

# Experimental Study on the Effect of Acupuncture on ERM/PI3/Akt Signal Pathway in Rats with Ischemic Stroke

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## Abstract

**Objective:** To observe the effect of acupuncture on the expression of ERM/PI3K/Akt signaling pathway in the brain tissue around the infarct focus of ischemic stroke model rats. **Methods:** According to the block randomization method, 80 male SD rats were divided into normal group (n = 10), sham operation group (n = 24), model group (n = 23) and acupuncture group (n = 23). The normal group was bred routinely without any intervention. In the sham operation group, only the skin was cut to find and the nerves and blood vessels were stripped and sutured. The model group and the acupuncture group were sutured to prepare the MCAO model. After the model was successfully established, acupuncture of the acupuncture group rats was given once a day, and the needles were kept for 30 minutes each time. The rats in the sham operation group and the model group only imitated capture and return, and were treated with acupuncture for 2 consecutive courses, each course was 5 days, and the two courses were rested for 2 days. After that, Western blot was used to detect ERM, changes in the concentration of PI3K and Akt proteins. **Results:** Compared with the normal group, the expression levels of ERM, PI3K, and Akt proteins in the sham operation group were reduced. Compared with the sham operation group, the expression levels of ERM, PI3K, and Akt proteins in the model group were significantly down-regulated. Compared with the model group, the protein expression levels of ERM, PI3K and Akt were significantly up-regulated in the acupuncture group, and the difference was statistically significant ( $P < 0.05$ ). **Conclusion:** Acupuncture can significantly up-regulate the expression of ERM, PI3K and Akt proteins, and its effect mechanism may be related to the transduction of ERM/PI3K/Akt signaling pathway in cells.

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## Keywords

Acupuncture, Ischemic Stroke, ERM/PI3/Akt Signaling Pathway

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## 1. Introduction

Ischemic stroke is a kind of irreversible brain damage caused by transient or continuous ischemia and hypoxia in the brain tissues. The development of this pathological process is often accompanied by the occurrence of key links such as apoptosis, autophagy, and oxidative stress [1]. Ezrin-root protein-membrane protein (ERM)/phosphatidylinositol-3-kinase (PI3K)/protein kinase B (PKB or Akt) signal transduction pathway is one of the pathways of intracellular and extracellular signal response, which can be used in a series of complex and rapid cascade reactions that occur in the process of brain injury after ischemia, and crosstalk produces intricate biological regulation in each link of the above-mentioned pathological process [2] [3] [4]. This experiment took this as the starting point, by establishing ischemic stroke model rats and giving acupuncture treatment, Western blot was used to detect the expression of ERM, PI3K, and Akt proteins in each group of rats, and to explore the effect of acupuncture on the expression of ERM, PI3K, Akt protein in the brain tissue around the ischemic focus of ischemic stroke model rats, and the relationship between its effect and the ERM/PI3K/Akt signal transduction pathway, which is the mechanism for the future acupuncture treatment of ischemic stroke. Research provides objective evidence.

## 2. Materials and Methods

### 2.1. Animals and Groups

Preliminary experiments have found that male rats are more adaptable to the environment and have a higher survival rate than female rats after modeling. Therefore, 80 SPF-grade adult male SD rats with a weight of 220 - 280 g and a temperature control of 18 were selected:  $-26^{\circ}\text{C}$ , humidity 50% - 60%. Provided by the Animal Experiment Center of Xi'an Jiaotong University, animal license number: SCXK (Shaanxi) 2018-001, according to the block randomization method, divided into normal group (n = 10), sham operation group (n = 24), model group (n = 23) and acupuncture group (n = 23).

### 2.2. Main Instruments and Reagents

Electrophoresis instrument (Beijing Liuyi Company), gel filling, vertical electrophoresis and transfer device (all from Shanghai Tianneng Company), Tanon 5200 automatic chemiluminescence imaging analysis system (Shanghai Tianneng Company), centrifuge (Shanghai Anting Company), Homogenizer, Ultrasonic Disintegrator (all from Ningbo Xinzhi Company), etc.

Primary antibody: ERM/ETV5 antibody (catalog number: A01809-2 manu-

facter: BOSTER dilution ratio: 1:1000), PI3KCA antibody (catalog number: PB0351 manufacturer: BOSTER dilution ratio: 1:1000), AKT1 antibody (catalog number: A00024 manufacturer: BOSTER Dilution ratio: 1:1000),  $\beta$ -Actin internal reference protein (Cat. No.: BM0627 Manufacturer: BOSTER Dilution ratio: 1:2000); Secondary antibody: HRP-Goat Anti-Rabbit (Cat. No.: BA1054 Manufacturer: Boster Dilution Ratio: 1:5000), HRP-Goat Anti-Mouse (Cat. No.: BA1050 Manufacturer: Boster Dilution Ratio: 1:5000); BCA Protein Quantitative Kit (Cat. No.: AR0146), SDS-PAGE Gel Preparation Kit (Cat. No.: AR0138), Western Dedicated primary antibody and secondary antibody diluent (catalog number: AR1017), ECL chemiluminescence reagent (catalog number: AR1196), etc.

