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Research report

Electroacupuncture improves neurobehavioral function and brain injury in rat model of intracerebral hemorrhage

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ABSTRACT

Acupuncture has been widely used as a treatment for stroke in China for a long time. Recently, studies have demonstrated that electroacupuncture (EA) can accelerate intracerebral hemorrhage (ICH)-induced angiogenesis in rats. In the present study, we investigated the effect of EA on neurobehavioral function and brain injury in ICH rats. ICH was induced by stereotactic injection of collagenase type I and heparin into the right caudate putamen. Adult ICH rats were randomly divided into the following three groups: model control group (MC), EA at non-acupoint points group (non-acupoint EA) and EA at Baihui and Dazhui acupoints group (EA). The neurobehavioral deficits of ICH rats were assessed by modified neurological severity score (mNSS) and gait analysis. The hemorrhage volume and glucose metabolism of hemorrhagic foci were detected by PET/CT. The expression levels of MBP, NSE and S100-B proteins in serum were tested by ELISA. The histopathological features were examined by haematoxylin-eosin (H & E) staining. Apoptosis-associated proteins in the perihematomal region were observed by immunohistochemistry. EA treatment significantly promoted the recovery of neurobehavioral function in ICH rats. Hemorrhage volume reduced in EA group at day 14 when compared with MC and non-acupoint EA groups. ELISA showed that the levels of MBP, NSE and S100-B in serum were all down-regulated by EA treatment. The brain tissue of ICH rat in the EA group was more intact and compact than that in the MC and non-acupoint groups. In the perihematomal regions, the expression of Bcl-2 protein increased and expressions of Caspase-3 and Bax proteins decreased in the EA group vs MC and non-acupoint EA groups. Our data suggest that EA treatment can improve neurobehavioral function and brain injury, which were likely connected with the absorption of hematoma and regulation of apoptosis-related proteins.

1. Introduction

Intracerebral hemorrhage (ICH), one of the most lethal stroke types, was characterized by high morbidity, mortality and disability rates (Adeoye and Broderick, 2010; Wang and Talkad, 2009). The incidence of ICH was about 24.6/100,000 people/year, even expected to double in the next 30 years (Aguilar and Brott, 2011; Aronowski and Hall, 2005). Beyond that, the mortality even approached 50% and about half of surviving patients were usually left with neurological disability (Qureshi et al., 2001). Despite conventional therapies including hematoma removal, edema attenuation and intracranial pressure reduction and promising preclinical assays consist of neuroprotective, anti-hypertensive and anti-inflammatory drugs are available, the effectiveness is far from satisfactory (Andaluz and Zuccarello, 2009).

Acupuncture, a traditional Chinese therapy methodolog with more than 3000 years history, has been widely applied for the treatment of various diseases, including stroke (Alexander et al., 2004; Bai et al., 2008). Electroacupuncture (EA), a novel, convenient and practical therapy based on acupuncture combining with modern electropathy, can not only quantify stimulus parameters but also enhance the curative effect, furthermore save manpower owing to the place of manipulating needles (Chang et al., 2014). So which has become more and more popular in both animal experiments and clinical practices. Previous studies have demonstrated that EA treatment exerted neuroprotective effects by suppressing neuron apoptosis (Cho et al., 2004; Yang et al., 2007), improving cognitive function (Lin et al., 2016) and accelerating angiogenesis (Luo et al., 2013) in ICH rat brains, but there have been few reports involving the effect of EA on ICH. In this study, we evaluate

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the treatment effect of EA stimulation at Baihui(GV 20) and Dazhui(GV 14) acupoints on ICH rats mainly by gait analysis system and ^{18}F FDG micro-PET/CT and investigate the corresponding mechanism preliminarily.

2. Materials and methods

2.1. Animal preparation

Adult male Sprague-Dawley rats, weighing 250–300 g and aged 8–10 weeks, were obtained from the SPF Laboratory Animal Center of Southwest Medical University. All of the animals were housed under controlled temperature (23 ± 2 °C) and lighting conditions (12 h light/12 h dark cycle) with free access to food and water. The experimental protocol was in accordance with the Guidance Suggestions for the Care and Use of Laboratory Animals, formulated by the Ministry of Science and Technology of the People's Republic of China.

2.2. Induction of intracerebral hemorrhage

ICH rats were established by injecting collagenase and heparin according to the previous studies (Chiu et al., 2012; Del Bigio et al., 1996). Briefly, rats were anesthetized with 10% chloral hydrate (0.5 ml/100 g) via intraperitoneal administration, and then fixed on a stereotaxic frame (Stoelting Co, USA) in the prone position. Following the scalp incision, a small burr hole, located 0.2 mm anterior and 3.0 mm lateral to the bregma, was drilled on the right cranial bone, then inserted a 5-μl Hamilton syringe with a deep of 6.0 mm to the bregma. Type I collagenase (2 μl, 0.125 U/μl, Sigma) and Heparin (1 μl, 2 U/μl) were slowly injected into the right caudate putamen at a speed of 0.03 μl/min by using the syringe. Following infusion, the syringe was maintained in the same place for 10 min and subsequently withdrawn slowly in order to minimize back-flow. The borehole was sealed with bone wax, and the wound was sutured.

