Effects of different frequencies of electro-acupuncture at Shuigou (GV 26) on recovery of motor function in rats with focal cerebral ischemic injury

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Abstract

OBJECTIVE: To investigate the effects of different frequencies of electro-acupuncture at Shuigou (GV 26) on the latent period and wave amplitude of motor evoked potentials (MEPs) in rats with focal cerebral infarction.

METHODS: Fifty healthy male Wistar rats were randomly divided into five groups: controls, model, 2 Hz Shuigou, 50 Hz Shuigou and 100 Hz Shuigou. There were 10 rats in each group. Using a modification of a technique for middle cerebral artery occlusion, focal cerebral ischemic injury was induced in all rats except those in the control group. The rats in the control group received no treatment. After behavioral deficit had been evaluated using the Zausinger 6-point neurological function score, the rats in the Shuigou groups underwent acupuncture and continuous wave stimulation at a frequency of 2 Hz, 50 Hz or 100 Hz (intensity 1 mA) for 10 min twice daily for 3 days. The control and model groups underwent no intervention. Zausinger 6-point neurological function score and MEPs were measured 72 h after the start of treatment.

RESULTS: The neurological function scores of the three Shuigou groups were significantly higher than those of the model group ($P<0.05$). There was no significant difference between sides in the latency and amplitude of MEPs in the model group ($P>0.05$). The latency on the affected side in the model group was significantly longer than that in the control group ($P<0.05$) and the amplitude on affected side was significantly reduced ($P<0.01$). After 3 days of electro-acupuncture, the latency on the affected side in the 2 Hz Shuigou group was significantly shortened ($P<0.05$) and the amplitude was significantly increased ($P<0.05$) compared with the model group.

CONCLUSION: Low frequency electro-acupuncture at Shuigou (GV 26) can promote recovery of motor function after focal cerebral ischemic injury in rats.

INTRODUCTION

In recent years, much research has shown that electro-acupuncture at Shuigou (GV 26) plays an impor
Motor evoked potentials (MEPs) are action potentials conducted along the descending tracts that reflect the excitability of the cortex and corticospinal tract. MEPs are commonly used in clinical practice and experimental research as an investigative tool to predict motor function recovery after sudden stroke and to determine the effects of different therapeutic approaches. In the present study, a model of middle cerebral artery occlusion (MCAO) was used to explore changes in motor cortex excitability after cerebral infarction, and the effect of electro-acupuncture at Shuigou (GV 26) on cortical excitability.

Materials and Methods

Experimental animals and groups

Adult male Wistar rats (specific pathogen-free animals), weighing 200 – 250 g and aged 4 – 8 weeks, were supplied by Wei Tong Li Hua Experimental Animal Technology Co., Beijing, China (Experimental Animal Quality Certificate Number: SCXK (Beijing) 2007-0001; Laboratory Animal License Number: Tianjin No. 007). Fifty experimental animals were divided randomly into a control group, a model group, and 2 subgroups. The rats in the control group did not receive any intervention. The animals were housed at a controlled temperature (20 ± 1°C), – 19:00), with free access to food and water.

Main instruments and reagents

Transcranial electrically stimulated MEPs were recorded using a Biopac MP150 multichannel physiological signal recorder (Biopac Systems, Inc., Goleta, CA, USA). Data and images were acquired and analyzed using Acknowledge 3.8.1 software with the Biopac MP System (Biopac Systems, Inc., Goleta, CA, USA). A HANS-200E acupoint nerve stimulator (YZB/SU0049-2008; Nanjing Ji-sheng Medical Technology Co. Ltd, Suzhou, China) was used for electro-acupuncture treatment. A stereotaxis instrument (MyNeuroLab; Leica Biosystems Richmond, Inc., Richmond, IL, USA), dental drill (Strong 90, Saeshin Precision Co. Ltd, Daegu, Korea), acupuncture needles (Hwato, 0.32×30 mm, Suzhou Medical Appliance Factory Co. Ltd, Suzhou, China) and chloral hydrate (AR; Chemical Regent Co. Ltd, Tianjin, China) were also used.

Establishment of MCAO model

An MCAO model was established using a modification of Longa’s method[6]. The rats were fasted overnight but allowed free access to water before the experiment. Anesthesia was induced with 10% chloral hydrate (3 mL/kg i.p.). The animals were fixed on the surgery board in the supine position and the surgical area on the neck was sterilized. A 1.5 – 2 cm incision was made on the left of the midline of the neck. The subcutaneous fascia was dissected to expose the left triangular area between the sternomastoid muscle and the sternohyoideus muscle. The left common carotid artery (CCA) and external carotid artery (ECA) were dissected carefully from the surrounding nerves and fascia. The ECA was ligated with No. 0 surgical suture and artery clips were used to occlude the bifurcation and the proximal end. The CCA was pricked on the proximal end with a 1 mL syringe, and then a nylon suture of 0.260 mm in diameter was inserted into the pinhole on the vessel. The suture was advanced towards the bifurcation; when the tip arrived at the bifurcation, the artery clip was loosened and the suture was advanced into the internal carotid artery until a slight resistance was felt. At this point, the suture entirely closed the opening of the MCA to a depth of 18 – 20 mm into the intracalvarium, blocking the MCA blood flow. After advancing the suture into the circle of Willis, the CCA and the pinhole were ligated to prevent bleeding, then the other artery clip was loosened and the operative wound was closed.

