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Effects of acupuncture on declined cerebral blood flow, impaired mitochondrial respiratory function and oxidative stress in multi-infarct dementia rats

Xuezhu Zhang [1], Bangqi Wu [1], Kun Nie, Yujie Jia, Jianchun Yu *

First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China

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ABSTRACT

Brain energy disorders and oxidative stress due to chronic hypoperfusion were considered to be the major risk factors in the pathogenesis of dementia. In previous studies, we have demonstrated that acupuncture treatment improved cognitive function of VaD patients and multi-infarct dementia (MID) rats. Acupuncture therapy also increased the activities of glycometabolic enzymes in the brain. But it is not clear whether acupuncture treatment compensates neuronal energy deficit after cerebral ischemic through enhancing the activities of glucose metabolic enzymes and preserving mitochondrial function, and whether acupuncture neuroprotective effect is associated with activations of mitochondrial antioxidative defense system. So, the effect of acupuncture therapy on cognitive function, cerebral blood flow (CBF), mitochondrial respiratory function and oxidative stress in the brain of MID rats was investigated in this study. The results showed that acupuncture treatment significantly improved cognitive abilities and increased regional CBF of MID rats. Acupuncture elevated the activities of total SOD, CuZnSOD and MnSOD, decreased the level of malondialdehyde (MDA) and superoxide anion, regulated the ratio of reduced glutathione (GSH) and oxidized glutathione (GSSG) in mitochondria, and raised the level of the respiratory control index (RCI) and P/O ratio and the activities of mitochondrial respiratory enzymes of MID rats. These results indicated that acupuncture treatment improved cognitive function of MID rats; and this improvement might be due to increased CBF, which ameliorated mitochondrial dysfunction induced by ischemia and endogenous oxidative stress system of brain.

1. Introduction

Vascular dementia (VaD) is one of the most common types of dementia in older adults, characterized by a progressive worsening of memory and other cognitive functions due to chronic, reduced blood flow in the brain. Besides cognitive impairment, patients with severe VaD present with disruption in their personal, social and vocational functioning (Roman, 2002). In recent years, the incidence of VaD rises exponentially for patients with cerebrovascular risk factors such as hypertension, cardiac disease, diabetes, smoking, alcoholism and hyperlipidemia. In the US and Europe, the ratio of VaD to AD (Alzheimer's disease) is thought to be 1:5, and dementia following stroke is thought to occur in one quarter to one third of cases of stroke. VaD is also a growing problem in China, with a prevalence of about 1–3%, similar to prevalence rates in the US and Europe (Dubois and Hebert, 2001).

Brain energy disorders and oxidative stress due to chronic hypoperfusion were considered to be the major risk factors in the pathogenesis of dementia. Hypoperfusion cause impairment of bioenergetics, which appears early and progresses rapidly, consistent with the primary defect. In addition, ischemia induces chronic hypoxic state, which causes the formation of a large amount of reactive oxygen species (ROS). Increased ROS production disrupts antioxidant defense and directly impairs mitochondrial homeostasis and energy production, which also appear to be a key factor in the development of VaD (Piantadosi and Zhang, 1996).

Glucose is the primary fuel for the brain and sufficient energy support is essential to maintain normal brain function (Laughlin, 2004). An interruption of blood flow to the brain causes energy shortage, resulting in neuronal injury and death, even long-term cognitive decline. GLUT1 mainly expresses on both luminal and abluminal faces of CNS endothelium and responsible for transporting glucose from blood into the brain across the blood–brain barrier (Dick et al., 1984). The level of GLUT1 is regulated by glucose concentration, ischemia and hypoxia (Baumann et al., 2002; Vemula et al., 2009). Enhanced GLUT1 expression has neuroprotective

* Corresponding author. Fax: +86 22 27432227.
E-mail address: zhangxuezhu1999@126.com (J. Yu).
[1] These authors contributed equally to this work.

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properties during cerebral ischemia due to transport more glucose into the brain to meet its metabolic needs (Espinoza-Rojo et al., 2010).

Acupuncture is a branch of traditional Chinese medicine and has been proven effective in treating VaD. Based on his clinical experience, Professor Jingxian Han thought that age-related decline in activity of "qi" within Sanjiao could cause abnormal changes in functions of many tissues and organs, which could eventually lead to dementia (Han, 2007). Therefore, he created the "Sanjiao" acupuncture method to regulate abnormal function of qi activity within Sanjiao and the prescription included "Danzhong" (CV17), "Zhongwan" (CV12), "Qihai" (CV6), bilateral "Xuehai" (SP10) and "Zusanli" (ST36) five acupoints. In the past few years, we gave acupuncture treatment to more than 200 VaD patients and most of them benefited from the therapy. Evaluated by MMSE, HDS-R and ADL exams, these patients showed remarkable improvements in memory, orientation, calculation and self-care abilities (Yu et al., 2006). In our previous studies, we have demonstrated that acupuncture therapy could improve the cognitive function of multi-infarct dementia (MID) rats, and increase the activities of glycometabolic enzymes (e.g., hexokinase, pyruvate kinase, and glucose 6 phosphate dehydrogenase) in the cortex (Zhao et al., 2011). However, it is not clear whether acupuncture treatment can compensate neuronal energy deficit through enhancing the activities of glucose metabolic enzymes and preserving mitochondrial function after cerebral ischemic, and whether acupuncture neuroprotective effect is associated with activations of mitochondrial antioxidative defense system. So, in this paper, the effect of acupuncture on the cerebral blood flow, mitochondrial respiratory function and oxidative stress of MID rats was studied to explore its anti-dementia mechanism further.

