

## BASIC INVESTIGATION

## Electroacupuncture effect on neurological behavior and tyrosine kinase-JAK 2 in rats with focal cerebral ischemia

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### Abstract

**OBJECTIVE:** Electroacupuncture effect on neurological behavior and the expression of tyrosine kinase Janus kinase 2 (JAK 2) of ischemic cortex in rats with the focal cerebral ischemia were investigated in this study.

**METHODS:** The model of focal cerebral ischemia was established by the heat-coagulation induced the occlusion of the middle cerebral artery. The electro-acupuncture was applied on Baihui (GV 20) and Dazhui (GV 14), and AG490 was applied by intracerebroventricular infusion. The expressions of JAK2 mRNA and phosphorylated JAK2 (p-JAK2) in the ischemic cortex were observed by in situ hybridization and western blotting.

**RESULTS:** The expressions of JAK2 mRNA and p-JAK2 were rarely found in sham surgery group. In model group, the expression of JAK2 mRNA and JAK2 phosphorylation had increased. After 1 day of cerebral ischemia, the expression had reached its peak. After cerebral ischemia, the expressions of JAK2 mRNA and p-JAK2 were consistent with the neurological deficit score. Electroacupuncture treatment and AG490 intervention were able to improve the neurological deficit score after cerebral ischemia, and down-regulate the expressions of JAK2 mRNA and JAK2 phosphorylation.

**CONCLUSION:** After cerebral ischemia, the excessive expressions of JAK2 and the JAK2 phosphorylation would be one of mechanisms by which the brain injury got worse. The therapy of electro-acupuncture could reduce the expression of JAK2, and inhibit JAK2 phosphorylated activation, so as to block the abnormal activation of signal transduction pathway which was induced by JAK2.

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**Key words:** Electroacupuncture; Brain ischemia; Neurobehavioral manifestations; Janus kinase 2; Alpha-cyano-(3,4-dihydroxy)-N-benzylcinnamide

### INTRODUCTION

Stroke is a leading cause of death and the most frequent cause of permanent disability worldwide.<sup>1</sup> Acupuncture has been a common therapy in Chinese medicine for ischemic stroke, and has a unique effect on saving the ischemic penumbra in the dying neurons and promoting functional recovery after nerve injury.<sup>2</sup> Present studies showed that apoptosis induced by cerebral

ischemia was associated with interleukin, tumor necrosis factor and other cytokines,<sup>3</sup> and the Janus kinase/signal transducer and activator of transcription (JAK/STAT) was one of the main pathways downstream of cytokine receptors and growth factor receptors by transducing signals from cell surface to the nucleus.<sup>4,5</sup> JAK2 as a member of Janus kinase family, its structure is highly homologous to the other members in the family, and participates in the immune, hematopoietic, nervous and other systems of signaling transduction. Cytokines (IFN $\gamma$ , IL6, OSM, EPO, etc) induce cell proliferation, differentiation and apoptosis via signal transduction pathways related to JAK2.<sup>6</sup> AG490 (an inhibitor for JAK2 phosphorylation) can inhibit the activation of JAK2 after cerebral ischemia, and down-regulate the phosphorylation of STAT3, so as to resist the neurons apoptosis, protect the neurons after cerebral ischemia and improve the neurological deficits.<sup>7</sup>

Previous studies suggested that focal cerebral ischemia caused the excessive expressions of p-JAK2 and p-STAT3 and the abnormal activation of JAK2-STAT3 signal transduction pathway. Electroacupuncture could reduce the protein expressions of p-JAK2 and p-STAT3, so as to block the abnormal activation of JAK2-STAT3 signal transduction pathway. It might be one of the important mechanisms of cerebral ischemia by which electric-acupuncture produced its effect.<sup>8</sup> In present study, the effects of electro-acupuncture on the expressions of JAK2 mRNA and p-JAK2 protein in rats with focal cerebral ischemia were observed by *in situ* hybridization and western blotting. We investigated JAK2 activation in focal ischemia-induced neuronal damage and the effect of electroacupuncture, so as to reveal the mechanism by which electroacupuncture exerted its effect on ischemic cerebral diseases.

