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Effects of Polysaccharide of Gastrodia Elata Blume and Electro-Acupuncture on Expressions of Brain-Derived Neurotrophic Factor and Stem Cell Factor Protein in Caudate Putamen of Focal Cerebral Ischemia Rats

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Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: The aim of this study was to explore the neural protective effect of polysaccharide of Gastrodia elata Blume (PGB) and electro-acupuncture (EA) on focal cerebral ischemia rats.

Material/Methods: A total of 40 Sprague-Dawley rats were randomly divided into 5 groups (normal group, model group, PGB group, EA group and PGB+EA group). The model was prepared by middle cerebral artery occlusion (MCAO). Two week after modeling, rats were given PGB, EA, or a combination of the 2 in continuous treatment for 2 successive weeks. 14 days after modeling, expressions of BDNF and SCF protein in the caudate putamen (CPu) were detected by immunohistochemistry.

Results: Positive expression of BDNF and SCF protein was found in the right caudate putamen of each group of rats. Expressions of BDNF and SCF in the CPu of the model group were higher than normal group ($P<0.05$). Compared with the model group, the expressions of BDNF and SCF in the CPu of the PGB group, the EA group, and the PGB plus EA group increased significantly ($P<0.05$). The expressions of BDNF and SCF obviously increased in the PGB plus EA group compared to those of the EA group and the PGB group ($P<0.05$).

Conclusions: PGB and EA up-regulated the expressions of BDNF and SCF protein in the CPu of focal cerebral ischemia rats, and the combination of PGB+EA has a synergistic effect on the recovery from cerebral ischemia.

MeSH Keywords: Brain Ischemia • Brain-Derived Neurotrophic Factor • Caudate Nucleus • Electroacupuncture • Gastrodia • Stem Cell Factor

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Background

As one of the main elements of the caudate putamen nucleus of rat middle cerebral artery occlusion of cerebral ischemia and penumbra, recovery of partly injured neurons located in the ischemic penumbra region plays an important role in neural function [1]. Studies have shown that acupuncture upregulates expression of endogenous brain-derived neurotrophic factor and stem cell factor in regions of the cerebral cortex, hippocampus, and caudate putamen after cerebral ischemia, which is conducive to the repair of neuronal damage [2,3]. Electro-acupuncture combined with traditional Chinese medicine is an effective mode for treating cerebral ischemia and is widely used in clinical applications, but the mechanism is still unclear. Gastrodia elata polysaccharide is the main active ingredient of the Chinese traditional medicine Gastrodia elata. It has anti-cerebral ischemia and other effects, but the exact mechanism remains unclear. A preliminary study by our group revealed Gastrodiae polysaccharide and electro-acupuncture can promote the up-regulation of expression of BDNF and SCF and repair the nerve function [4,5]. The present study explored Gastrodia elata polysaccharide and the effect of electro-acupuncture combined with focal cerebral ischemia in rat ischemic caudoputamen expression of BDNF and SCF in order to perfect the polysaccharide.

Material and Methods

The experimental animals and grouping

We purchased 40 male SD rats, weight 200±20 g, from the Experimental Animal Center of Zhejiang province (Animal certificate No.: SCXK (Zhejiang) -2008-0033]. Rats were randomly divided into 5 groups: a normal group, a model group, a polysaccharide group, an electro-acupuncture group, and an acupuncture-drug combination group.

The main reagents and instruments

We used rabbit anti-BDNF, rabbit anti-SCF, and SABC Immunohistochemistry kits from Wuhan Boster Biological Engineering Company. The low-frequency electronic pulse therapeutic apparatus was from Shanghai Huayi Medical Instrument Co. (G6805-2). We also used an OLYMPUS BX51 fluorescence microscope and the ImageJ image analysis system (Japan). Gastrodia elata polysaccharide was produced by peeling and slicing Gastrodia elata Blume, using a homogenizer to make a paste, distilled water extraction, and extraction with 95% pure alcohol, followed by DEAE-52 cellulose column (2.6×50 cm) chromatography separation and purification for the preparation of PGB.

The experimental method

Animal treatment was according to the Zea Longa [6] method for establishing a rat model of unilateral middle cerebral artery occlusion cerebral ischemia. Rats were anesthetized with pentobarbital sodium 30 mg/kg by intraperitoneal injection after being fixed in supine position. We made a median incision in the skin of the neck and performed blunt separation of the layers of tissue, exposing the right common carotid artery and internal carotid artery, separated from the external carotid artery bifurcation. A glass needle was used to carefully separate the vagus nerve and trachea. We tightened the proximal end of the internal carotid artery, and inserted fishing line (diameter 0.26 mm) into the trocar. We inserted the trocar into the internal carotid artery with 0 silk ligation. Trocar insertion continued out of the intracranial insertion depth of about 18.5–0.5 mm to the micro-sense of resistance, so that the line end went through the middle cerebral arteries, anterior cerebral artery, and ligation of the internal carotid artery, to the suture layer. At 2 weeks after modeling, rats were treated with Gastrodia elata polysaccharide 100 mg/kg by gavage treatment (1 time/day for 2 weeks). Rats in the electro-acupuncture group were treated with “Baihui” and “Zusanli” acupuncture points and given 2-Hz treatment (strength 3V, pulse width 1 ms, 30 min, 1/day, for 2 weeks). Combined acupuncture and medicine group rats were given electro-acupuncture therapy and treatment with Gastrodia elata polysaccharide.

