EFFECT OF FAST ROTATING-LONG RETAINING ACUPUNCTURE THERAPY ON CEREBRAL CONTENTS OF GLUTAMIC ACID AND GAMMA-AMINOBUTYRIC ACID IN VESTIBULE NUCLEUS OF RATS WITH POSTERIOR CIRCULATION ISCHEMIC VERTIGO

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ABSTRACT

Objective*: This study is to investigate the effects of Fast rotating-Long retaining Acupuncture Therapy on cerebral Contents of Glutamic Acid and Gamma-aminobutyric Acid in Vestibule nucleus of Rats with Posterior circulation ischemic vertigo.*

Method*: The rats with Posterior circulation ischemic vertigo were randomly divided into model group(M), common acupuncture group(CA), Fast rotating-Long retaining Acupuncture method group(FRA),and sham operation group(SO). The two acupuncture group selected "Baihui acupoint", "Shuaigu acupoint " and "Fengchi acupoint " treatment, 1 times a day. One group was given conventional acupuncture and another was given Fast rotating-Long retaining Acupuncture Method treatment.The treatment must last 10 days. After 10 days, the rats were killed and the contents of glutamic acid and gamma-aminobutyric acid were measured.*

Result*: Compared with sham operation, Glu levels in model group were significantly higer(P<0.01),while GABA levels significantly lower (P<0.01). Compared with model group,Glu levels in common acupuncture group were lower(P<0.05), and GABA levels in common acupuncture group were higer(P<0.05); GLU levels in Fast rotating-Long retaining acupuncture method group were significantly lower(P<0.01), GABA levels were significantly higer(P<0.01).*

Conclusion*: The fast rotating-Long retaining acupuncture method can alleviate the "excitability toxicity" after ischemia, regulate the content of GLU and GABA, so as to improve the cerebral ischemia state and alleviate the vertigo symptoms.*

Keywords: *Fast rotating-Long retaining acupuncture, Poster circulation ischemia vertigo, GLU, GABA.*

DOI: 10.19193/0393-6384_2021_4_350

Received *October 15, 2020;* ***Accepted*** *March 20, 2021*

Introduction

Posterior circulation refers to the vertebrobasilar artery system, which is composed of vertebral artery, basilar artery and posterior cerebral artery. Posterior circulation ischemia (PCI) is a common ischemic cerebrovascular disease, accounting for about 20% of ischemic cerebrovascular diseases. Clinical take vertigo as main symptom, so we call it ischemic vertigo[(1)]. The pathogenesis of vertigo is complex, and it is manifested as rotating sensation or dumping sensation, paroxysmal, accompanied by nystagmus, balance disorder and vegetal nervous system symptoms[(2)]. The pathological mechanism is mainly related to vestibular lesion, which is mainly caused by vestibular system ischemia, including vestibular receptors, vestibular nerve and corresponding central center[(3)]. After the occurrence of cerebral ischemia, it can cause neuronal excitation, thus triggering "excitatory toxicity", leading to the increase of glutamic acid (GLU) content and the decrease of the inhibitory gamma-aminobutyric acid (GABA[(4)]. The purpose of this study was to investigate the therapeutic mechanism of fast rotating-Long retaining acupuncture on posterior circulation ischemic vertigo by measuring the content of GLU

and GABA in Poster circulation ischemia vertigo rats.

Material and methods

Animals and groups

There were 56 healthy SD rats with SPF grade, half male and half female, with body mass of 180 ~ 200g.Provided by Beijing Vitonlihua Laboratory Animal Technology Co., LTD., Certificate No.11400700246872, Animal License No. SCXK(Beijing)2016-0011. The experiment began one week after the rats were adapted to feeding. The indoor temperature was controlled at 20 ~ 25 °C and the humidity was 40% ~ 70%. According to the random number table method, the rats were divided into model group, sham operation group, ordinary acupuncture group and fast twist long-stay group, with 14 rats in each group.The experiment was conducted in accordance with the Guidelines on treating Experimental Animals issued by the Ministry of Science and Technology in 2006. According to the random number table method, the rats were divided into model group, sham operation group, ordinary acupuncture group and Fast rotating-Long retaining acupuncture group, with 14 rats in each group.The experiment was conducted in accordance with the uidelines on treating Experimental Animals issued by the Ministry of Science and Technology in 2006.