MCAO surgery was performed in the rats in the model group and the Shuigou groups. The rats in the control group did not receive any intervention. The animals were housed at a controlled temperature (20 – 25°C), and under diurnal lighting conditions (07:00 – 19:00), with free access to food and water.

Neurological function score

Neurological deficits were assessed by a person blinded to the MCAO surgery and the electro-acupuncture treatment. The neurological function of all animals was evaluated after the MCAO surgery and after 3 days of electro-acupuncture using the Zasungger[7] 6-point neurological function score. The criteria for scoring were as follows: 0 points – rats without any spontaneous motion; 1 point – rats that could move freely with circling behavior towards the paretic side; 2 points – rats
that moved on the floor with circling behavior towards the paretic side if the tail was held; 3 points – resistance to lateral pressure toward the paretic side was decreased; 4 points – the forelimb of the paretic side could not be flexed or the whole body was flexed to the paretic side; 5 points – no neurological deficit. Rats that scored 1–3 were regarded as having a focal cerebral ischemic injury and were used in the subsequent experiments.

**Electro-acupuncture treatment**

Shuigou (GV 26) was located at the cleft lip 1 mm below the nasal tip with reference to the "Animal Points Atlas" of the Chinese Acupuncture and Moxibustion Association. It was punctured with a needle inserted obliquely upward into the nasal septum for 3.0 mm, and electro-acupuncture was administered using an acupoint nerve stimulator (HANS-200E; Nanjing Jensun Medical Technology Co. Ltd., Nanjing, China; continuous-wave; stimulating voltage 1.5 V; current 0.1 mA; frequency 2 Hz, 50 Hz or 100 Hz). The duration of stimulation was 10 min, twice daily for 3 days. The rats in the control group and the model group received no intervention.

**Transcranial electrical stimulation and recording of MEPs**

Seventy-two hours after confirmation of MCAO, the rats were anesthetized with 10% chloral hydrate (3 mL/kg i.p.) and fixed on a stereotaxic apparatus. After conventional disinfection, the skull was exposed and, using a desktop dental drill, holes 2 mm in diameter were drilled on both sides, 1.0 – 2.0 mm posterior to the coronal suture and 1.0 – 2.0 mm to the sagittal suture. An adjustable heating pad was placed under the chest and abdomen of the rat; meanwhile, the back of the rat was illuminated with an incandescent light to maintain the body temperature. Monopolar transcranial stimulation was performed to obtain MEPs. A silver chloride electrode of 1 mm in diameter was placed perpendicularly on the surface of the left motor cortex as the stimulus anode. A cathode electrode was held to the tip of the tongue by a fish tail clip. A concentric needle electrode was then inserted into the gastrocnemius muscle belly perpendicular to the muscle fibers as the recording electrode. The electrode handle was fixed on the stent of the micro-thruster and the concentric needle was advanced about 2 – 5 mm into the gastrocnemius muscle. A metal 6-syringe needle was inserted across the cervical subcutaneous tissue as the grounding electrode. DC square wave electrical pulses were delivered from the stimulation module for transcranial stimulation. Parameter settings were: filter band 1.0 – 5 kHz; amplification 1000; sample rate 20 kHz; pulse duration 2 ms; stimulus interval 4 s; stimulating voltage 0.4 V; and current 20 mA. The main outcome measures were the waveform of the MEPs, latency and amplitude. Both left and right sides were stimulated and recorded.

**Signal analysis**

Latency was represented by the period from the beginning of electrical stimulation to the moment that the signal deviated from the baseline (Figure 1, T). The amplitude was represented by the first peak-to-peak amplitude (Figure 1, P-P). A cascade of 10 MEPs evoked by transcranial electrical stimulation was recorded on each side. The latencies and amplitudes of the 10 waveforms were measured and the average values were calculated for the experimental analysis.

**Statistical methods**

SPSS 13.0 statistical software was used for processing the data, which were expressed as mean ± standard deviation (x ± s). One-way analysis of variance was used for multiple comparisons among sample means. The least significant difference t-test was used for comparison between two experimental groups. The rank test was used for neurological function scores. P<0.05 was regarded as statistically significant.

**RESULTS**

**Quantitative analysis of experimental animals**

The experiment studied 50 healthy male Wistar rats. There was no loss of animals in the control group. However, five rats died (two in the model group and three in the Shuigou groups) during the experiment; these were replaced by a further five randomly sampled animals.

**Effect of electro-acupuncture at Shuigou (GV 26) on neurological function score**

There was no significant difference in neurological function score between the model group and the three Shuigou groups before the electro-acupuncture treatment (P=0.081), indicating that all rats had the same baseline neurological deficit. After 3 days, neuronal function scores had increased in the model group and the three Shuigou groups, but the latter were all significantly higher than the former (all P<0.05) (Figure 2).