Immunohistochemical staining method

After the experiment, the rats were injected with 30 mg/kg sodium and intraperitoneal injection anesthesia, 1% degrees of 4 degrees of polyformaldehyde phosphate-buffered solution to the brain, and then were fixed in 4% formalin-fixed liquid for 6 h, followed by conventional paraffin embedding. We removed the caudate putamen segment containing brain tissue serial sections (thickness 5 mm) and divided them into 2 groups. Sections were dewaxed to 3% H_2O_2 for removal of endogenous peroxidase for 5–10 min, followed by hot repair. We used 5% normal sheep serum closed after 37°C in a temperature box 1H dropping BDNF, SCF antibody (dilution ratio 1: 100), according to SABC kit instructions, and the stained them with DAB and mounted them. In each group, we selected the same 16 coronal slices (each of which are randomly selected from 2). The average gray count and measurement of immunoreactive products of BDNF and SCF immunoreactive cells were 400 times under the vision of the right caudate putamen region in intact cell membrane by using the image analysis system values.

Statistical analysis

The experimental data were analyzed using SPSS 13 statistical software. Single-factor analysis of variance was used for

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Table 1. Comparison of number of BDNF positive cells and gray value among different groups (χ±s).

Group	Count of BDNF positive cell	Mean gray value
Normal group	8.31±0.95	148.13±5.26
Model group	14.38±1.96*	130.06±3.79*
PGB group	16.44±1.71**,***	124.56±4.99**,***
Electro-acupuncture group	17.63±2.03**,***	122.50±4.94**,***
Acupuncture drug combination group	26.69±2.27**	114.81±6.15**

* Compared with the model group, P<0.05; ** compared with the combination of acupuncture and medicine, P<0.05; *** compared with the acupuncture drug combination group, P<0.05.

Table 2. Comparison of number of SCF positive cells and gray value among different groups (χ±s).

Group	Count of SCF positive cell	Mean gray value
Normal group	7.56±1.03	154.63±6.74
Model group	10.63±1.09*	143.56±5.72*
PGB group	15.31±1.40**,***	131.81±4.52**,***
Electro-acupuncture group	14.63±1.41**,***	133.19±4.21**,***
Acupuncture drug combination group	24.56±1.36**	122.31±4.60**

* Compared with the normal group, P<0.05; ** compared with the model group,P<0.05; *** compared with the acupuncture drug combination group, P<0.05.

comparison of the number of BDNF and SCF positive cells and gray value among different groups. *P*<0.05 was defined as a statistically significant difference.

Results

Expression of BDNF protein

In the normal group, the right caudate putamen expressed little BDNF protein; BDNF immunoreactive products were brown-yellow colored, mainly in cytoplasm and cell membrane. Compared with the normal group, the number of BDNF-positive cells in the model group was increased, and the dyeing depth, the average gray value was decreased, and the difference was statistically significant (P<0.05). Compared with the model group, there were significantly more BDNF-positive cells, the number of positive cells was significantly increased, the average gray value was significantly lower, and the difference was statistically significant (P<0.05). The number of BDNF-positive cells in the combination of acupuncture and medicine group was higher than that of the polysaccharide group and the electro-acupuncture group; the average gray value was decreased, and the difference was statistically significant (P<0.05) (Tables 1, Figure 1).

SCF protein expression

In rats in the normal group, the right caudate putamen had a small amount of lightly stained (pale yellow) SCF-positive cells, mainly located in the cell membrane and cytoplasm. Compared with the normal group, the number of SCF-positive cells in the model group was increased and the average gray value decreased; the differences were statistically significant (P <0.05). Compared with the model group, there were significantly more SCF-positive cells in the PGB group, electro-acupuncture group, and the acupuncture combined with medicine group, and the average gray value was decreased significantly; the differences were statistically significant (P<0.05). Compared with the polysaccharide group, the combined acupuncture and medicine group and the electro-acupuncture group had more SCF-positive cells and the average gray value decreased; the differences were statistically significant (P<0.05) (Table 2, Figure 2).