Experimental apparatus

Disposable millineedle (0.35mm × 25mm, Wuxi Jiajian Medical Device Co., LTD.); Ultraviolet spectrophotometer (752, Shunping, Shanghai); Miniature high-speed centrifuge (C2500-R-230V, Labnet, USA); Three-dimensional brain locator (DW-2000D, Chengdu Taimeng Software Co., LTD.); Laser doppler blood flow meter (Moorvms - Ldf, British Moor insturements company); Multiskan MK3 (Thermo Scientific); Glutamate Test Box (A074, Nanjing Jiancheng Institute of Bioengineering); Gamma-aminobutyric acid test box (H168, Nanjing Jiancheng Institute of Biological Engineering).

Methods

Model of Poster circulation ischemia vertigo rats

Rats were anesthetized with 10% chloral hydrate (0.35 mL/100g body weight), fixed in supine position, neck skin was cut, blunt tissue was separated, right CCA was exposed and separated, and thread was threaded for later use.The bifurcation of the right SCA was found in the thoracic cavity along the proximal end of the right CCA, and the right SCA was gouged out with the curved tweezers to peel off the surrounding nerves and tissues, and then threaded for use. The rats on the 3 d brain stereotaxic instrument, adopt the cranial head, is apart from the former 10.2 mm, anterior fontanelle raphe on the right side of 1.1 mm to 2.5 mm, 4.5 mm to 5.5 mm deep place to locate on the right side of vestibular nuclei in rats, cut in the middle of the scalp is about 1.5 cm incision, burning with hydrogen peroxide, subcutaneous tissue before exposure, the anterior fontanelle in anterior fontanelle before 1.5 mm each side open and down with dental drill to drill a diameter of 1.5 mm round hole, just can accommodate laser probe, try to make the surface smooth and not drilled through. Then, the blood flow of the right vestibular nucleus tissue was measured with laser doppler flowmeter, and the cerebral blood flow map was recorded 5min after the cerebral blood flow value was stable.After the blood flow was stable, CCA and SCA were ligated on the right side, and the blood flow curve was continued to be recorded for 10min.After the operation the rats were injected penicillin 40,000 units. After the modeling, the cerebral blood flow was measured by laser Doppler flowmeter, which decreased by more than 20%, and the movement was unstable, the walking was deviated, and the spontaneous activity was reduced. The positive rats were deemed as the modeling success[5]. In the sham group, only the right CCA and SCA were separated without ligation and suture directly.

Intervention method

Model group: After the success of the model group, the model group did not do other treatment, free feeding water feeding.

Ordinary acupuncture group: acupuncture treatment was given. Referring to literature[6-7], "Baihui", "Pinggu" and "Fengchi" points of rats were selected for routine hair shearing disinfection. After flexible fixation, 0.35mm × 25mm needle was quickly inserted under the skin and then inserted at an oblique Angle to the scalp with a depth of 8mm, and the needle was retained for 30 min, once a day for 10 consecutive days.

Fast rotating-Long retaining acupuncture: Point selection and needle insertion methods were the same as those in the ordinary acupuncture group.

Immediately after the insertion, the needle handle was gripped by the right thumb and the radial edge of the index finger and the needle was rotated back and forth, with a frequency of 200 ~ 300 times / min. The needle was successively performed for 1 min. Sham operation group: The rats had no other treatment, free feeding and drinking water feeding.

Index detection

After the intervention, the rats in each group were anesthetized. Their heads were quickly severed and the brain stem tissues were removed on ice trays. According to the brain mapping map of rats[8], vestibular nuclei were taken out and fixed in 4% paraformaldehyde buffer. The brain tissue was taken out in the proportion of 1:9 (g:ml) by weight and volume, 9 times of normal saline was added. Homogenate was homogenized in a homogenate under ice bath condition, and centrifuged at 2500 RPM for 10min. Take 0.2ml supernatant and add 0.6ml GLU reagent (1:3 ratio).The mixture was thoroughly mixed, centrifuged at 3000 ~ 3500 RPM for 10 minutes, and 0.5ml supernatant was taken for testing.After 40 minutes in a 37°C water bath, the product was removed and placed at 340nm with an optical diameter of 1cm. The double steam water was set to zero, and the absorbance A2 value of each tube was measured.

