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Electroacupuncture-regulated neurotrophic factor mRNA expression in the substantia nigra of Parkinson's disease rats^{*****☆}

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Abstract

Acupuncture for the treatment of Parkinson's disease has a precise clinical outcome. This study investigated the effect of electroacupuncture at *Fengfu* (GV16) and *Taichong* (LR3) acupoints in rat models of Parkinson's disease induced by subcutaneous injection of rotenone into rat neck and back. Reverse transcription-PCR demonstrated that brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor mRNA expression was significantly increased in the substantia nigra of rat models of Parkinson's disease, and that abnormal behavior of rats was significantly improved following electroacupuncture treatment. These results indicated that electroacupuncture treatment upregulated brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor mRNA expression in the substantia nigra of rat models of Parkinson's disease. Thus, electroacupuncture may be useful in the treatment of Parkinson's disease.

Key Words

neural regeneration; acupuncture and moxibustion; neurodegenerative diseases; electroacupuncture; brain-derived neurotrophic factor; glial cell line-derived neurotrophic factor; substantia nigra; rotenone; Parkinson's disease; rats; reverse transcription-PCR; grants-supported paper; neuroregeneration

Research Highlights

- (1) Electroacupuncture at *Fengfu* (GV16) and *Taichong* (LR3) acupoints upregulated brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor mRNA expression in the substantia nigra of rat models of Parkinson's disease.
- (2) Results suggest that electroacupuncture can protect dopaminergic neurons in the substantia nigra of rat models of Parkinson's disease.

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INTRODUCTION

Parkinson's disease is characterized by specific, progressive dopaminergic neuronal loss in the substantia nigra, high disability rate and long course of disease. Madopar is commonly used to supplement dopamine and amantadine, and monoamine oxidase inhibitors are used to indirectly promote dopamine production or reduce dopamine decomposition, respectively. Western medicine can improve the symptoms of Parkinson's disease, but patients are required to be treated for long periods increasing the risk of adverse events. Moreover, in some cases the efficacy of drugs can be reduced over time.

Acupuncture is simple to perform, is not toxic, does not have adverse effects, and has been extensively used in the clinic. Acupuncture for the treatment of Parkinson's disease has a precise clinical outcome^[1-2]. Numerous studies have confirmed that acupuncture can improve the abnormal behavior of Parkinson's disease in mice, reduce the loss of dopaminergic neurons in the substantia nigra, relieve mitochondrial injury, suppress the decrease in mitochondrial complex activities and protect mitochondrial function^[3-6]. Acupuncture can regulate monoamine neurotransmitter levels, elevate decreased dopamine, norepinephrine and serotonin levels, improve regional blood flow, and exert therapeutic effects.

There has been an increased focus on studies of neurotrophic factors, because they may be useful in treating Parkinson's disease^[7]. In addition, brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor have specific effects on dopaminergic neurons.

This study established a rat model of Parkinson's disease by subcutaneous injection of rotenone in the neck and back, and investigated the effect of acupuncture on brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor mRNA expression in the substantia nigra using reverse transcription-PCR, and its efficacy in the treatment of Parkinson's disease in rats.

RESULTS

Quantitative analysis of experimental animals

A total of 40 Sprague-Dawley rats were randomly assigned to a blank group (normal), sham-surgery group

(subcutaneous injection of sunflower oil), Parkinson's disease model group (subcutaneous injection of rotenone) and electroacupuncture group (subcutaneous injection of rotenone + electroacupuncture at *Fengfu* (GV16) and *Taichong* (LR3) acupoints). Two rats in the model group and two rats in the electroacupuncture group died of poisoning. A total of 36 rats were included in the final analysis.

Behavioral changes in rats with Parkinson's disease

Rats experienced tremor, rigor and slow movement at 7–10 days following rotenone injection. Moreover, reduced resistance to arresting movement, piloerection, stooping and yellow and dirty hair was observed. In accordance with previously published criteria^[8], the symptoms of Parkinson's disease models were typical. After electroacupuncture treatment, the above-described behavior of Parkinson's disease rats was dramatically improved. No significant change was detectable in the blank and sham-surgery groups.

Brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor mRNA expression in the rat substantia nigra

A 219-bp fragment of brain-derived neurotrophic factor mRNA and a 242-bp fragment of glial cell line-derived neurotrophic factor mRNA were obtained by PCR amplification (Figure 1).

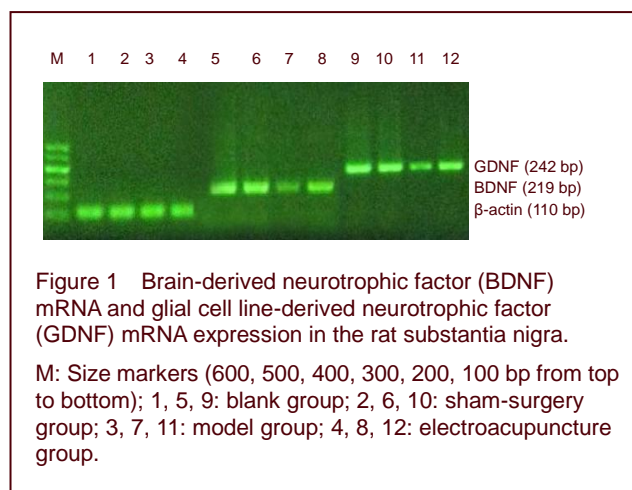


Image analysis system demonstrated that brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor expression was lower in the Parkinson's disease model group compared with the blank and sham-surgery groups ($P < 0.01$). However, brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor mRNA expression was significantly greater in the electroacupuncture group than in the model group ($P < 0.01$; Table 1).

Table 1 Effects of electroacupuncture at *Fengfu* (GV16) and *Taichong* (LR3) acupoints on brain-derived neurotrophic factor (BDNF) mRNA and glial cell line-derived neurotrophic factor (GDNF) mRNA expression in the substantia nigra of Parkinson's disease rats

Group	n	BDNF	GDNF
Blank	10	145.25±6.84 ^a	127.22±11.45 ^a
Sham-surgery	10	144.12±7.28 ^a	120.32±13.48 ^a
Model	8	56.30±12.13	59.26±15.44
Electroacupuncture	8	107.02±14.25 ^a	103.02±26.62 ^a

^a*P* < 0.01, vs. model group. Data represent the ratio of absorbance of target gene (BDNF, GDNF) and β -actin (%). Measurement data were expressed as mean \pm SD. Multigroup comparison was tested by one-way analysis of variance. Paired comparison of intergroup mean was made by independent samples *t*-test.

DISCUSSION

Parkinson's disease is a neurodegenerative disease that commonly occurs in the nervous system of middle aged and elderly people. After Alzheimer's disease, Parkinson's disease has the next highest incidence rate. Clinical symptoms of Parkinson's disease include tremor, myotonia, bradykinesia, gait disturbance, unstable posture and reduced voluntary movement. The major pathological change is progressive dopaminergic neuronal loss in the substantia nigra. Rotenone, an insecticide used for agriculture and fishponds, has strong liposolubility and can traverse the blood-brain barrier, where it exerts a cytotoxic effect^[9]. Recently, rotenone has been used to establish a rat model of Parkinson's disease. A previous study revealed that rotenone denatured dopaminergic neurons in the substantia nigra and striatum, which are associated with the onset of Parkinson's disease^[10]. Sherer *et al*^[11] suggested that dopaminergic neuron loss was observed in the substantia nigra of Lewis rats following subcutaneous injection of rotenone. In this study, Parkinson's disease models were established by subcutaneous injection of rotenone at a low dose in the neck and back over a long period of time. Behavioral observations demonstrated that rats developed tremor, rigor, reduced movement, slow movement and unstable gait after long-term injection of low-dose rotenone. Chen *et al*^[12] suggested that above-described abnormal behaviors of animals were induced by dopaminergic neuron damage and deletion in the substantia nigra.