Discussion

The caudate putamen is the main blood supply region of the rat middle cerebral artery after middle cerebral artery occlusion. Effective treatment is important for recovering neural function after cerebral ischemia. Our study showed that with

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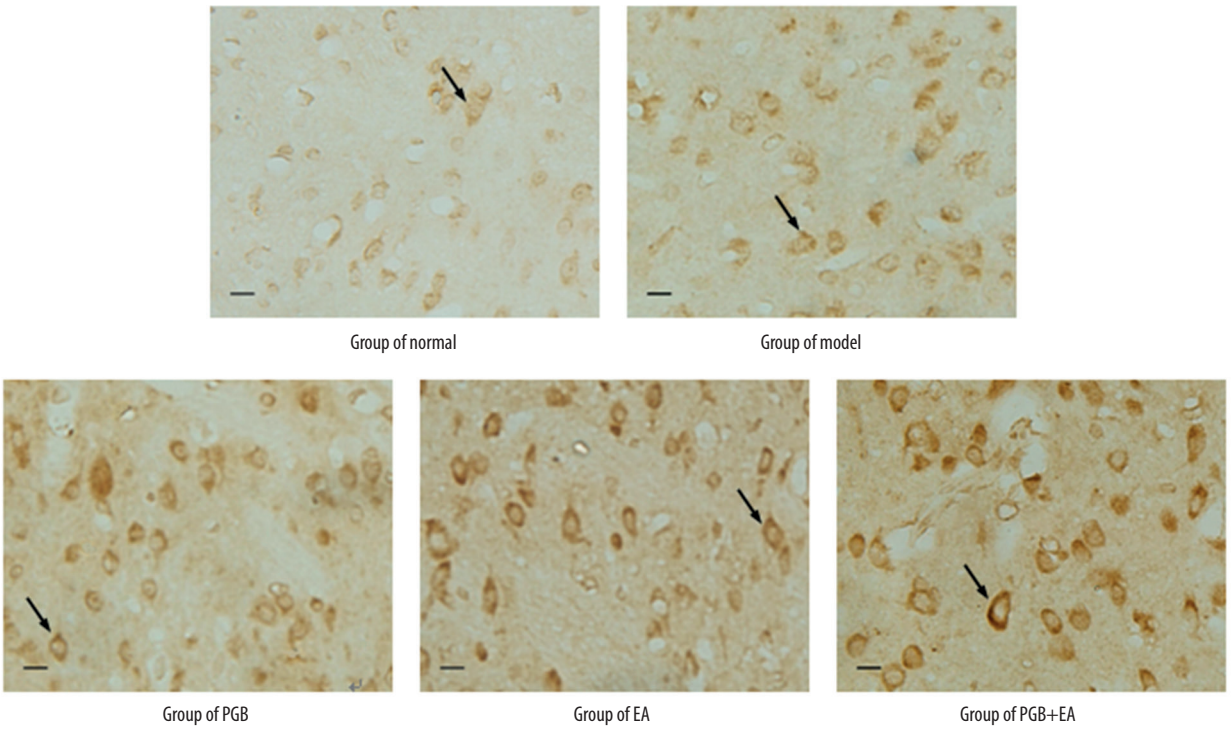

Figure 1. The expression of BDNF IR-positive cells in right caudate putamen in each group of rats (IR, ×400).

