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Research article

Effects of alpha-lipoic acid on spatial learning and memory, oxidative stress, and central cholinergic system in a rat model of vascular dementia

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HIGHLIGHTS

- Alpha-lipoic acid ameliorated learning and memory impairment in vascular dementia rats.
- Alpha-lipoic acid reduced oxidative stress in hippocampus of vascular dementia rats.
- Alpha-lipoic acid restored central cholinergic system in vascular dementia rats.

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ABSTRACT

Brain oxidative stress due to chronic cerebral hypoperfusion was considered to be the major risk factor in the pathogenesis of vascular dementia. In this study, we investigated the protective efficacy of alpha-lipoic acid, an antioxidant, against vascular dementia in rats, as well as the potential mechanism. Bilateral common carotid arteries occlusion (BCCAO) induced severe cognitive deficits tested by Morris water maze (MWM), along with oxidative stress and disturbance of central cholinergic system. However, administration of alpha-lipoic acid (50 mg/kg, i.p.) for 28 days significantly restored cognitive deficits induced by BCCAO. Biochemical determination revealed that alpha-lipoic acid markedly decreased the production of malondialdehyde (MDA) and the generation of reactive oxidative species (ROS), and increased the level of reduced glutathione (GSH) in the hippocampal tissue. Additionally, alpha-lipoic acid raised the level of acetylcholine (ACh) and choline acetyltransferase (ChAT) and decreased the activity of acetylcholinesterase (AChE) in the hippocampus. These results indicated that treatment with alpha-lipoic acid significantly improved behavioral alterations, protected against oxidative stress, and restored central cholinergic system in the rat model of vascular dementia induced by BCCAO.

Vascular dementia (VD), a brain disorder in which cognitive decline is attributable to cerebrovascular pathologies, is the second most common form of dementia after Alzheimer's disease [1]. VD leads to unremitting and largely irreversible deterioration in quality of life, imposing a tremendous burden on families and a financial cost to society [2]. However, the pathogenesis of VD is not yet entirely clear and there are no drugs especially effective for the treatment of vascular cognitive impairment. Considerable evidence indicates that chronic cerebral hypoperfusion (CCH) is a major cause of VD [3–5] and demonstrated that CCH can lead to oxidative stress, which resulted in the cognitive damage [6,7]. Previous studies have demonstrated that CCH may induce central cholinergic dysfunction in the cerebral cortex and hippocampus of rats with BCCAO [7,8]. Considering the importance of central cholinergic system in the processing of information related to cognitive function [9,10], it is reasonable that central cholinergic deficiency was considered to play a critical role in the cognitive deficits of subjects with VD [11] and AD [12]. Thus, protecting against the oxidative injury and

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central cholinergic dysfunction induced by chronic cerebral hypoperfusion may be crucial for managing VD.

Alpha-lipoic acid (ALA), a naturally occurring disulfide, has been identified as an ideal neuroprotective antioxidant based on its ability to cross the blood-brain barrier and its uniform uptake by the various parts of the central and the peripheral nervous system [13]. The pharmacologic effects of ALA include: increasing Ach production, increasing glucose uptake, scavenging ROS and lipid peroxidation products, and inducing syntheses of antioxidant protective enzymes and of glutathione [14].

Protective effects of ALA against nerve degeneration have been shown in models of Alzheimer's disease [15], Parkinson [16], and diabetes [17]. Additionally, ALA has been shown to exert neuroprotective effects in animal models of seizure [18], cerebral ischemia [19], autoimmune encephalomyelitis [20], subarachnoid hemorrhage [21], and traumatic brain injury [22]. Furthermore, in a rat model of pilocarpine-induced seizure [23] and in a high-fat diet model with impaired spatial learning [24], ALA can reverse cognitive impairment observed in seizure rats, as well as increase the ChAT activity in hippocampus of rats. However, no information is available with regard to the possible protective effect of ALA on cognitive deficits induced by CCH.

In the light of these findings, the present study was designed to evaluate the role of exogenous ALA on CCH induced cognitive impairment, oxidative brain injury and central cholinergic dysfunction.