## MATERIALS AND METHODS

### *Animal grouping*

A total of 250 adult, male, specific pathogen-free, SD rats, weighing 180-240 g, were provided by the Experimental Animal Center of Guangzhou University of Traditional Chinese Medicine. All experimental procedures were performed in accordance with the Guidance Suggestions for the Care and Use of Laboratory Animals, formulated by the Ministry of Science and Technology of the People's Republic of China.<sup>9</sup> The 250 SD rats were randomly assigned to sham surgery group, model group electro-acupuncture (EA) group, AG490 group and EA+AG490 group ( $n=50$ ). In terms of the time period after cerebral ischemia, each group could be further divided into five subgroups of 2 h, 1, 3, 7, 21 days.

### *Establishment of focal cerebral ischemia model*

Heat coagulation-induced occlusion of the middle cerebral artery was used to establish a model of focal cere-

bral ischemia.<sup>10</sup> Rats were initially intraperitoneally anesthetized with a 10% chloral hydrate (330 mg/kg) and then fixed in a right upper lateral position on the surgical table. An incision was made at the median position between the ear and eye to isolate the temporal muscle. The middle cerebral artery was exposed and occluded using burned stainless steel wire. In the sham surgery group, the middle cerebral artery was exposed without occlusion.

### *Intracerebroventricular infusion of AG490*

The JAK2 phosphorylation inhibitor AG490 (Merck, Darmstadt, German) (250  $\mu$ m in 3% DMSO) was continuously infused into the lateral ventricles of rats. The drugs were filled into 25  $\mu$ L osmotic mini-pumps which contained 10  $\mu$ L solution. Each pump was connected to a stainless steel cannula (Alzet brain infusion) with peristaltic tubing and primed overnight at 37°C to ensure immediate delivery after implantation. The cannula was stereotaxically implanted into the lateral ventricle (bregma; 0.8 mm posterior, -4.8 mm dorsoventral, -1.5 mm lateral)<sup>11</sup> and secured to the skull with needle of syringe. The pump was placed in the skin fold on the neck of the rat. The cannula and pump implantation was conducted under chloral hydrate anesthesia. The time of intracerebroventricular infusion was 10 min, and the needle stayed for 2 min. Penicillium was used for disinfection after infusion. The AG490 group and EA + AG490 group were occluded the middle cerebral artery after 20 min when finished intracerebroventricular infusion.<sup>12</sup>

### *Electroacupuncture treatment*

The acupoints of Baihui (GV 20) and Dazhui (GV 14) in Du meridian were chosen according to document.<sup>13</sup> Baihui is located in the median of the parietal bone, and Dazhui is located in the median of the back, below the spinous process of the seventh cervical and first thoracic vertebrae. A 0.3 mm  $\times$  25 mm needle (Suzhou Medical equipment Factory, Suzhou, China) was inserted into Baihui approximately 15 mm backward and another into Dazhui vertically at a depth of 7.5 mm. The acupoints were stimulated with electro-acupuncture (G-6805 electric acupuncture apparatus, Qingdao Xingsheng Medical Instrument Factory, Qingdao, China, 4/20 Hz, 1-2 mA for 30 min, QD)

### *Neurological evaluation*

Neurological deficits were evaluated on a 5-point scale (Zea Longa *et al.* 1989)<sup>14</sup> before middle cerebral artery occlusion (MCAO) and after 2 h, 1, 3, 7, 21 days of focal cerebral ischemia by an investigator blinded to the study groups. A score of 0 suggested no neurological deficit (normal), 1 suggested mild neurological deficit (failure to extend left forepaw fully), 2 suggested moderate neurological deficit (circling to the left), 3 suggested severe neurological deficit (falling to the left), and 4 suggested obviously severe neurological deficit (the rat

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