In this study, Parkinson's disease model rats underwent electroacupuncture at *Fengfu* and *Taichong* acupoints. We confirmed that electroacupuncture therapy could significantly improve the abnormal behavior of Parkinson's

disease rats and increase brain-derived neurotrophic factor mRNA and glial cell line-derived neurotrophic factor mRNA expression in the rat substantia nigra. Neurotrophic factors regulate mature neuron function, protect neuronal survival and promote neuronal regeneration. Brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor are important factors that promote dopaminergic neuron survival and differentiation in the midbrain, repair dopaminergic neurons, prevent and reduce dopaminergic neuron degeneration and loss, as well as elevate dopamine levels^[13].

The transplantation of brain-derived neurotrophic factor-positive fibroblasts in the corpus striatum of rats with 6-hydroxydopamine-induced Parkinson's disease significantly reduced the death of dopaminergic neurons in the substantia nigra, and decreased the loss of nerve endings^[14]. Brain-derived neurotrophic factor treatment protected dopaminergic neurons, promoted their repair, effectively prevented dopaminergic neuron degeneration and death induced by 6-hydroxydopamine, MPP⁺ and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, enhanced dopaminergic neuron function, elevated dopamine levels, increased the number of tyrosine hydroxylase-positive cells and their processes, all resulting in the improvement of Parkinson's disease symptoms. A previous study showed that brain-derived neurotrophic factor suppressed the decrease of dopaminergic neurons, but improved cognitive functions^[15]. Glial cell line-derived neurotrophic factor administration significantly protected dopaminergic neurons and improved functional disturbances in Parkinson's disease models. Ding *et al*^[16] found that glial cell line-derived neurotrophic factor remarkably promoted the differentiation of midbrain neural stem cells into mature dopaminergic neurons in a hypoxic environment. Exogenous glial cell line-derived neurotrophic factor infusion in the corpus striatum decreased the apoptotic rate of dopaminergic neurons, increased tyrosine hydroxylase immunoreactivity and tyrosine hydroxylase-positive axons and synapse blebbing processes, increased the number, volume and process length of dopaminergic neurons and the ability to reuptake dopamine. Glial cell line-derived neurotrophic factor pretreatment reduced 6-hydroxydopamine and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine damage effects in dopaminergic neurons of mice^[17]. In addition, it is an effective method to increase brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor contents in the brain for the treatment of Parkinson's disease.

Our previous studies demonstrated that

electroacupuncture therapy could enhance the expression of brain-derived neurotrophic factor and its receptor TrkB and glial cell line-derived neurotrophic factor and its receptor Ret, in the substantia nigra of rat models of Parkinson's disease induced by 6-hydroxydopamine, as determined by immunohistochemistry^[18-19]. Moreover, related studies suggested that electroacupuncture exerted a protective effect on dopaminergic neurons in the substantia nigra of rat models of Parkinson's disease, and that its mechanism may be associated with the excitatory neurotrophic effects of brain-derived neurotrophic factor^[20-21]. In summary, electroacupuncture at *Fengfu* and *Taichong* acupoints can relieve the abnormal behavior of Parkinson's disease in rats by increasing brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor mRNA expression in the substantia nigra.

MATERIALS AND METHODS

Design

A randomized controlled animal experiment.

Time and setting

Experiments were performed at the Acupuncture Complex Laboratory, Hubei University of Chinese Medicine, China from March 2010 to December 2011.

Materials

A total of 40 clean, healthy, male Sprague-Dawley rats, aged 3 or 4 months and weighing 200–250 g, were purchased from the Experimental Animal Center, Tongji Medical College, Huazhong University of Science & Technology, China (license No. SCXK(E)2004-0007). Protocols were conducted in accordance with *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of China^[22].

Methods

Establishment and intervention of rat models of Parkinson's disease

As previously described^[23-25], 2 mg/mL rotenone sunflower oil emulsion (2 mg/kg per day) was subcutaneously injected into the neck and back of rats from the Parkinson's disease model and electroacupuncture groups for 4 consecutive weeks to induce Parkinson's disease-like symptoms. Rotenone and sunflower oil were purchased from Sigma (St. Louis, MO, USA). Sunflower oil at a dose of 2 mg/mL without

rotenone (2 mg/kg per day) was subcutaneously injected into the neck and back of rats from the sham-surgery group. Rats in the blank group were untreated. Rats experiencing tremor, rigor, reduced movement, slow movement and unstable gait were positively identified as having Parkinson's disease-like symptoms^[8].