1. Materials and methods

1.1. Animals

Adult male Wistar rats weighing 300 ± 20 g were obtained from the Hebei Medical University and housed in groups of five per cage under standard laboratory conditions. They were kept at constant room temperature (25 ± 1 °C) and humidity (40–60%). The rats were kept on a 12 h light/dark cycle, with lights on at 08:00 am and with free access to food and water. Animal experiments were performed according to the regulations of laboratory animal management promulgated by the Ministry of Science and Technology of the People's Republic of China [1988] No. 134, which coincides with internationally recognized NIH guidance. The rats were acclimatized to housing conditions for 7 days before surgery.

1.2. Drugs and chemicals

All standard chemicals used in this study were of analytical grade. ALA and 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Sigma (St. Louis, MO, USA). GSH, MDA, Ach, ChAT, and AchE detection kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). ALA was dissolved in physiological saline freshly prior to the injections.

1.3. Surgical procedure

The surgical procedure was performed as described previously [25]. Briefly, after animals were anesthetized with 10% chloral hydrate (300 mg/kg, i.p.), the bilateral common carotid arteries were exposed via a ventral midline incision and were double-ligated with silk sutures. The sham-operated rats were treated in a similar way without any artery occlusion. During the operation, a heating lamp was used to maintain body temperature of animals at 37 ± 0.5 °C.

1.4. Drug administration

Animals were randomly divided into four groups with eight in each: SHAM (sham-operated) group, BCCAO (BCCAO + normal saline) group, BCCAO + ALA (BCCAO + alpha-lipoic acid 50 mg/kg i.p.) group, SHAM + ALA (sham-operated + alpha-lipoic acid 50 mg/kg i.p.) group. Starting from the day after the surgery, all rats received ALA dissolved in normal saline or normal saline according to the experimental plan every 24 h up for a total of 28 injections.

1.5. Learning and memory assessment

MWM was used for learning and memory behavior assessment [26]. MWM was done 24 h after the last administration of ALA, and was performed in a 180-cm diameter water pool and virtually divided into four quadrants. The pool was filled with water (22 ± 1 °C). A colorless escape platform (10 cm in diameter) was submerged 2 cm beneath the water surface, located in a designated target quadrant. The maze was located in a quiet test room, surrounded by many visual cues outsides of the maze, which were visible from within the pool and could be used by the rats for spatial orientation.

Each test consisted three parts, learning trials (existed platform), probe trials (without platform), and visible platform tests. The acquisition test was performed for five consecutive days of training with four trials per day. Animals were given 120 s to locate the hidden platform, and any animals that did not find the platform within 120-s period were placed on the platform for 30 s. The acquisition time was recorded at the time the animal got into the water and ending at the time the animal reached the submerged platform. On the sixth day, probe trials without platform were assessed with only one starting point, and the time spent in the target quadrant where the platform had been located was recorded. To test the visual, motor, and motivation skills of rats, the visible platform tests were performed for 2 days with four trials per day 24 h after the probe trial. The escape platform was positioned 1 cm above the water surface. The duration to reach the visible platform and the swimming speed were recorded and analyzed.

1.6. Tissue dissection

Following the behavioral tests, animals were sacrificed and brains were quickly removed, and then hippocampi were dissected on an ice-cold surface. The structure was weighed and homogenized in 10 volumes (1:10, w/v) of ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 3000 rpm for 10 min at 4 °C, and the supernatant was used to determine the content of ROS, MDA, GSH, Ach, ChAT, and AchE.

1.7. Determination of ROS

ROS was measured by using the oxidant sensing fluorescent probe, DCFH-DA. The intensity of fluorescence was determined by flow cytometry (BDFACSAria II) at 488 nm excitation and 530 nm emission. Cell Sorter software (version 7.9, BD Biosciences) was used for both data acquisition and analysis.

1.8. Determination of MDA

MDA, an index of lipid peroxidation, was measured based on the reaction with thiobarbituric-acid (TBA) reaction described by Okhawa et al. [27]. MDA reacts with TBA as a thiobarbituric acid reactive substance to produce a pink complex with a peak absorbance at 532 nm. The quantification of MDA was determined by comparing the absorption to the standard curve of MDA equivalents.

1.9. Determination of GSH

Assay of GSH was performed in tissue homogenates by the method of Ellman [28]. Supernatants were incubated with DTNB