### 2.3. Preparation and Evaluation of Ischemic Stroke Model

Using the focal cerebral ischemia model of S. Tchen *et al.* [5] of the Department of Neuropathology, South Carolina School of Medicine, USA: 1) Ligation of the common carotid artery (CCA): intraperitoneal anesthesia with 10% chloral hydrate (4 ml/kg-bw). Make a 1.5 cm incision at the midline of the neck, separate the CCA on both sides, and ligate the CCA on the right side. The left side was clamped with a non-invasive arteriole clip for 1 hour and then opened. 2) Block the middle cerebral artery (MCA): fix the rat with a stereotaxic device, make a 1.5 cm incision on the connection line between the right eye and the right ear, separate the temporal muscle, expose the temporal bone, and use an operating microscope to place a dental drill on the scales. At the junction of the zygomatic bone and the zygomatic bone, a 0.5 × 0.5 cm bone window was drilled on the squamous bone facing the mouth side 1 mm. Use a No. II scalpel to carefully cut the dura mater to expose the main middle cerebral artery, block it with a bipolar coagulation knife, reset the temporal muscle, and suture the wound to simulate the acute stage of clinical ischemic stroke. The signs of the model's success are the Horner sign on the ipsilateral side and hemiplegia with heavy forelimbs on the contralateral side after the experimental rat wakes up. After the modeled animals wake up naturally, their neurological disorders are scored according to Zea-Longa's 5-level 4-point scoring standard [6]. Grade 0 (0 points) no neurological loss; Grade 1 (1 point) left forelimb inability to fully extend; Grade 2 (2 points) circular movement to the left, mild focal neurological loss; Grade 3 (3 points) to the left Side down, moderate focal neurological loss; grade 4 (4 points) unable to walk naturally, severe focal neurological loss; only rats with neurological dysfunction above grade 1 can be retained. The rest were included in the model group; the sham operation group: no thread plugs were inserted, and the remaining operations were the same as the model group.

### 2.4. Treatment Methods

Choose acupoints: Baihui, Fengchi, Quchi, Hegu, Zusanli, Yanglingquan, Sanyinjiao as the selected acupuncture points for this topic.

Point selection basis: Ischemic stroke belongs to the category of "stroke" in

traditional Chinese medicine. It takes imbalance of yin and yang and qi and blood as the basic pathology. Deficiency of the liver and kidney and the lack of water retention are the basic pathogenesis, which ultimately leads to poor circulation of qi and blood. The skin tendons and veins are lost in the pathological process of nurturing. Wind, fire, phlegm, and blood stasis can cause disease alone or cause and effect each other, and they are comprehensively intertwined. Acupuncture and moxibustion treatment of stroke has always taken the meaning of more qi and more blood in the Yang Meridian. Hegu is the original point of the Yangming Large Intestine Meridian of the Hand. It is the mark of the body's vitality and blood, which can pass the menstruation and activate the blood, and it is good at extinguishing the wind, suppressing the spasm, and refreshing the brain and resuscitating. Quchi point is the combined point of the five points of the Yangming meridian of the hand, from which the qi of the Yangming meridian enters and merges with the viscera, which can pass up and down to regulate the qi of the Yangming meridian. Zusanli is the combined point of the five acupoints of the foot-yangming meridian. The wind pool is a meeting of Shaoyang and Yangwei, which can be refreshing and refreshing except for the wind inside and outside. Yangyang Lingquan is the meeting point of the tendons and the joint points of the Shaoyang meridian. Sanyinjiao is the intersection point of the Zusanyin meridian, which can nourish the three yin, fill the brain, harmonize qi and blood, and calm the mind. Since stroke is located in the brain, the Du Channel acupoint Baihui starts from the brain, and it is used to "open the resuscitation, open and close, and improve the home of the soul", and the Du Channel enters the collateral brain. To use, take the meaning of "seeking yang in yin".

In previous clinical studies, it has been confirmed that the above-mentioned acupoints have achieved better effects in the treatment of ischemic stroke [7] [8] [9].

### **Acupuncture Positioning and Operation**

The above acupuncture points are strictly based on the standard of "The Atlas of Common Animal Acupoints" formulated by the Experimental Acupuncture Research Association of the Chinese Acupuncture and Moxibustion Society [10]. Acupuncture operations are completed by acupuncturists who have received uniform training. The acupuncture needles are disposable sterile Huatuo brand acupuncture needles produced by Suzhou Medical Supplies Factory, specifications: 0.40 mm × 13 mm, executive standard number: GB2024-1994; operation method: routine disinfection of acupoints, using flattening and reducing acupuncture methods, Once a day, the needles are retained for 30 minutes each time, and the needles are given once every 15 minutes. The 5th is a course of treatment. After a course of treatment, rest 2 days, and then proceed to the next course of treatment for a total of 2 courses. Rats in the non-acupuncture group are routinely raised until the prescribed acupuncture course for the other groups ended.