Statistical method

SPSS13.0 statistical software was used for data processing, and the experimental data were expressed as ( ). Analysis of variance (ANOVA) was used for comparison between groups, and pairwise comparison was conducted. $P < 0.05$ was considered statistically significant.

Results

The general condition of the rats

In the sham operation group, 12 rats were set, 56 rats were successfully constructed, and randomly divided into the model group, the ordinary acupuncture group and fast rotating-Long retaining acupuncture group, 14 rats in each group. During the treatment, 3 rats died in the model group, and no rats died in the two acupuncture groups. The sham operation group was in good mental state and could move freely.The other groups of rats were lethargic and their reaction ability decreased, spontaneous activity decreased, and water intake decreased,too. After treatment, the both acupuncture group were better than the model group. The rats in acupuncture group were all in better condition.

Effect on the content of GLU and GABA in vestibular nucleus in ischemic vertigo model rats (Table 1).

Group	n	GLU (μmol/L)	GABA (μmol/L)
SOG	12	157.09±18.34	10.36±2.12
MG	11	326.70±10.44	14.07±6.03
OAG	14	$250.49±7.15^{\Delta *}$	$18.13±5.23^{*}$
FRRG	14	$219.19±11.12^{\Delta *}$	$21.92±4.56^{*\bullet}$

Table 1: Effect on the content of GLU and GABA in vestibular nucleus in ischemic vertigo model rats.
Note: Comparison with sham operation group: $^{\Delta}P < 0.01$;Comparison with model group: $P < 0.01$; Comparison with common acupuncture group: $^{\bullet}P < 0.05$.

Discussion

Since the vestibular system is sensitive to ischemia[9], a certain degree of posterior circulation ischemia will lead to insufficient perfusion of vestibular receptors, vestibular nerve and corresponding central nerves, whick leading to vestibular system dysfunction. Body balance instability, dizziness and other symptoms will appear. Therefore, the improvement of vestibular nerve and blood supply of corresponding central center in patients with ischemic vertigo can alleviate vertigo symptoms and is beneficial to the treatment of the disease.

According to traditional Chinese medicine, the pathogenesis of vertigo is deficiency of qi and blood, deficiency of liver and kidney, phlegm and dampness blocking collaterals, and vertigo caused by qingyang not rising. Therefore, it is necessary to use the methods of filling marrow, activating blood and removing blood stasis, and dredging meridians and collaterals for treatment. In clinic, the treatment of this disease is more effective. In this experiment, acupuncture at various points of the rat's head can play the role of vacuity and purging, replenishing cerebral marrow, activating blood and removing blood stasis, and dredging meridians. Baihui acupoint will be the meeting of all the Yang of the body.It can lift the spirit of a body and has the ability to calm the mind set dazzle. Fengchi acupoint is the point of the Gallbladder meridian of Foot Shaoyang and it is also the intersection point of the foot Gallbladder meridian of Foot Shaoyang and

the Yang wei meridian. The acupoint can promote Yang Jing qi and blood so that it can be good for the brain.The dizziness will be eliminated[10-11]. Shuigu acupoint, located above the ear tip, belong to the Gallbladder meridian of Foot Shaoyang and the Bladder Meridian of Foot-Taiyang. It can clear wind, activate collateral, redue moisture and turbidity.Fast rotating -Long retaining acupuncture can stimulate the qi of the meridians in order to improve the curative effect in a greater extention.

After cerebral ischemia occuring, the excitability of neurons is often increased, which can lead to the release of a large number of excitatory GLU and result in neurotoxicity and vertigo. GABA is an inhibitory neurotransmitter and has a neuroprotective effection[10-11]. In this experiment, comparing with the model group. both the ordinary acupuncture group and fast rotating-Long retaining acupuncture group can effectively reduce the content of GLU and increase the content of GABA .So both the acupuncture therapy can cure the vertigo caused by post - circulatory ischemia. The effect of the fast rotating-Long retaining acupuncture group is better than the ordinary acupuncture group. Therefore, it can be concluded that acupuncture therapy can treat vertigo by correcting posterior circulation ischemia and improve blood supply to vestibular nucleus, while the fast rotating-long retaining acupuncture therapy can enhance the curative effect.

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