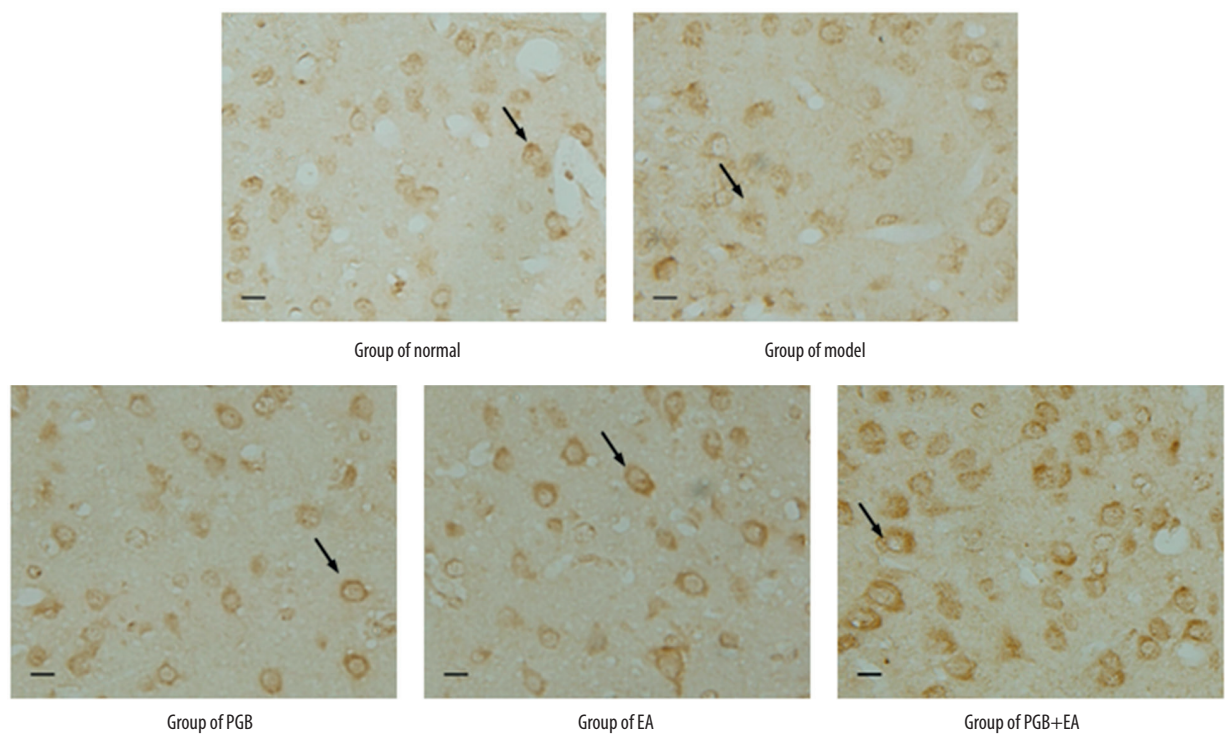

Figure 2. The expression of SCF IR-positive cells in right caudate putamen in each group of rats (IR, ×400).

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14-day brain artery embolization, the expression of protein BDNF and SCF in rats in the model group was higher than those in the normal group, which suggests that ischemic lesions putamen neurons are protected by the effect of BDNF and SCF, and may have the potential to repair neurological function and remodeling.

In recent years, with the development of extraction and purification of polysaccharide components in traditional Chinese medicine and other technologies, research shows that many traditional Chinese medicines, such as astragalus, Chinese wolfberry, and Chinese angelica polysaccharide, have neuroprotective effects on ischemic brain injury [7–9]. PGB is an effective active ingredient of traditional Chinese medicine, which can significantly promote the expression of BDNF and SCF of cerebral ischemia in rats, further enhancing the proliferation of endogenous neural stem cells [4,5]. After cerebral ischemia, the expression of endogenous BDNF increased, which plays an important role in the process of neuronal repair and reconstruction [10,11]. Up-regulating the expression SCF exerts important regulatory roles in proliferation, migration, differentiation, and survival of neural stem cells [5]. In this study, we treated rats with PGB. After 14 days, we observed that the expression of BDNF and SCF protein in rat ischemic caudate putamen in the PGB group was significantly higher than in the model group, suggesting that PGB exerts neuroprotective effects through the up-regulation of BDNF and SCF expression in brain tissues around the ischemic lesion. We found that low-frequency electro-acupuncture and PGB have similar effects on the up-regulation of BDNF and SCF protein expression in ischemic caudate putamen, but the combined treatment was more effective.

At present, acupuncture treatment for cerebral apoplexy has good clinical efficacy. Electro-acupuncture is more objective and controllable than manual acupuncture, and it has become the primary means for studying the mechanism of acupuncture in the laboratory [12]. It can significantly enhance the BDNF expression of cerebral cortex ischemia in rats and also protects against cerebral ischemic injury. After cerebral ischemia, the up-regulated expression of neurotrophic factors is important for proliferation, migration, and differentiation of neural stem cells [13]. Acupuncture can promote long-term BDNF increase and may be an important mechanism for electrical stimulation to exert brain-protective effects [14]. Clinical observations suggest that the combination of scalp and body acupuncture improves blood circulation and promotes the recovery of limb function. Combined use of acupuncture treatment in improvement of neurological can obtain better effects [15].

Recent studies have shown that the acupuncture-drug combination can reduce excitatory amino acid toxicity and calcium influx by inhibiting the increase of free radicals and cell apoptosis, improving blood rheology and other aspects to treat cerebral ischemia [16], but due to many influencing factors the mechanism has not been elucidated. Further experimental and clinical studies are needed to determine how to combine acupuncture and drug treatment of cerebral ischemia, thereby obtaining better clinical efficacy.

Conclusions

PGB and electro-acupuncture can up-regulating the expression of BDNF and SCF in CPu of focal cerebral ischemia rats. The combination of PGB and EA has a synergistic effect. This may be one of the mechanism of acupuncture combined with medicine for treatment of cerebral ischemia. This study provides a strategy for clinical use of the combination of polysaccharide components in Chinese traditional medicine and electro-acupuncture in treatment of patients with cerebral infarction.

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