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Acupuncture reversed hippocampal mitochondrial dysfunction in vascular dementia rats

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ABSTRACT

Hippocampal mitochondrial dysfunction due to oxidative stress has been considered to play a major role in the pathogenesis of vascular dementia (VD). Previous studies suggested that acupuncture could improve cerebral hypoperfusion-induced cognitive impairments. However, whether hippocampal mitochondria are associated with this cognitive improvement remains unclear. In this study, an animal model of VD was established via bilateral common carotid arteries occlusion (BCCAO) to investigate the alterations of cognitive ability and hippocampal mitochondrial function. BCCAO rats showed impairments in hippocampal mitochondrial function, overproduction of reactive oxygen species (ROS) and learning and memory deficits. After two-week acupuncture treatment, BCCAO-induced spatial learning and memory impairments as shown in Morris water maze were ameliorated. Hippocampal mitochondrial respiratory complex enzymes (complex I, II, IV) activities and cytochrome c oxidase IV expression significantly increased, which might contribute to the reduction of hippocampal ROS generation. In addition, acupuncture significantly improve mitochondrial bioenergy parameters such as mitochondrial respiratory control rate and membrane potential not PDH A1 expression. Placebo-acupuncture did not produce similar therapeutic effects. These findings suggested that acupuncture reversed BCCAO-induced hippocampal mitochondrial dysfunction, which might contribute to its prevention on cognitive deficits.
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1. Introduction

Vascular dementia (VD), a syndrome characterized by progressive cognitive decline, is second to Alzheimer's disease (AD). It is estimated that 35.6 million people suffered from dementia (World Health Organization, 2012). And the total cost in 2010 was between $157 billion and $215 billion in US, which surpassed that of cancer and heart diseases (Hurd et al., 2013). Accumulated evidence indicated that oxidative stress is a risk factor contributing to the development of neurodegenerative diseases including VD (Iadecola, 2010). Mitochondria are both the primary source of reactive oxygen species (ROS) and first targets of oxidative stress, the damage of which may play a vital role in the pathogenesis of VD. Oxidative stress-induced brain mitochondrial dysfunction destroyed Ca^{2+} homeostasis and neurological function, leading to cognitive impairments in animal models of cerebral ischemia (Christophe and Nicolas, 2006).

Acupuncture, as an important non-drug therapy, has been progressively accepted by both practitioners and patients worldwide. Previous clinical and experimental studies demonstrated that acupuncture could improve cerebral ischemia-induced cognitive impairments (Zhang et al., 2015; Liu et al., 2013). Moreover, the cognitive improvement might be achieved by reversing brain mitochondrial dysfunction in multi-infarct dementia rats (Zhang et al., 2014). Reduction in cerebral blood flow resulted from cerebral ischemia could lead to selective neuronal injuries in vulnerable regions of the brain, especially the hippocampus, which has been shown to be involved in learning and memory processes, and to be particularly important for spatial navigation and spatial memory (Román et al., 2010). However, whether hippocampal mitochondria are associated with this reversal effect in bilateral common carotid arteries occlusion (BCCAO) model remains unclear. Therefore, we used

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BCCAO model to investigate whether acupuncture-induced cognitive improvement might be associated with amelioration of hippocampal mitochondrial dysfunction in VD rats.

2. Experimental procedures

2.1. Animals

Adult male Wistar rats weighing 270–320 g, purchased from Vital River Laboratories (Beijing, China), were housed in a temperature-controlled environment on a 12-h light/12-h dark cycle, with access ad libitum to food and water. All experiments were approved by the Laboratory Animal Care Committee of the Tianjin University of Traditional Chinese Medicine and complied with the requirements of the Provisions and General Recommendations of Chinese Experimental Animal.

2.2. Bilateral common carotid arteries occlusion

The vascular dementia model was established by BCCAO. Rats were anesthetized with chloral hydrate (35 mg/100 g intraperitoneal injection). A ventral midline skin incision with 2–3 cm was made in the neck area and the bilateral common carotid arteries were then exposed respectively and gently separated from the vagus nerve. After that, each artery was double ligated with silk suture (5–0). Rats of sham-operated group ($n = 14$) were subject to the same operation without artery occlusion as a control. After surgery, rats were placed under a heating lamp for the prevention of hypothermia until these rats regained consciousness. In our trials, all the death of rats occurred during the first 3 days after surgery and the mortality of animals was 30%.

