100 mg) of the forebrain (cerebral cortex) were dissected, homogenized, and deproteinized in 3 ml of ice-cooled lysis solution (Trichloroacetic acid, 6%, w/v). After centrifugation (12,000*g*, 10 min, 4 °C), the supernatant was collected and neutralized with $KHCO_3$ (2 mol/l, pH 7). Afterward, brain ammonia content was measured using standard kits [45].