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Alpha lipoic acid inhibits neural apoptosis via a mitochondrial pathway in rats following traumatic brain injury

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ABSTRACT

Alpha lipoic acid (ALA) is a powerful antioxidant that has proven protective effects against brain damage following a traumatic brain injury (TBI) in rats. However, the molecular mechanisms underlying these effects are not well understood. This study investigated the effect of ALA on neural apoptosis and the potential mechanism of these effects in the weight-drop model of TBI in male Sprague-Dawley rats that were treated with ALA (20 or 100 mg/kg) or vehicle via intragastric administration 30 min after TBI. Brain samples were collected 48 h later for analysis. ALA treatment resulted in a downregulation of caspase-3 expression, reduced the number of positive cells in the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay and improved neuronal survival. Furthermore, the level of malondialdehyde and glutathione peroxidase activity were restored, while Bcl-2-associated X protein translocation to mitochondria and cytochrome c release into the cytosol were reduced by ALA treatment. These results demonstrate that ALA improves neurological outcome in rats by protecting neural cell against apoptosis via a mechanism that involves the mitochondria following TBI.

1. Introduction

There are currently no effective treatments for traumatic brain injury (TBI) that improve clinical outcome, leading to high health care costs, morbidity, and mortality (Toklu et al., 2009). Secondary brain damage seems to contribute to the poor outcome of TBI patients (Jin et al., 2008; Park et al., 1999; Yan et al., 2008a), which, resulting from neural apoptosis, undermines short- as well as long-term outcome (Hu et al., 2009). Oxidative stress plays a major role in the pathogenesis of secondary brain injury following TBI (Ansari et al., 2008a). Mitochondria are the main cellular source of reactive oxygen species (ROS) and their impairment increases ROS production, damaging mitochondrial proteins, DNA, and lipids, disrupting cellular Ca^{2+} homeostasis, inducing apoptosis, and causing metabolic failure (Crow et al., 2004; Lemasters et al., 1999; Paradies et al., 2014; Robertson et al., 2009). Apoptotic mechanisms must be tightly regulated to prevent neuronal death after TBI (Keane et al., 2001); this includes the control of the mitochondrial pathway of apoptosis (Crow et al., 2004; Simpkins et al., 2010).

Antioxidant therapy represents a viable treatment approach for TBI. Alpha-lipoic acid (ALA) is a naturally occurring substance that acts as an essential cofactor for various enzymes required for oxidative metabolism (Smith et al., 2004), and also has therapeutic potential for treating mitochondria-related disorders (Reed, 1998; Tirosh et al., 2003). However, to date there have been no studies investigating the effect of ALA on the related mitochondrial pathway following brain injury. In this study, the role of ALA in the modulation of mitochondrial function and apoptotic mechanisms was examined in a rat model of TBI.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats weighing 250–280 g were obtained from the Animal Center of Jinling Hospital. The animals were maintained on a 12:12 h light/dark cycle under conditions of controlled temperature and humidity. All procedures were approved by the Animal Care and Use Committee of Southern Medical University and conformed to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Abbreviations: ALA, alpha lipoic acid; TBI, traumatic brain injury; ROS, reactive oxygen species; MDA, malondialdehyde; GPx, glutathione peroxidase; Bax, Bcl-2 associated X protein; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

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Rats were randomly assigned to five experimental groups: sham + vehicle, sham + ALA (100 mg/kg), TBI + vehicle, TBI + ALA (20 mg/kg) and TBI + ALA (100 mg/kg). Animals in the TBI + ALA and TBI + vehicle groups were subjected to TBI and treated with ALA or vehicle, respectively. Animals in the sham + ALA and sham + vehicle groups were subjected to a procedure similar to TBI but without brain perforation, followed by ALA or vehicle treatment. In addition, the ALA dose (20 mg/kg), chosen in a preliminary study, was found to be without serious systemic effects on the normal rats. Due to animal welfare and reduction of the number of rats in the experiments, we did not include any additional sham + ALA (20 mg/kg) group. Animals were sacrificed 48 h after the surgery. A total of 12 animals per group were used for Western blotting; 6 animals per group were used for the analysis of brain water content and neurological function; 6 animals per group were used for biochemical analyses; and 6 animals per group were used for immunohistochemistry, Nissl staining, and the terminal deoxynucleotidyl transferase-mediated biotinylated deoxyuridine triphosphate nick-end labeling (TUNEL) assay.

2.2. Drug administration

ALA was purchased from Sigma-Aldrich (catalogue number: T5625; St. Louis, MO, USA), dissolved in the vehicle solution (10% dimethyl sulfoxide in corn oil), and administered by oral gavage once daily for 2 days starting 30 min after the induction of TBI (Toklu et al., 2009). Animals in the sham + ALA group received ALA and those in the sham + vehicle and TBI+ vehicle groups received equivalent volumes of vehicle at the same time points.