2.3. Acupuncture treatment

Three days after undergoing surgery, the surgery group was randomly divided into the following three groups ($n = 14$ rats per group): impaired group; acupuncture group, and placebo-acupuncture group. Acupuncture and placebo-acupuncture groups were given acupuncture treatment once daily for 2 weeks, with a rest on the seventh day, for a total 12 treatments. For acupuncture rats, one-off sterile, 0.3 mm × 40 mm, acupuncture needles (Hwato, China) were penetrated into *Baihui* (GV-20, midline of the head and approximately midway on the line connecting the apices of the auricles) and bilateral *Zusanli* (ST-36, 5 mm distal to the head of the fibula beneath the stifle and 2 mm lateral to the tibial tuberosity). The needles were twisted 2 times per second for 30 s respectively. Placebo-acupuncture rats were given acupuncture treatment at the bilateral hypochondrium (10 mm above iliac crest) for 45 s. The stimulation duration of placebo-acupuncture rats was equal to that of acupuncture rats. The rats in sham-operated group and impaired group were performed to same amount of capture stimulation as the acupuncture treatment.

2.4. Morris water maze test

The Morris water maze (MWM) test, performed as previously described (Morris, 1984), was widely used to evaluate the spatial learning and memory function. It consists of a large circular pool with 160 cm in diameter, 60 cm in height and water at 23 ± 1 °C. The pool was divided into four quadrants (northeast, northwest, southeast and southwest). A removable hidden platform (10 cm in diameter) located in the target quadrant (northwest) at a depth of 2 cm below the surface of the water. Each rat was subjected to three training trials per day for five consecutive days. It was given 90s to search for the platform. Once the rat located at the submerged platform, it was allowed to remain on it for 10 s and the latency to escape was recorded. If the rat failed to find the platform within 90 s, it was guided to the platform and placed onto it for 10 s, and the latency time was recorded as 90 s. After each trial the rats were placed back into their home cages and then relaxed for 10 min before the next trial began. On day 6, the probe trial was conducted by removing the platform and the rats were permitted to swim freely for 90 s. Quadrant dwell time in the target quadrant was recorded.

On day 7, the visible platform test was performed to assess animals' visual abilities. The platform was placed 1.5 cm above the surface of water.

2.5. Measurement of ROS level

As described previously (Vergun et al., 2001), ROS level was detected using the oxidative fluorescent dye, DHE (GENMED). After anesthesia, the brain was rapidly removed and placed on the ice bag. A bamboo chip was used to separate hippocampus from the brain. Hippocampus tissue samples were placed in a manual teflon homogenizer to homogenize with 10 ml saline solution. After centrifugation, DHE reagent was added in the obtained supernatant. The resulting suspension should be protected from light and incubated at 37 °C for 30 min. The fluorescence intensity was determined in a fluorescence spectrophotometer with the wavelength at 540 nm excitation and 590 nm emission.

2.6. Mitochondrial isolation

Hippocampal mitochondria were isolated from rats as previously described (Irwin et al., 2008). The hippocampus in the brain were rapidly separated and minced with scissor. After that, the pieces were placed in a manual teflon homogenizer to homogenize with ice-cold mitochondrial isolation buffer (70 mM sucrose, 210 mM mannitol, 1 mM EDTA, 2 mM HEPES and 0.5 g/L BSA, pH 7.4). The resulting hippocampal homogenates were centrifuged at 1500 × g for 15 min at 4 °C. The supernatant was centrifuged at 12,000 × g for 15 min at 4 °C, and the obtained mitochondrial pellet was resuspended in 300ul of BSA-free isolation buffer. The mitochondrial protein content was measured by the BCA method. The suspended mitochondrial samples were used immediately for measurements of respiratory function and membrane potential or stored at −80 °C for respiratory complex enzymes activities and protein expression.

2.7. Measurement of mitochondrial respiration

Mitochondrial oxygen consumption was measured at 25 °C using Oxygraph-2k (Oroboros, Austria). A suspension of brain mitochondria containing 100ug protein was placed in 2 ml respiratory buffer (250 mM sucrose, 10 mM HEPES, 1 mM EGTA, 1 mM EDTA, 10 mM succinate, 10 mM K_2HPO_4, 5 mM $MgCl_2$, 2 g/L BSA, pH 7.4) to yield a final concentration of 50ug/ml. After 40 min baseline recording, mitochondria were energized by the addition of succinate (10 mM) as substrates. State 3 (ST3) respiration was stimulated by the addition of ADP (90 uM). When all the ADP was converted to ATP, state 4 (ST4) respiration was measured. The oxygen consumption was expressed in nmol oxygen/min/mg protein. Mitochondrial respiratory control rate (RCR) was determined as the ratio between oxygen consumption/min in ST3 and oxygen consumption/min in ST4.

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