2.3. EA stimulation

After the operation, the animals with functional deficits were randomly divided into the following three groups (n = 5 in each group): model control group (MC), EA at non-acupoint points group (non-acupoint EA) and EA at Baihui (GV20) and Dazhui (GV14) acupoints group (EA). At 2 days following ICH, rats of EA group received EA treatment for 10 min once daily and 14 consecutive days. Acupoints were selected according to Experimental Acupuncture (Li, 2003). The acupoint Baihui (GV20), which is located in the center of the parietal bone, and Dazhui (GV14), which is located on the posterior midline and between the seventh cervical vertebra and the first thoracic vertebra. In EA group, two acupuncture needles were obliquely inserted into GV20 with a depth of 2 mm and vertically inserted into GV14 with a depth of approximately 5 mm respectively, and the needles were connected to the EA apparatus (G6805; SMIF, Shanghai, China), delivering a continuous wave at 3 Hz and 1 mA. In non-acupoint EA group, needles were inserted into right brachium and hip, received the same electrical stimulation.Rats of MC group were not treated with EA.

2.4. Behavioral testing

Modified Neurological Severity Scores (mNSS) (Schallert et al., 1997; Shohami et al., 1995) was used to evaluate the neurologic deficits of the rats at 2 h following the induction of ICH, 1d, 3d, 5d, 7d and 14d after treatment by an observer who was blinded to the experimental groups. The score is made up of motor, sensory (visual, tactile and proprioceptive), balance, and reflex tests. The degree of neurological deficits was graded from 0 to 18 (normal score, 0; maximal score, 18). The criteria were set as follows: 13–18, serious injury; 7–12, moderate injury; 1–6, slight injury. One point is awarded for the inability to perform the test or the lack of a tested reflex when scoring the severity of injury. That is to say, the higher the score, the more severe the injury.

2.5. Gait analysis

The Tread-Scan Gait Analysis System (Clever Sys. Inc., Reston, VA, USA) was used to obtain and evaluate the gait behaviors of the ICH rats on day 14 following treatment. In order to reduce lighting fluctuation, the lights on the TreadScan™ was turned on 3–5 min prior to testing. A background image was captured for data analysis before each rat was placed into the treadmill. Following the training (20s) and rest (1 min), the rat continued running in the TreadScan™ system with the speed set at 8 cm/s and the video of footprints of the rat was recorded for 20 s at 100 frames per second. Then the recorded file was analyzed with the Tread Scan software and the data were exported to Microsoft Excel.

The gait parameters measured in this study are as follows: stride length, the running speed, stance time and swing time, base of support (BOS), print area, foot pressure and stride number.

2.6. Positron emission tomography/computed tomography, PET/CT

PET/CT imaging was performed at day 14 after treatment by micro PET/CT (Siemense, German). All the rats were forbidden drinking and fasting overnight before PET/CT scanning. After anesthesia administration, the rats were injected with 0.5–0.8 mCi of 18F-fluorodeoxyglucose (^{18}F-FDG) via tail vein and returned to an warm cage for 30 min to allow for the brain uptake of ^{18}F FDG. Subsequently, they were placed in the prone position in a rat holder for brain PET imaging. Then the rat holder was placed on the PET/CT scanner bed and all animals had a CT scan after a PET scan immediately (the brain as the center). Lastly, the hemorrhagic lesion volume and its glucose metabolism was detected by analyzing the PET and CT imagings of rats.

2.7. Specimen preparation

Following final behavioral assessments and PET/CT images, the rats were sacrificed at day 14 post-treatment. Firstly, animals were deeply anesthetized with 10% chloral hydrate via intraperitoneal administration. Then, their hearts were exposed. Venous blood (3–5 ml each rat) was collected from the right atrium and placed for 30 min at 4 °C. The serum was separated from blood by centrifugation at 1500 rpm for 5 min at 4 °C and then kept at −20 °C for ELISA. The rats were perfused transcardially with 0.9% saline (200–250 ml) followed by ice-cold 4% paraformaldehyde (PFA) (200–250 ml) after serum collection. The brains were dissected and post-fixed in 4% PFA for 24 h, then sequentially dehydrated in 15% sucrose solution for 2d and 30% sucrose solution for 5–7d at 4 °C until sinking. The brains were cut into 1 cm segments coronally centered on the needle track. For frozen sections, tissues were embedded in tissue freezing medium (Sakura Finetek USA, Inc, Torrance), then quickly frozen and cut into serial coronal sections (8 μm thickness) with a freezing microtome (Leica, CM 1950) and were kept in frige at −20 °C.

2.8. ELISA

The contents of Myelin basic protein (MBP), Neuron-specific enolase (NSE) and Soluble protein–100 B (S100-B) in serum have a close relation with acute brain injury and can represent the severity of brain injury after ICH. The concentrations of the three proteins in serum were detected via the colorimetric ELISA kits method at day 14 post-treatment. The kits were taken out from the refrigeration environment and balanced 15–30 min in the room temperature. Different dilution of Standard and testing samples (5-fold dilution) were added to the ELISA plates coated with MBP, NSE and S100-B (Beijing Chenglin Biological