**Effect of electro-acupuncture at Shuigou (GV 26) on MEP latency**

There was no significant difference in MEP latency between the two sides in the control group rats (P>0.05). The latencies on both sides were longer in the model group than in the control group, with a significant diff-
ference on the affected side \((P<0.05)\). After 3 days of electro-acupuncture at Shuigou (GV 26), the latency on the affected side was significantly decreased in the 2 Hz group compared with the model group \((P<0.05)\).

There was no significant difference in latency between the 50 Hz and 100 Hz groups and the model group (both \(P>0.05\)) (Table 1).

**Effect of electro-acupuncture at Shuigou (GV 26) on MEP amplitude**

There was no significant difference in MEP amplitude between the two sides in the control rats \((P>0.05)\). On both sides, the amplitude in the model group was significantly lower than that in the control group \((P<0.05)\). After electro-acupuncture at Shuigou (GV 26), the amplitude on the affected side was significantly increased in the 2 Hz group compared with the model group \((P<0.05)\). There was no significant difference in amplitude on either side in the 50 Hz and 100 Hz groups compared with the model group (both \(P>0.05\)) (Table 2).
DISCUSSION
Most researchers consider transcranial electrically stimulated MEPs to be action potentials conducted along the corticospinal tract and rubrospinal tract in the anterior and anterolateral funiculus of the spinal cord. Latency reflects nerve conduction velocity and the wave amplitude reflects the number of neurons discharged. Previous studies indicate that injury to the corticospinal tract after MCAO not only damages the conductive structure, but also changes its electrophysiological properties. Ischemic injury can lead to abnormal MEPs due to effects on the function of motor cortical neurons and the pyramidal tract. Abnormal MEPs manifest in two principal forms: 1) defects of waveform or reduction of amplitude, indicating decreased cortex stimulus-response sensitivity; and 2) prolongation of conduction time, indicating obstruction of the central motor conduction pathway.

MEPs comprise a series of unstable waveforms of varying latency and amplitude (Figure 1). Latency is shortened and amplitude increased with increasing intensity of stimulation, but both are relatively stable near the threshold for electrical stimulation. Our study showed that the first negative peak (N1) and the following positive peak (P1) were stable when the voltage reached 0.4 mV and the current 20 mA. It is speculated that when the intensity of an electrical stimulus is near the threshold for stimulation, the descending tracts in the spinal cord are in a saturated excited state, and the number of excited axons and neuron conduction velocity are unable to increase significantly with further increases in the intensity of stimulation.

Motor function was improved significantly in the 2 Hz Shuigou group compared with the other Shuigou groups (P<0.05), indicating that low frequency electro-acupuncture at Shuigou (GV 26) can, with time, improve the latency and amplitude of MEPs after cerebral infarction in rats. However, MCAO not only inhibited MEPs on the affected side, but also inhibited those on the healthy side to some extent. Bilateral MEP abnormality caused by a lesion on one side only is possibly due to the following. 1) Lesions in different locations and of differing extent produce different MEP abnormalities. If only nervous tissue is damaged, nerve impulses can still be conducted by other paths. However, lesions affecting the motor center and motor pathway will lead to MEP abnormalities. 2) Because the two sides of the cerebral hemisphere are connected by the corpus callosum, injury to the cerebral hemisphere on one side will cause dysbolism and impairment of brain electrical activity.

According to the "Handbook of Prescription for Emergency", Pinching Shuigou (GV 26) is the best way to save acute stroke patients. Shuigou (GV 26) is an acupoint of The Governor Vessel and crosses the brain, which is the Sea of Yang meridian; thus, it is a key point with a specific role in the treatment of stroke. Our research demonstrates that both latency and amplitude were improved to varying degrees after 3 days of treatment at Shuigou (GV 26), suggesting that electro-acupuncture at Shuigou (GV 26) can improve the electrophysiological characteristics of the motor tract. Other scholars have also found that electro-acupuncture has a therapeutic effect on cerebral ischemic injury. Studies of the mechanisms show that electro-acupuncture at Shuigou (GV 26) can stimulate the excitability of cortical neurons, facilitate synaptic transmission in the motor cortex and improve axonal transport in the subcortical pyramidal tract, reduce inhibition of the conduction of excitability, increase the number of neurons or nerve fibers participating in bio-electrical activity, and improve the excitability of brain nerve cells. Low frequency electro-acupuncture can regulate the release of cerebral neuropeptides, increase cerebral blood flow, reduce the ischemic penumbra of cerebral cortex neuron damage, and promote neuron repair and healing after ischemia, playing an important role in protecting the cortical center system.

Neuronal repair can reduce inhibition of transcranial electrical stimulation signals in nerve conduction, thereby shortening latency, and increase the number of firing neurons, resulting in a corresponding increase of amplitude.

In summary, preliminary studies suggest that low frequency electro-acupuncture can alleviate injury induced by cerebral ischemia, protect motor neurons and promote recovery of useful limb function in stroke patients, providing objective support for the use of acupuncture during the early stage of brain injury.

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