ORIGINAL ARTICLE

Study on the effect of electroacupuncture on the mechanism of action in rats with ischemic stroke

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Jia Q, Yinlan H, Jialu L, et al. Study on the effect of electroacupuncture on the mechanism of action in rats with ischemic stroke. J Biomol Biochem. 2019;3(1):1-4.

Objective: Hematoxylin and Eosin (H&E) staining and flow cytometry were used to observe the pathological morphology and apoptosis of rat neurons in different functional states to explore the mechanism of electroacupuncture treatment of ischemic stroke.

Method: Thirty male Sprague-Dawley rats were divided into three groups according to the random number table method: normal blank group, model control group, and acupoint group (Baihui, Hegu, Quchi, Zusanli, Sanyinjiao), 10 in each group. The model control group and the acupoint group were treated with focal internal cerebral ischemia model. Rats in the normal blank group and the model control group were fixed without acupuncture during treatment, once a day for 30 minutes, 10 times in a row, and samples were taken after 10 days. The acupoint group was treated with electroacupuncture at 48 hours after operation. The prescriptions were Baihui, Hegu, Quchi, Zusanli and Sanyinjiao. The other acupuncture points except Baihui were connected to the electroacupuncture instrument. Stimulation for once a day, 30 minutes each time, continuous acupuncture for 10 days, 10 days after the death to take the brain.

Results: Compared with the normal apoptotic rate in the normal blank group, the apoptosis rate of the model control group and the acupoint group increased at an early stage, and the expression of the model control group was higher than that of the acupoint group. The difference was statistically significant (P<0.05); Compared with the normal apoptotic rate in the normal blank group, the apoptosis rate of the model group and the acupoint group increased, and the expression of the acupoint group was lower than that of the model control group, the difference was statistically significant (P<0.05); Compared with the early and late apoptotic rate of the model control group, the apoptotic rate of the early acupoint group decreased, but the difference was not statistically significant (P>0.05), the late apoptosis rate decreased and the difference Statistical significance (P<0.05).

Conclusion: Electroacupuncture can treat ischemic stroke by inhibiting the apoptosis of nerve cells in the brain.

Key Words: *Electroacupuncture; Stroke; Apoptosis*

INTRODUCTION

Stroke is a syndrome of sputum, unconsciousness, half-length, slanting eyes, and unfavorable speech as the main symptoms [1,2]. Among them, ischemic stroke accounts for about 75% of the incidence of stroke, its onset is sudden, the onset is rapid, and the condition is very varied. Surviving patients often leave different levels of disability, which brings a heavy burden to society and families [3,4]. A large number of clinical practices have confirmed that electroacupuncture has a significant effect in the treatment of stroke sequelae, and acupuncture treatment has the characteristics of economy, green and safety [5-9]. However, there are few experimental studies on the mechanism of action of its treatment. H&E staining and flow cytometry were used to observe the differential expression of neuronal apoptosis in healthy rats, ischemic stroke rats and ischemic stroke after acupuncture treatment from multiple angles and qualitative aspects.

MATERIAL AND METHODS

Main materials

Thirty male Sprague-Dawley rats (SPF grade), weighing 200-250 g, were provided by the Experimental Animal Center of Ningxia Medical University. Standard rat granules are fed, sterile water is fed, and 12 hr light is alternated day and night, and animal litter is used. Single cage feeding, ambient temperature 18-25°C, relative humidity of 40-70% is maintained. Minimize the pain of experimental animals in the experiment. Minimize the pain of experimental animals in the experiment. The experimental process will be reviewed and approved by the Ethics Committee of Ningxia Medical University. Formaldehyde and anhydrous ethanol were purchased from Sinopharm Chemical Reagent. Hematoxylin, eosin, and neutral resin were purchased from bioswamp. The apoptosis detection kit was purchased from Bebo Biotech and the fetal bovine serum was purchased from Tianhang Biotechnology. 0.25% trypsin digest was purchased from Solaibao Technology and acupuncture needle and electronic needle therapy instrument were provided by Suzhou Medical Products Factory.