Electroacupuncture therapy

In accordance with acupoint patterns^[26], rat acupoints were localized in combination with comparative anatomy. Rat models in the electroacupuncture group received electroacupuncture at *Fengfu* (in the posterior region of the neck, directly inferior to the external occipital protuberance) and *Taichong* (in the groove between the first and second metatarsal bones) acupoints. Rats were subjected to electroacupuncture at *Fengfu* acupoint at an angle of 45° and at *Taichong* acupoint at an angle of 90° using a 0.3 mm × 25.0 mm stainless steel needle (Huatuo, Suzhou, China). The depth of needle insertion was 4 mm. G6805-2 electroacupuncture therapeutic apparatus (Shanghai Medical Instruments Co., Ltd., Shanghai, China) was set for a continuous wave and frequency of 2 Hz. The intensity was at the point where rat limbs trembled and could be tolerated.

Electrode conjugation procedure: the positive electrode was connected to the *Fengfu* acupoint, and the negative electrode was connected to the *Taichong* acupoint (bilateral *Taichong* acupoints were selected on alternate days). Electroacupuncture was performed at the same time point every day for 20 minutes, once a day, for 7 days as a treatment course, for 3 consecutive courses. Rats in the blank, sham-surgery and model groups underwent grasping and fixation similar to those in the electroacupuncture group but without acupuncture.

Isolation of substantia nigra of rat midbrain

Rats were intraperitoneally anesthetized with 10% chloral hydrate (35 mg/100 g) and decapitated. The substantia nigra of midbrain was dissociated on ice, placed at –70°C in a nitrogen canister for detection of brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor mRNA.

Reverse transcription-PCR of brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor mRNA expression in the rat substantia nigra

Total RNA extraction in the substantia nigra: total RNA was extracted by one-step TRIzol method according to RNA extraction kit (Invitrogen Life Technologies, Carlsbad, CA, USA), and then stored at –80°C.

Upstream and downstream primers of β -actin, brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor were synthesized by Wuhan Guge Biological Technology Co., Ltd., China.

Primer sequence:

Gene	Sequence (5'-3')
β -actin	Upstream: CGT TGA CAT CCG TAA AGA CCT C Downstream: TAG GAG CCA GGG CAG TAA TCT
BDNF	Upstream: GTG TGA CAG TAT TAG CGA GTG GG Downstream: GAT TGG GTA GTT CGG CAT TG
GDNF	Upstream: TGG GAT GTC GTG GCT GTC TG Downstream: GCC GCT TGT TTA TCT GGT GAC

BDNF: Brain-derived neurotrophic factor; GDNF: glial cell line-derived neurotrophic factor.

Reverse transcription: 2 μ L of RNA, 1 μ L of upstream primer and 1 μ L of downstream primer were added in a 20- μ L reverse transcription reaction system. Reverse transcription cDNA kit, Taq enzyme and dNTP were purchased from Toyobo, Shanghai, China.

PCR amplification: 0.125 μ L of Takara Taq, 2.5 μ L of buffer, 2 μ L of dNTP, 2 μ L of upstream primer, 2 μ L of downstream primer, and 2 μ L of cDNA were added in reaction system for amplification. Reaction conditions are as follows: 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 1 minute, followed by 72°C for 10 minutes. All specimens were stored at 4°C.

PCR product detection and data processing: PCR products were determined by 2% agarose gel electrophoresis containing ethidium bromide. Absorbance was monitored by a gel-scanning system (Bio-Rad, Hercules, CA, USA). The absorbance ratio of target gene to β -actin was calculated.

Statistical analysis

The data were analyzed using SPSS 19.0 software (SPSS, Chicago, IL, USA), and expressed as mean \pm SD. Multigroup comparison was performed by one-way analysis of variance. Intergroup mean paired comparison was done by independent samples *t*-test. A value of $P < 0.05$ was considered statistically significant.

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Conflicts of interest: None declared.

Ethical approval: All experimental procedures were approved by the Animal Ethics Committee of Hubei University of Chinese Medicine in China.

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