Complementary Therapies in Medicine 32 (2017) 85–90

Contents lists available at ScienceDirect

Complementary Therapies in Medicine

journal homepage: www.elsevier.com/locate/ctim

Scalp acupuncture attenuates neurological deficits in a rat model of hemorrhagic stroke

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ARTICLE INFO

Keywords:
Scalp acupuncture
Hemorrhagic stroke
Neurological deficit

ABSTRACT

Background: Hemorrhagic stroke accounts for approximately 15% of all stroke cases, and is associated with high morbidity and mortality. Limited human studies suggested that scalp acupuncture could facilitate functional recovery after cerebral hemorrhage. In the current study, we used an animal model of cerebral hemorrhage to examine the potential effects of scalp acupuncture.

Methods: Adult male Sprague-Dawley rats received autologous blood (50 μL) into the right caudate nucleus on the right side under pentobarbital anesthesia, and then received scalp acupuncture (DU20 through GB7 on the lesion side) or sham acupuncture (1 cm to the right side of the acupoints) (n = 10 per group). A group of rats receiving autologous blood into the caudate nucleus but no other intervention, as well as a group of rats receiving anesthesia but no blood injection to the brain (n = 10 per group) were included as additional controls. Composite neuroscore, corner turn test, forelimb placing test, wire hang task and beam walking were used to evaluate the behavior of rats. Hematoxylin and Eosin (HE) staining was used to observe the histopathological changes. Western blot was used to detect the content of tumor necrosis factor alpha (TNF-α) and nuclear factor-KappaB (NFκB) protein expression.

Results: Scalp acupuncture attenuated neurological deficits ($p < 0.01$ or < 0.05 vs. sham acupuncture using a variety of behavioral tests) at 1–7 days after the treatment. The brain content of TNF-α and NFκB was decreased ($p < 0.01$ for both).

Conclusions: Scalp acupuncture could improve neurological deficits in a rat model of hemorrhagic stroke.

1. Introduction

Hemorrhagic stroke accounts for 15% of all stroke cases.[1] About 75% of the survivors have severe neurological deficits.[2] Patients with a small amount of bleeding in the basal ganglia (putamen, caudate and thalamus) typically survive, but are left with neurological deficits of varying degrees.[3] To date, no effective treatment has been shown to improve patient prognosis.[4]

Acupuncture is widely used as an alternative treatment of stroke patients in China. Acupuncture has multiple biological responses, including circulatory and biochemical effects.[5,6] The effects are mediated mainly by sensory neurons to many structures within the central nervous system.[7] Upon a literature search, we 13 randomized controlled trials (a total of 1575 participants) [8–20] that documented improvement of neurological deficits by acupuncture treatment; 5 of these studies were conducted in patients with ischemic stroke exclusively,[8,9,13,16,20] 3 were conducted in patients with hemorrhagic stroke,[10–12] and the remaining 5 including patients with ischemic as well as hemorrhagic stroke.[14,15,17–19] The quality of these trials, however, is generally poor. Only 1 trial had a sham acupuncture arm.[9] In the remaining 12 trials, effects of acupuncture were deduced from comparison with the baseline. The intervention was also heterogeneous: 1 trial used electrical stimulation, 7 trials used manual stimulation and the remaining 5 used a combination of manual and electrical stimulation. In addition, acupoints vary significantly across these studies. For studies involving scalp acupuncture (SA), the acupoints included DU20,[8,10–13,16–18,20] DU24[17,18,20] and EX-HN1.[12,13,15–17,19,20] Heterogeneity in other aspects also existed. Nevertheless, in the 5 trials that assessed neurological function using the Barthel index[8,13,16,17,20] (an index that focus on dependency) (involving 1124 participants), significant improvement in dependency by acupuncture was noted ($p < 0.05$ or $p < 0.01$). The largest trial by

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http://dx.doi.org/10.1016/j.ctim.2017.03.014
Received 5 February 2017; Received in revised form 21 March 2017; Accepted 21 March 2017
Available online 19 April 2017
0965-2299/

Zhang, et al.[8] trial showed a tendency of decreased mortality and morbidity in the acupuncture plus standard care arm (80/385, 20.7%) than in the standard care arm (102/396, 25.8%) at 6 months (odds ratio, 0.75; 95% confidence interval, 0.54–1.05).

For acupuncture treatment of hemorrhagic stroke in China, Baihui (DU20) is the most commonly used site. A previous study in a rat model of hemorrhagic stroke indicated that SA could improve neurological function by affecting blood flow in various organs.[21] A meta-analysis of the studies in rat models of stroke indicated that SA at Baihui (DU20) could attenuate brain edema, protect neurons from insult, and ultimately attenuate neurological deficits.[22] These past studies indicated that SA could alleviate neurological deficits after hemorrhagic stroke. In this study, we used a rat model of hemorrhagic stroke (autologous blood injection into the brain) to examine the potential effects of SA (from DU20 through GB7 on the lesion side) on neurological functions.

2. Materials and methods

2.1. Animals

All experiments were approved by the Animal Care and Use Committee of Heilongjiang University of Chinese Medicine and conducted in compliance with the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH; publication Nos. 85-23; 1996). All efforts were made to minimize animal suffering. The experiments were conducted using 40 adult male Sprague-Dawley rats (300 ± 10 g; purchased from Harbin Veterinary Research Institute). Rats were maintained under a 12 h/12 h light cycle at 21 ± 2 °C and 50 ± 5% relative humidity, with unlimited access to standard food and water.