Method

Experimental grouping: Rats were randomly divided into normal blank group, model control group and acupoint group, with 10 rats in each group. Except the normal blank group, the other groups used the international common internal carotid artery suture method for focal permanent cerebral ischemia in rats [10].

Establishment of animal models: Before the experiment, the rats in each group were numbered and weighed. The rats in the model control group and the acupoint group were anesthetized with 10% chloral hydrate and fixed in the supine position. In the middle of the neck, a small mouth of about 1.5-1.8 cm was cut longitudinally, and the subcutaneous tissue and muscle were gently peeled off with a scalpel to fully expose and separate the common carotid artery, external carotid artery and internal carotid artery. Care should be taken to protect the vagus nerve during the separation, then the common carotid artery and the external carotid artery were ligated, and a small incision was made about 4 mm from the bifurcation of the common carotid artery, and the suture was inserted into the internal carotid artery. When the insertion depth of the suture is about 18 mm, the thin wire of the distal end of the common carotid artery is tightened to suture the muscle and the skin. After 2 hours of ischemia, the needle was pulled back to the common carotid artery without re-anesthesia. Postoperative animals

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Received: *June 26, 2019,* ***Accepted:*** *June 28, 2019,* ***Published:*** *July 05, 2019*

were housed in single cages and given adequate food and water. The success criteria for rat modeling are based on the Longa neurobehavioral scoring standard [10]. When the rat showed undamaged side buckling or tail-hanging to the rear, tumbling, tilting, etc. to the uninjured side, it suggested that the modeling was successful. If the rat model is unsuccessful or died during the modeling process, the number of rats in each group is supplemented by random principle.

Treatment plan: Rats in the normal blank group and the model control group were fixed only without acupuncture intervention, once a day, 30 min each time, and the samples were directly tested after 10 consecutive times. The rats in the acupoint group were treated with electroacupuncture at 48 hours after operation. The acupoint location was based on the acupoint localization method of the experimental acupuncture and moxibustion. The prescriptions are the Baichi acupoint and the affected side of the forelimb of the Quchi, Hegu and the hind limbs of the Zusanli and Sanyinjiao. The two acupoints of the forelimb are a group, and the two acupoints of the hind limb are a group. The electro-acupuncture instrument is given a sparse wave, frequency 5/45 Hz, moderate stimulation, intensity is measured by the limb vibration of the rat, once a day, each time 30 min, and continuous acupuncture 10 times, 10 days after taking samples.

Experimental materials: After 10 interventions in each group of rats, they were weighed and sacrificed. First intraperitoneal injection of 10% chloral hydrate (300 mg/kg), after successful anesthesia, the rats were killed by decapitation, the head was cut off the epidermis, the skull was removed, and the entire rat brain was removed with a scalpel handle (The left cerebral cortex and hippocampus of the mouse). The cerebral cortex was fixed in 4% paraformaldehyde for 48 hours, and then dehydrated, embedded, sectioned and H&E stained to observe the pathological changes of the cerebral cortex neurons in the ischemic side of the rat. The cell suspension was used to observe the apoptosis rate of hippocampal neurons in the ischemic area by flow cytometry.

Statistical processing: Experimental data was processed using SPSS 21.0 statistical software. Measurement data are expressed as mean ± standard deviation (X ± S). For each group of data, normal distribution and homogeneity test of variance were firstly performed. When the variance was not uniform, the logarithmic transformation was used to make it normal distribution and the variance was uniform. The comparison of multiple groups of samples was analyzed by one-way ANOVA. The pairwise comparison uses the LSD-t test. Taking α =0.05, the difference was statistically significant at P<0.05.

RESULTS

Behavioural changes in rats in each group

Before the model was established, the rats in each group had good mental state, the skin was shiny and the state was active, and the diet was normal. Compared with the normal blank group, the model control group rats had poor mental state, dull hair color, like quietness, lethargy, poor diet, loose stools, the affected side eyes could not be completely opened, and the affected side limbs were weak, limp, gait is unstable, feeling slow. After electroacupuncture treatment, the rat's diet and body weight increased with the acupoint group and the model control group, the hair color returned to natural, and all the symptoms of action agility were alleviated and improved.