## 2.5. Index Detection Method

Western blot was used to detect the expression levels of ERM, PI3K, and AKT proteins in brain tissue. Firstly, the brain was decapitated and the brain was extracted, and the total protein in the brain on the side of the infarct was extracted to determine the protein concentration. After the protein was separated by SDS-PAGE electrophoresis gel, the protein on the gel was transferred to the PVDF membrane, and then blocked with 5% BSA. Add the primary antibody, ERM dilution ratio is 1:1000, PI3K dilution ratio is 1:1000, AKT dilution ratio is 1:1000,  $\beta$ -actin dilution ratio is 1:2000, and incubate at 4°C for 12 hours on a shaker. Wash with TBST 3 times, 10 minutes each time, incubate in a proportionally diluted secondary antibody for 2 hours at room temperature, and then wash 3 times with TBST, 10 minutes each time. Finally, configure the ECL working solution according to the proportion, add the ECL working solution to the blotting membrane for 30 - 60 seconds, blot the luminescent liquid, place the blotting membrane on the imaging analyzer for automatic imaging, and analyze the gray value of the protein band by the software ImageJ.

## 2.6. Statistical Methods

Use Excel form to register the experimental research data, use SPSS 22.0 statistical software to analyze the data, the data are expressed as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ), and the data are tested for normal distribution, and t test is used for normal distribution. Does not conform to the normal distribution with the rank sum test, and  $P < 0.05$  is considered as the difference is statistically significant.

## 3. Results

### The Effect of Acupuncture on the Expression of ERM, PI3K and Akt Protein in Rats with Ischemic Stroke

Western blot analysis showed that the expression levels of ERM, PI3K, and Akt proteins in the sham operation group were lower than those in the normal group ( $P < 0.05$ ); compared with the sham operation group, the expression levels of ERM, PI3K, and Akt proteins in the model group were significantly lower Decrease ( $P < 0.05$ ); Compared with the model, the protein expression of ERM, PI3K and Akt in the acupuncture group were significantly increased ( $P < 0.05$ ). The details are shown in **Figures 1-3**.

## 4. Discussion

At present, with the increase of living standards, ischemic stroke is on the rise, and the research on its mechanism of stroke is also intensified. Among them, the research of damaged neuron signal transduction pathway has attracted the attention of scholars at home and abroad [11]. Ezrin-root protein-membrane protein (ERM), as a cell-skeleton membrane junction protein, has a regulatory effect on the transduction of multiple signal pathways, and participates in and regulates cell growth, migration, membrane transport and other biological functions [12].



Some scholars have found *in vitro* cell culture experiments to simulate ischemic stroke [14] [15] [16] [17] that threonine residues at the carboxyl end of activated ERM produce phosphorylation, and then undergo conformational changes, exposing binding sites, and further phosphorylation PI3K regulatory subunits, activated subunits can initiate PI3K activation, prompting it to issue complex recruitment exercises, and then be called and migrated to the adjacent plasma membrane to undergo phosphorylation, making 3,4-bisphosphate phosphatidylinositol (PIP2) convert to phosphatidylinositol 3,4,5-triphosphate (PIP3). As a second messenger transduction molecule, PIP3 continues to perform phosphorylation and transmits its activation signal to the core signal protein Akt in the membrane. Phosphorylated Akt further participates in cellular processes including apoptosis and glucose metabolism, and can directly phosphorylate more a kind of transcription mediators, by regulating these transcription media, can inhibit the expression of apoptotic genes and enhance the expression of anti-apoptotic genes, thereby promoting cell survival [18]. In this experiment, Western blot method was used to detect the protein concentrations of ERM, PI3K, and Akt. The results showed that: compared with the normal group, the expression levels of ERM, PI3K, and Akt protein in the sham operation group were lower ( $P < 0.05$ ); compared with the sham operation group, the expression levels of ERM, PI3K, and Akt proteins in the model group were significantly reduced ( $P < 0.05$ ); compared with the model, the expression levels of ERM, PI3K, and Akt proteins in the acupuncture group were significantly increased ( $P < 0.05$ ). It can be seen that acupuncture can significantly up-regulate the protein expression levels of ERM, PI3K, and Akt in the brain tissue around the infarcted area of ischemic stroke model rats. The effect mechanism may be related to the activation of the ERM/PI3K/Akt signal transduction pathway and the inhibition of neuronal apoptosis in the brain tissue around the infarct.

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### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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