

Expression of SOCS1 in Rats of Sciatic Nerve Injury after EA Treatment

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ABSTRACT

Objective: To investigate the efficacy and mechanism of electroacupuncture treatment in rats of sciatic nerve injury.

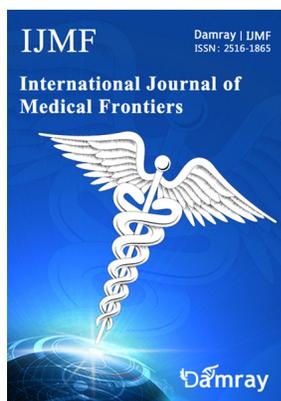
Methods: Seventy five male SD rats were randomly divided into control group, model group and electro-acupuncture (EA) group. In the EA group, "Zusanli" points and "Yanglingquan" points of the left lower extremity were selected. The frequency of EA was 5 Hz, and EA performed for 15 minutes a day. The sciatic nerves were collected at 1 week, 2 weeks, 3 weeks, 4 weeks and 6 weeks, respectively, and the expression of SOCS1 protein was detected by Western blot.

Results: The results of gait analysis showed that the electro-acupuncture group tended to be more in the control group than in the model group. The wet weight of triceps surae in rats was closer to 1 than that in EA group and model group. Nissl staining showed that the number of Nissl bodies in EA group was significantly higher than that in model group. The expression of SOCS1 protein in the model group was significantly higher than that in the EA group and the control group.

Conclusion: EA may repair peripheral nerve injury by inhibiting the expression of SOCS1 in sciatic nerve.

Key words: EA; Sciatic nerve; Peripheral nerve; SOCS1

Electro-acupuncture (EA) as one of the main rehabilitation methods for peripheral nerve injury, has obvious clinical effect and long effective period [1,2]. In recent years, many clinical and experimental studies have shown that EA has significant therapeutic effects on peripheral



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nerve injury in human and animal models [3], but its specific mechanism is not completely clear [1]. Suppressor of cytokine signaling (SOCS) family is a class of negative regulators produced by cells and fed back to block cytokine signaling [2] they are involved in the regulation of many cytokine responses in the process of human immune regulation and various inflammatory diseases. In this experiment, the rat model of sciatic nerve injury was treated by EA and the expression of SOCS1 was observed. The possible mechanism of EA in treating peripheral nerve injury was discussed.

MATERIALS AND METHODS

Grouping and model preparation

Seventy-five adult male SD rats (Jinan Saifu Laboratory Animal Breeding Co., Ltd.) of clean grade, weighing 180-220 g, were randomly divided into blank group, model control group and EA group. The experiment was approved by the Animal Protection and Use Committee of Qingdao University, and the operation met the requirements of animal ethics. The model preparation method was used to establish a rat model of sciatic nerve injury [3]. The simple steps were as follows: 8% chloral hydrate (40 mg/kg) was injected into the abdominal cavity for anesthesia, the left hind legs of the rats were depilated for skin preparation, routine disinfection, incision of the skin along the lower edge of the left femur, blunt separation of muscles, exposure of the sciatic nerve, and 4.0 surgical suture thread in the sciatic bone. Three loosening knots with an interval of 1 mm were made before the nerve bifurcation to compress the epineurium slightly, with a slight twitch of the toe as the degree, and then the muscle fascia and skin were sutured.

After the establishment of the sciatic nerve injury model, the rats limped, licked, suspended and other hindlimb protection phenomena, indicating that the chronic crush injury model of sciatic nerve was successfully prepared. The blank group included rats were untreated.

Fixation and EA

The patented flexible rat fixator (patent No. 2011100 21482.5) was used to fix the rats, and the rats were placed on the fixator 5 minutes before each EA treatment to promote its adaptability to the fixator. After modification of Huatuo electronic physiotherapy instrument, the left lower limb Zusanli and suspension bell were taken by EA. The left lower limb Zusanli and suspension bell were inserted vertically into the needle for 2-3, the density wave with frequency of 5 Hz, the current intensity of 2 mA (slight twitch of the left lower limb muscle), which lasted 15 minutes, and EA was performed at the

same time every afternoon for 5 consecutive days and 2 days off for a course of treatment.