Pathological changes of cerebral cortical neurons in each group

In the normal blank group, the nerve cells of the rats were arranged neatly, the structure was clear, the nuclear membrane was intact, the nucleolus was obvious, and the structure was normal. In the model control group, the cells were arranged in disorder, a large number of cells were apoptotic and necrotic, the cell gap was widened, the nucleus was broken or reduced, and the cell morphology was incomplete. The necrosis and apoptosis of nerve cells in the acupoint group were significantly improved compared with the model control group, but there were still some incomplete cells and nuclear pyknosis (Figure 1).

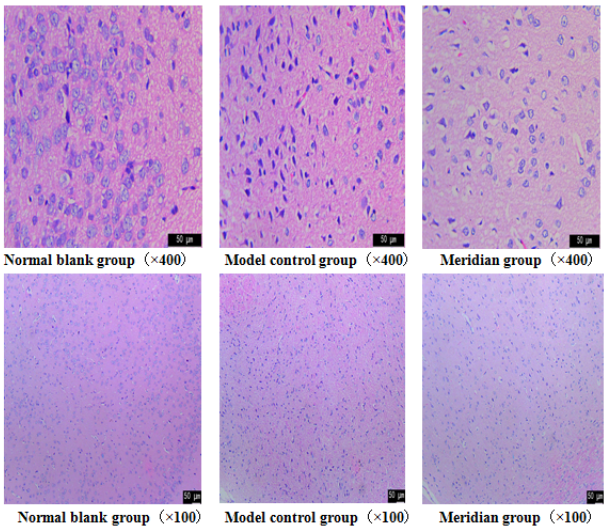

Figure 1) *Cerebral cortex H&E staining to observe the pathological changes of nerve cells*

The apoptosis of hippocampus in each group of rats

Compared with the early apoptotic rate of the normal blank group, the apoptosis rate of the model control group and the acupoint group showed an increasing trend, and the expression of the model control group was higher than that of the acupoint group, the difference was statistically significant (P<0.05). Compared with the normal apoptotic rate in the normal blank group, the apoptosis rate of the model group and the acupoint group increased, and the expression of the acupoint group was lower than that of the model control group, the difference was statistically significant (P<0.05). Compared with the early and late apoptotic rate of the model control group, the apoptotic rate of the early acupoint group decreased, but the difference was not statistically significant (P>0.05),The rate of late apoptosis was decreased and the difference was statistically significant (P<0.05). Apoptosis of cerebral cortical neurons in each group (x ± s) (unit: 100%) and Apoptosis of cerebral cortical neurons in each group after logarithmic transformation (x ± s) (unit: 100%) are shown in Table 1 and Table 2.

TABLE 1
Apoptosis of cerebral cortical neurons in each group (x ± s) (unit: 100%)

Grouping	N	Early apoptosis	Late apoptosis
Normal blank group	8	0.7938 ± 0.3782	0.0388 ± 0.0146
Model control group	8	7.9950 ± 1.2436	0.7238 ± 0.1151
Meridian group	8	5.2475 ± 1.8533	0.4100 ± 0.0769

Logarithmically transform all of the above data to conform to the normal distribution and the variances for subsequent comparisons

TABLE 2
Apoptosis of cerebral cortical neurons in each group after logarithmic transformation (x ± s) (unit: 100%)

Grouping	N	Early apoptosis	Late apoptosis
Normal blank group	8	-0.2016 ± 0.4071	-1.4426 ± 0.1815
Model control group	8	0.8981 ± 0.0685[a]	-0.1453 ± 0.0701[b]
Meridian group	8	0.6907 ± 01801[a]	-0.3942 ± 0.0841[b,c]

Note: One-way ANOVA is used, and the two pairs are compared using the LSD-t test.