2.2. General experiment design

Rats were anesthetized with pentobarbital (60 mg/kg; intraperitoneal injection), and fixed on a stereotactic frame (STW-3X, Chengdu Instrument Factory, Sichuan, China). A midline incision was made to the scalp to expose the anterior fontanelle and coronal sutures. A 1-mm diameter burr hole (0.2 mm posterior and 3.5 mm lateral to bregma) was made using a dental drill. Autologous blood (50 μL) was injected at a speed of 20 μL/min into the right caudate putamen at the following coordinates: AP: −0.24 mm, L: 3.5 mm, D: 6 mm[23] The microinjector needle was left in place for 5 min prior to withdrawal. The burr hole and incision were closed with dental cement and suture.

Starting from post-operative day 1, rats received SA (DU20 through GB7 on the lesion side; distance: 1.5 cm) or sham SA (from 1 cm to the right of DU20, for 1.5-cm along the nostril direction; Fig. 1) (n = 10 per condition). Two sessions of SA (each lasting 30 min) were conducted daily for 7 consecutive days. The SA was conducted manually using a stainless steel needle (0.35mm × 40 mm, HuaTuo Brand, Suzhou Medical Appliance, Suzhou, Jiangsu, China) in fully-awake rats with minimal restraint. For each 30-min session, needling (approximately180–200 r/min) was carried out for 3 bouts, each lasting for 5 min. A group of rats receiving blood injection into the brain but no acupuncture at all, as well as a group of rats receiving sham surgery but no blood injection into the brain were included as additional controls (n = 10/group).

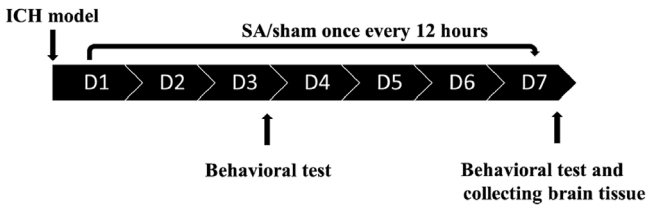

Fig. 1. The general design of experiment. Experiment period of each rat in the four groups was 7 days; Behavioral tests were evaluated at two time points: d3 and d7. SA or sham SA was administered from the d1 to d7 once every 12 h. On the seventh day, after the second administration of behavior test, rats were perfused and then the brain tissues were collected for western blot analysis and histopathologic examination. SA, scalp acupuncture; sham SA, sham scalp acupuncture.

2.3. Behavioral testing

Behavioral testing included a composite neurological scale, corner turn test, forelimb placing test, wire hang task and beam walking, as described by Krafft et al. [24]

The composite neuroscore consisted of the following 7 items, with a total score in the range from 3 to 21 (Table 1): I) Spontaneous activity: rats were observed for 5 min in home cage, assessing their ability to approach all four walls of the cage; II) Symmetry of limb movement: rats were suspended by the tail to observe movement of the limbs; III) Lateral turning: rats were suspended by the tail to observe the turning reaction upon stroking each side of the body; IV) Forelimb outstretching: rats were suspended by the tail to assess walking on forelimbs; V) Axial sensation: tactile stimuli were employed on each side of the rat trunk using a cotton swap to observe response on both sides; VI) Vibrissae proprioception: a cotton swap was touched the vibrissae gently on each side from the rear of the animal towards its head; VII) Climbing: rats were placed on a rough surface (22 × 44 cm) at a 45° angle.

For the corner turn test, rats were allowed to proceed into a 30 °corner. To exit the corner, the rat could turn either to the right or left. The turning was assessed 10 times with 30-s interval. The score was calculated as number of left turns/all turns ×100. Only turns involving full rearing along either wall were included.

For the forelimb placing test (also known as vibrissae-elicited forelimb placing test), rats were held by the trunk, and slowly moved up and down to facilitate muscle relaxation. The vibrissae were induced by brushing on the edge of a table surface. Healthy rats place the forelimbs to stimulate ipsilateral vibrissae. The procedure was repeated 10 times. The score was calculated as number of successful forelimb placements/all times × 100.

In the wire hang task, rats were placed midway on a wire (50 × 0.15 cm) mounted between two platforms at 40 cm above the ground. The performance was observed for a maximum of 1 min, and scored using a six-point scale. Each testing session consisted of 3 trials.

For the beam walking, rats were placed midway on a horizontal rod (90 × 1 cm) mounted on two platforms at 40 cm above the ground. The performance was observed for a maximum of 1 min and scored using a six-point scale. Each testing session consisted of 3 trials.

2.4. Histopathologic examination

Upon the completion of behavioral experiments, rats were anesthetized with pentobarbital overdose. The brain was removed rapidly for Western blot analysis (n = 5/group) or perfused with 4% paraformaldehyde and cut into 4-μm slices for routine hematoxylin and eosin staining and subsequent observed the cell structure in the semi dark area under a microscope (400×).

2.5. Western blot assay

Proteins were extracted from tissue samples using a RIPA lysis buffer, separated with SDS-PAGE, and transferred to PVDF membranes. After blocking with 5% skimmed milk, membranes were incubated with a rabbit TNF-α polyclonal antibody (1:1000; Abcam, ab6671, Cambridge, MA, USA), a rabbit NFκB monoclonal antibody (1:1000; Abcam, ab32360) or a rabbit GAPDH polyclonal antibody (1:2000; Abcam, ab9485) overnight at 4 °C, and then with a goat-anti-rabbit IgG labeled with HRP (1:2000, Abcam, ab6721) at room temperature for 2 h. After extensive washing, the blots were developed using an ECL method (Beyotime, Jiangsu, China) and visualized using a chemilumi-