2.3. Rat TBI model

The TBI model used in this study was a modified version of the weight-drop model (Feeney et al., 1981). As described in our previous study (Yan et al., 2008b), rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg), and a 6-mm diameter hole was made through the exposed skull using a dental drill on the left (parietal) side; the center of the hole was 1.5 mm posterior and 2.5 mm lateral to bregma. The dura was left intact during the operation. TBI was induced by a 40 g weight dropped from a height of 25 cm along a stainless steel string, which translated into 1000× *g*/cm. Sham rats were subjected to identical treatment but without injury. The rats were returned to their cages after the operation.

2.4. Neurological evaluation

Neurological function was evaluated 24 and 48 h after TBI using the previously described scoring system (Feeney et al., 1982; Su et al., 2011). Briefly, beam-walking performance was rated using a seven-point rating scale, as follows: 1, the animal is unable to place the affected hind limb on the horizontal surface of the beam; 2, the animal places the affected hind limb on the horizontal surface of the beam and maintains balance but is unable to traverse the beam; 3, the animal traverses the beam dragging the affected hind limb; 4, the animal traverses the beam while on one occasion placing the affected hind limb on the horizontal surface of the beam; 5, the animal crosses the beam while placing the affected hind limb on the horizontal surface of the beam to aid fewer than half of its steps; 6, the animal uses the affected hind limb to aid more than half of its steps; and 7, the animal traverses the beam with no more than two foot slips. Testing was performed by an investigator who was blind to the treatment conditions. The sequence of the behavioral tasks was randomized. A lower score represented neurological deficits.

2.5. Brain water content

The brain water content was measured as previously described (Hu et al., 2009; Xu et al., 2014). After the neurological function test 48 h after TBI, animals were sacrificed and their brains quickly dissected. After removal of the brainstem and cerebellum, the right and left cortical tissue was harvested. Each sample was immediately weighed to determine the wet weight, then dried for 72 h at 80 °C and weighed to obtain the dry weight. The brain water content was calculated with the formula: [(wet weight – dry weight)/wet weight] × 100%.

2.6. Determination of malondialdehyde (MDA) content and glutathione peroxidase (GPx) activity

MDA content and GPx activity were determined as previously described (Ding et al., 2014). Left cortical tissue samples were homogenized in 2 ml phosphate-buffered saline (PBS, pH 7.4), then centrifuged at 12,000× *g* for 20 min at 4 °C. MDA content and GPx activity were determined using commercial kits (Nanjing Jiancheng Biochemistry Co., Nanjing, China) according to the manufacturer's instructions and measurements were made using a spectrophotometer. Total protein concentration was determined by the Bradford method. MDA level and GPx activity are expressed as nmol/mg protein and U/mg protein, respectively.

2.7. Immunohistochemistry

Caspase-3 expression was evaluated by immunohistochemistry. Brain tissue samples were fixed in formalin for 24 h and embedded in paraffin. Sections were cut at a thickness of 5 μm and deparaffinized in xylene, dehydrated in a graded series of ethanol, subjected to antigen retrieval in citrate buffer (pH 6.0) for 30 min in a 37 °C chamber, and washed in PBS. The sections were quenched in 3% hydrogen peroxide and blocked with PBS containing 10% goat serum (Sigma-Aldrich) for 1 h at 37 °C, then incubated with a rabbit monoclonal anti-caspase-3 antibody (1:300; Cell Signaling Technology, Boston, MA, USA) overnight at 4 °C, followed by three 15-min washes in PBS and incubation with horseradish peroxidase (HRP)-conjugated IgG (1:500; Bioworld Technology, Inc., St. Louis Park, MN, USA) for 60 min at room temperature. After three washes in PBS, sections were counterstained with hematoxylin, dehydrated, and cleared with xylene before mounting. Control tissue was subjected to the same procedure but without the primary antibody step.

2.8. Nissl staining

Coronal sections of brain tissue (5 μm thick) were stained with cresyl violet as previously described (Zhuang et al., 2012). Normal neurons had large cell bodies and cytoplasmic volume, with one or two large, round nuclei. In contrast, damaged cells were identified as those with shrunken cell bodies, condensed nuclei, and dark cytoplasm containing many empty vesicles. Histological examination was performed by two observers who were blinded to the group assignment.

2.9. TUNEL assay

Paraffin-embedded brain tissue sections (5 μm thick) were assessed for apoptotic cells using a TUNEL detection kit (Roche, Indianapolis, IN, USA) as described in previous studies (Xie et al., 2014; Zhang et al., 2013; Zhuang et al., 2012). Briefly, sections were deparaffinized and subjected to antigen retrieval, and endogenous peroxidase activity was quenched. The diaminobenzidine reaction was followed by re-staining, hydrochloric acid/alcohol differentiation,