2. Material and methods

2.1. Animals

Sixty healthy male Wistar rats (300–320 g) were randomly assigned into the following three groups: normal control group (Gn, $n = 10$), sham-operated group (Gs, $n = 10$) and operated group ($n = 40$). The animals were housed under standard conditions and allowed free access to water and food. All animals were maintained in accordance with principles of laboratory animal care (*NIH publication No. 86-23, revised 1985*) and *Guide for the Care and Use of Laboratory Animals, revised 200*6 (*Ministry of Science and Technology of the People's Republic of China*), and animal protocols were approved by the Laboratory Animal Care Committee of the Tianjin University of Traditional Chinese Medicine.

Before the surgery, 10 mL whole blood was collected from a Wistar rat, dried at 37 °C and ground into microemboli with diameter of 100–200 μm. After anesthetized, the bifurcation of the right common carotid and external carotid artery of rats was exposed and a temporary clip was applied to the external carotid artery just above its origin. The embolic insult was induced in rats in operated group by injecting 0.3 mL of 3% microemboli saline suspension into the internal carotid artery over 1–2 mins. Sham-operated rats received carotid injections of 0.3 mL of physiologic saline. Rats were allowed to recover for 1 week and three animals were dead during the recovery period.

One week later, all the animals were given hidden platform trials for five consecutive days. And the operated rats were further randomly divided into impaired group (Gi), acupuncture group (Ga) and sham-acupuncture group (Gsa) (ten rats in each group) according to their performance in Morris water maze test. After that, all the animals were handled according to their groups. The Ga group was given treatment with the "Sanjiao" acupuncture method once daily for 21 days and the location of acupoints was shown in Table 1. One-off sterile acupuncture needles (Huatuo, Suzhou Medical Instruments Factory, Suzhou, China), with a length of 13 mm and diameter of 0.14 mm, were used in the trial. After sterilization with iodine, needles were inserted horizontally 2–3 mm into CV17; 3–4 mm perpendicularly into CV12, CV6 and ST36; 2–3 mm obliquely into SP10, and rotated 2–3 times per second clockwise for 30 s respectively. The Gsa rats were needled at the two points located at the hypochondrial region of the body for 105 s per point in the same way as the Ga group. The rats in the other groups were grasped in the same manner and for the same periods of time as the Ga group.

At the end of the treatment, all the animals were trained in Morris water maze again to evaluate their cognitive function, including two hidden platform trials per day for 2 days and a probe trial. The details of surgical procedure, Morris water maze test, the anatomical location of acupoints and acupuncture treatment have been described in our previous studies (Liu et al., 2006; Yu et al., 2005).

2.2. Determination of regional cerebral blood flow

After Morris water maze test, all the rats were initially anesthetized with isoflurane in a mixture of 70% N_2 and 30% O_2 (5% induction, 2% maintenance). Anesthesia depth was assessed by testing the corneal reflexes and motor responses to the tail pinch. Femoral artery was catheterized for arterial pressure and heart rate (PowerLab, ADInstruments) and for blood sampling. Animals were artificially ventilated with an O_2–N_2 mixture and the O_2 concentration in the mixture was adjusted to provide an arterial PO_2 of 120–150 mm Hg. End-tidal CO_2, monitored by a CO_2 analyzer (Capstar-100, CWI), was controlled at 33–35 mm Hg. A heating lamp thermostatically controlled by a rectal probe was used to maintain the rat body temperature at 37 ± 0.5 °C. Throughout the experiment, two samples (50 μL) of arterial blood were collected for blood gas analysis.

Regional CBF was measured with a DRT4 laser Doppler blood flow and temperature monitor (Moor Instruments Inc). A small area of skull approximately 2 mm posterior to the bregma and 2 mm lateral to the sagittal suture was thinned to allow placement of the laser-Doppler probe. The tip of the probe (Φ 2 mm) was stabilized to the thin-skull window using a tissue adhesive (Aron Alpha; Toa, Tokyo, Japan). After achieving a stable baseline, the CBF was continuously measured for 10 min. Zero value for CBF was determined by the end of the experiment, when the heart was stopped with an overdose of isoflurane.

2.3. Studies in isolated mitochondria

After the measurement of CBF, the brain was removed from rats, washed and minced in isolation buffer (25 mM sucrose, 75 mM mannitol, 1 mM EGTA) before being homogenized. Mitochondria were obtained by differential centrifugation, and the protein content was measured by the BCA method. Markers of oxidative damage and activity of antioxidant enzymes in isolated mitochondria were determined using the respective kits (Nanjing Jiancheng, China). Oxygen consumption was measured using a Clark type oxygen electrode (YSI 5300, Yellow Springs, Ohio, USA). State 4 respiration was evaluated in the presence of succinate plus rotenone or with sodium glutamate and sodium malate. State 3 respiration was stimulated by the addition of ADP. Respiratory control index (RCI) was calculated as the ratio of state 3/state 4. The P/O ratio was calculated from the added amount of ADP and total amount of oxygen consumed during state 3. The activities of mitochondrial respiratory chain enzyme complexes [complex I (NADH-ubiquione oxidoreductase), complex II (succinate-ubiquinone oxidoreductase), and