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Research report

Neuroprotective actions of taurine on hypoxic-ischemic brain damage in neonatal rats

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ABSTRACT

Taurine is an abundant amino acid in the nervous system, which has been proved to possess antioxidation, osmoregulation and membrane stabilization. Previously it has been demonstrated that taurine exerts ischemic brain injury protective effect. This study was designed to investigate whether the protective effect of taurine has the possibility to be applied to treat neonatal hypoxic-ischemic brain damage. Seven-day-old Sprague-Dawley rats were treated with left carotid artery ligation followed by exposure to 8% oxygen to generate the experimental group. The cerebral damage area was measured after taurine post-treatment with 2,3,5-triphenyltetrazolium chloride (TTC) staining, Hematoxyline-Eosin (HE) staining and Nissl staining. The activities of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), myeloperoxtidase (MPO), ATP and Lactic Acid productions were assayed with ipsilateral hemisphere homogenates. Western-blot and immunofluorescence assay were processed to detect the expressions of AIF, Cyt C, Bax, Bcl-2 in brain. We found that taurine significantly reduced brain infarct volume and ameliorated morphological injury obviously reversed the changes of SOD, MDA, GSH-Px, T-AOC, ATP, MPO, and Lactic Acid levels. Compared with hypoxic-ischemic group, it showed marked reduction of AIF, Cyt C and Bax expressions and increase of Bcl-2 after post-treatment. We conclude that taurine possesses an efficacious neuroprotective effect after cerebral hypoxic-ischemic damage in neonatal rats.

1. Introduction

Neonatal hypoxic-ischemic brain damage (HIBD) is a relatively common malignant complication caused by clinical perinatal asphyxia in infants and young children (Chen et al., 2015; Thatipamula et al., 2015), which occurs in 1–6 of every 1000 live term births (Gu et al., 2016; Koonrungsesomboon et al., 2014). Statistics suggests that approximately 40% of the affected infants die in the neonatal period and an additional 30% have lifelong neurological deficits including cerebral palsy, epilepsy and cognitive disabilities (Hristova et al., 2016). The treatment and care for the sequelae of HIBD require extensive resources. What is unfortunate is that current treatment regimens are not optimal, even remained ineffective. Moreover, even with the best care, these children only have little improvement in the overall ability. Accordingly, HIBD is a major public health issue which globally leads to substantial socioeconomic burden of the individual, family and healthcare system (Ding et al., 2016). Altogether, efficient pharmacological strategies for the sanitation and therapy of HIBD are restricted by safety and toxicity considerations. Given the magnitude of the problem, it is urgent and unmet to focus on how to supply safe and effective neuroprotective medicine, which would promote prognosis of HIBD infants by promoting after-injury repair.

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Although the precise pathogenesis of HIBD is still inconclusive, it is valid that oxidative stress, oxidative metabolism and apoptosis are the significant components of cell death following HIBD (Taylor et al., 1999; Ding et al., 2016). Oxidative stress and oxidative metabolism play a pivotal role in the procedure of pathogenesis after the occurrence of HIBD which ultimately trigger cell death. Additionally, on account of the immature brains possess fairly high polyunsaturated fatty acid concentration, meanwhile low concentration of antioxidants, they are considered to be particularly prone to tissue damage due to oxidative stress after hypoxia-ischemia injury (Zhang et al., 2014). Pathological accumulation of excessive reactive oxygen species (ROS) and subsequent oxidative stress results in brain necrosis and apoptosis (Chang et al., 2016). Endogenous antioxidants such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), possess the ability of scavenging overproduction of oxidants to prevent deleterious ROS generation (Chen et al., 2015; Zhao et al., 2015).

Apoptosis is a form of cellular suicide which is essential for development and tissue homeostasis of all metazoan organisms (Guo et al., 2002). Mitochondrion as a key organelle is the main source of cellular ATP which may also regulate cell death (Granville et al., 2001). It is demonstrated that mitochondria are involved in cell death based on experimental stroke (Sun et al., 2011). A mechanism pertaining to the death of immature neurons is the accumulation of AIF and Cyt c, which is safely sequestered within the mitochondrial intermembrane space in non-apoptotic cells (Guo et al., 2002). On the other hand, anti-apoptotic protein Bcl-2, also known as a mitochondrial membrane protein, blocks the apoptotic death of many cell types (Taylor et al., 1999; Gu et al., 2016), it plays a crucial role in regulation of mitochondria-mediated cell death. Therefore, anti-apoptotic therapies via inhibiting AIF and Cyt c expression and modulating the actions of Bcl-2 family proteins have been proposed to be useful in ameliorating neonatal HIBD.