[a]Compared with early apoptosis in normal blank group P<0.05

[b]Compared with late apoptosis in the normal blank group P<0.05

[c]Compared with late apoptosis in the model control group P<0.05

DISCUSSION

Stroke, whose disease is in the brain, belongs to the Du Meridian, belongs to the meeting of Zhuyang, and dominates the life activities of the human body. The related theory of acupuncture and moxibustion believes that "the meridians have passed, the attending center and the hospital", the "Sanyang Wuhui" Baihui acupoint is located in the human dome, which is the foot Shaoyang bile, the foot and the yin and the liver, the foot and the bladder [11]. Hand, Shaoyang Sanjiao Jing and Du Meridian point's studies have shown that acupuncture or moxibustion can be applied to Baihui acupoints, which has the effect of opening up collaterals, replenishing cerebral palsy, rejuvenating yang, and promoting yang. Zhong Zang Jing's "There are five deaths in the wind" article puts forward that "the heart and the spleen are in the middle of the wind, then the tongue cannot be said to be strong; the liver and kidney are all strokes, then the hands and feet are not swearing". Therefore, acupuncture points selected Sanyinjiao, which is located at the junction of spleen, liver and kidney. It can strengthen the spleen, tonify kidney, benefit Qi and make Yin liquid to increase liver blood function. Quchi and Hegu are both the hand-yangming large intestine meridian points, and the Zusanli, which is compatible with the foot-yangming stomach, is the same as the Yangming Jingyu point, the multi-qi and blood-stained, the main Run Zongjin, the Zongjin main beam and the organs. The Yangming Jing and the Taiyin Meridian are in the table. Through this method of first and second days, they can play the role of refining the marrow, resolving the disease, and treating the disease. Therefore, these above acupuncture points can be used as a treatment point for ischemic stroke.

Apoptosis is a way of death that is different from cell necrosis. It means that cells under certain conditions, by following their own procedures, end their own life, and finally the cells are shed or broken down into a few apoptotic bodies. It is engulfed by other cells, which aggravates the lesions in the lesion [12]. In this study, the presence of ischemic stroke cell apoptosis was confirmed by H&E staining and flow cytometry. The results showed that the early and late neuronal apoptosis rates of the model control group were higher than those of normal healthy rats after modeling, and the mechanism may be: after cerebral ischemia, energy production is blocked, energy metabolism disorder induces mitochondrial damage and produces oxidative free radicals, and a large number of free radicals destroy the integrity of cell membrane, which in turn causes neuronal apoptosis and necrosis [13-18]. At the same time, H&E staining also showed that the model control group after modeling was compared with the normal blank group, and the neuronal integrity of the ischemic area was destroyed, showing nuclear fragmentation or reduction, nuclear membrane separation and necrosis. After 10 days of treatment with electroacupuncture, the nerve cells in the ischemic area were counted and observed again. Research shows: In the ischemic stroke rats after electroacupuncture, the early and late withering in the ischemic area compared with the untreated ischemic stroke rats, the necrosis and apoptosis of nerve cells were significantly improved. After cerebral ischemia, a large number of excitatory neurotransmitters are released from the axon terminals. These transmitters interact with their respective receptors to cause excitotoxicity, and a large number of nerve cells gradually become apoptotic [19-21]. After acupuncture intervention, the release of excitatory neurotransmitters was inhibited, thereby suppressing the excitatory toxicity. The destroyed ions gradually reached steady state, and the influx of Na^+, Cl^-, and Ca^{2+} was controlled. The release of induced inflammatory factors is reduced; ultimately reducing the number of neuronal apoptosis and necrosis [22-26]. Therefore, electroacupuncture treatment after ischemia can effectively control the apoptosis of ischemic stroke, suggesting that electroacupuncture can protect the brain by inhibiting the occurrence of neuronal apoptosis. This study is for ischemic stroke. Clinical and basic research provides a theoretical basis.

Conclusion

This study used H&E staining and flow cytometry to detect neuronal pathological morphology and apoptosis in rats with different functional states. The study found that electroacupuncture can inhibit the neuronal cells in the brain. It has a therapeutic effect on ischemic stroke.

ACKNOWLEDGMENT

The work was made possible because of Ningxia Education Department Ningxia Medical University China's first-class domestic medicine project (No.NXYLXK2017A06).

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