Taurine, a β-amino acid, presents high concentration in the mammalian tissues, which possesses a number of cytoprotective properties through its actions as neurotransmitter, neuromodulator, osmoregulator, anti-oxidant, membrane stabilizer, anti-inflammation and neuroprotection (El Idrissi, 2008; Schuller-Levis and Park, 2004; Haas and Hosli, 1973; Hussy et al., 1997; Huxtable, 1989; Huxtable, 1992). In recent years, taurine has been demonstrated to function neuroprotective activity in various kinds of in vitro and in vivo brain injury models. It has been reported that taurine reduces caspase-8 and caspase-9 expression in ischemia injury (Taranukhin et al., 2008) and intracellular calcium elevation, as well as depresses calpain activation, thereby attenuating glutamate-induced apoptotic neuronal death and enhancing Bcl-2:Bax ratio. Furthermore, in vivo taurine has been verified to protect brain against experimental stroke in a dose dependent manner with marked protection (Sun et al., 2011, 2012a,b; Wang et al., 2007).

However, no information is available on possible effects of taurine in neonatal brain injury induced by hypoxia-ischemia. It was speculated that taurine might exert a protective effect on neonatal HIBD under this background. To test the hypothesis, the present experiment was designed to investigate the potential neuroprotective effects of post-insult administration of taurine on neonatal HIBD using the neonatal hypoxic-ischemic rat model as well as to further identify its underlying mechanisms.

2. Materials and methods

2.1. Experiment animals

Female Sprague-Dawley rats with 7-day-old neonates were supplied by the Experimental Animal Center of Ningxia Medical University (Certificate number was SYXK Ningxia 2015-0001). The animals were housed in the temperature-controlled environment (22–24° C) under 12 h light and dark cycles and animals had access to food and water ad labium. The experimental designs and all procedures were in accordance both with the National Guidelines for Care and Use of Laboratory Animals, together with the Animal Care Guidelines issued by the Animal Experimental Committee of by Ningxia Medical University. All surgeries were performed under diethyl ether anesthesia. All efforts were made to minimize suffering, thereby reducing the number of animals used.

2.2. Construction of HIBD model

The HIBD model was established according to literatures. The method to create hypoxic-ischemic brain damage in the 7-day-old rat is based on the Levine preparation in the adult rat (Vannucci and Vannucci, 2005) except for rat in sham group, the concrete processes were operated as hereunder mentioned. The newborn rats of both sexes were narcotized by ether inhalation, a small incision was made in the left side of the neck, the thyroid, vein, and nerve tissues were stripped to expose the left common carotid artery, cut between double ligatures with 6-0silk surgical suture, and then sutured. Each surgery was completed within 5 min. The neonates were sent back to their cages with their mothers for 1.5 h. After recovery, pups were placed in a lower oxygen tank to maintain constant temperature (37 °C) under continuous hypoxia with 8% oxygen/balance nitrogen gas in the container for 2 h. After hypoxic exposure, all surviving pups were returned to their cages with their mothers until they were sacrificed. Animals in sham group were randomly selected from the same litters of hypoxic-ischemic rats, and then treated with anesthesia followed by exposing their left carotid artery without undergoing hypoxic-ischemic.

2.3. Drug administration

For evaluating the effects of taurine on neurological deficits, brain swelling, neutrophil infiltration and infarct volume, the rats were randomly assigned to five groups treated with taurine (Sigma, St. Louis, MO, U.S.A.) dissolved in normal saline (0.9%NaCl) before using. Drugs were administrated intraperitoneally (i.p.) in volume of 0.1 mL/10 g body weight and administered 15 min prior to testing. Pups of mixed sex from different litters were randomly divided into the following groups (n = 48, for each group):

- Sham (sham surgery) with NS group;
- HI (cerebral hypoxic-ischemic) with NS group;
- HI (cerebral hypoxic-ischemic) with taurine (30 mg/kg) group;
- HI (cerebral hypoxic-ischemic) with taurine (60 mg/kg) group;
- HI (cerebral hypoxic-ischemic) with taurine (120 mg/kg) group;

Taurine was given by intraperitoneal injection every 12 h for two consecutive days after HI. Sham and HI groups were treated with saline under the same conditions.

2.4. Measurement of infarct volume

Pups (n = 6, for each group) were anesthetized and euthanized 48 h after HI treatment. Rat brains were removed and sectioned coronally with a self made blade at 2-mm intervals and incubated for 30 min at 37 °C in a 2% solution of 2, 3,5-triphenyltetrazolium chloride (TTC) (Sigma, St. Louis, MO, U.S.A.) followed by overnight immersion in 4% formaldehyde solution. The TTC stained sections were recorded with a digital camera to measure the infarct volumes. Areas which were not stained red with TTC were considered injured, calculated by the image-analysis software (Image-Pro plus, USA). Since brain edema might significantly affect the accuracy