Behavioral indicators

Sciatic nerve index (SNI) was used to test the rats' nerve function. Rats were coated with ink on their hind claws and placed in a self-made open wooden groove with length of 60 cm, width of 10 cm and height of 10 cm. The white paper was cut into the same width as the wooden groove and laid on the bottom of the groove. After the rats' hind limbs were stained with pigment, the rats were placed at one end of the groove and made to walk on the other side of the groove, leaving 5-6 footprints on each hind limb [4]. The distance from the heel to the third toe (PL), the distance from the first toe to the fifth toe (TS) and the distance from the second toe to the fourth toe (ITS) were measured. Sciatic nerve function index (SFI) was calculated according to the formula. The sciatic nerve function index SFI = 0 was normal and - 100 was complete injury [5,6].

Sampling and tissue sections

After 1 week, 2 weeks, 3 weeks, 4 weeks and 6 weeks of EA the rats were subjected to deep anesthesia: (1) The left hind legs of the rats were depilated and skinned, routinely disinfected, the skin was cut along the lower edge of the left femur, the muscles were bluntly separated, the sciatic nerve was exposed, and the sciatic nerve was cut along the bifurcation of the sciatic nerve for about 2 cm. The sciatic nerve was quickly placed at -80°C . (2) After thoracotomy, left ventricular catheterization and rapid infusion of 200 ml normal saline, the tissues were immobilized with 200 ml paraformaldehyde solution containing 4%. L4-6 tissue of spinal cord segment was quickly removed and placed in the above fixative solution at 5°C for 48 h. After fixation, it was placed in the embedding box with slow flushing (6-8) h. Different concentrations of ethanol (100%, 95%, 85%, 75%, 50%) and xylene were dehydrated layer by layer and then Nissl staining was conducted.

Nissl staining for the detection of Nissl bodies in the posterior horn of spinal cord

Slices ($8\ \mu\text{m}$) were baked at 70°C for 30 minutes in a constant temperature oven, then dewaxed and rehydrated in xylene, ethanol (100%, 95%, 85%, 75%) and double steaming water respectively. Nissl dye solution was added to the slices for dyeing for 10 minutes. The excess dyes were washed out with double steaming water. Nissl methyl violet was used for differentiation (6-8)s, 100% ethanol was used for dehydration for 5 min, and xylene was used for transparent for 5 min. Observation, if necessary, repeated differentiation, neutral gum sealing, the positive staining results were analyzed [7].

Protein extraction and Western blot detection of SOCS1 protein changes

Protein was extracted for 10 minutes at 5°C and washed by PBS for 10 minutes. 70 μm solution was added to the grinder for 5 minutes, 80 μm solution was added to the grinder, then the grinder was rinsed with 80 μm solution, then broken for 15 minutes at 5°C, centrifuged with 12,000 R for 15 minutes to extract total protein, and then quantified. Protein was stirred by BCA method, mixed with appropriate BCA working solution. After 10 min of boiling water bath, the supernatant was centrifugally cooled and stored in a refrigerator at - 80°C. According to the molecular weight of target protein (35), Western blot was selected to gel at 12% colloid. 5% skim milk powder was sealed at room temperature at 1 h. Samples were incubated with primary antibodies over night following with secondary antibody: COCS-1 1:1000, GAPDH 1: 1500 at room temperature for 2

h. Samples were then washed with TBST for 3 times, 5 min each time. ECL was used for color rendering and imaging system for scanning imaging. The image Pro 6.0 instrument was used for stripe protein gray value analysis, GAPDH was used as an internal reference protein calibration.

Data processing and statistical analysis

All the data were processed by SPSS 22.0 software, and using one way ANOVA analysis for multiple groups. $P < 0.05$ was considered as statistical significant.

RESULTS

Gait analysis results (sciatic nerve index)

After EA for 1 W and 2 W, there was significant difference in gait analysis between the control group and the model group and the EA group ($P < 0.05$, Table 1).

Table 1. Gait Analysis

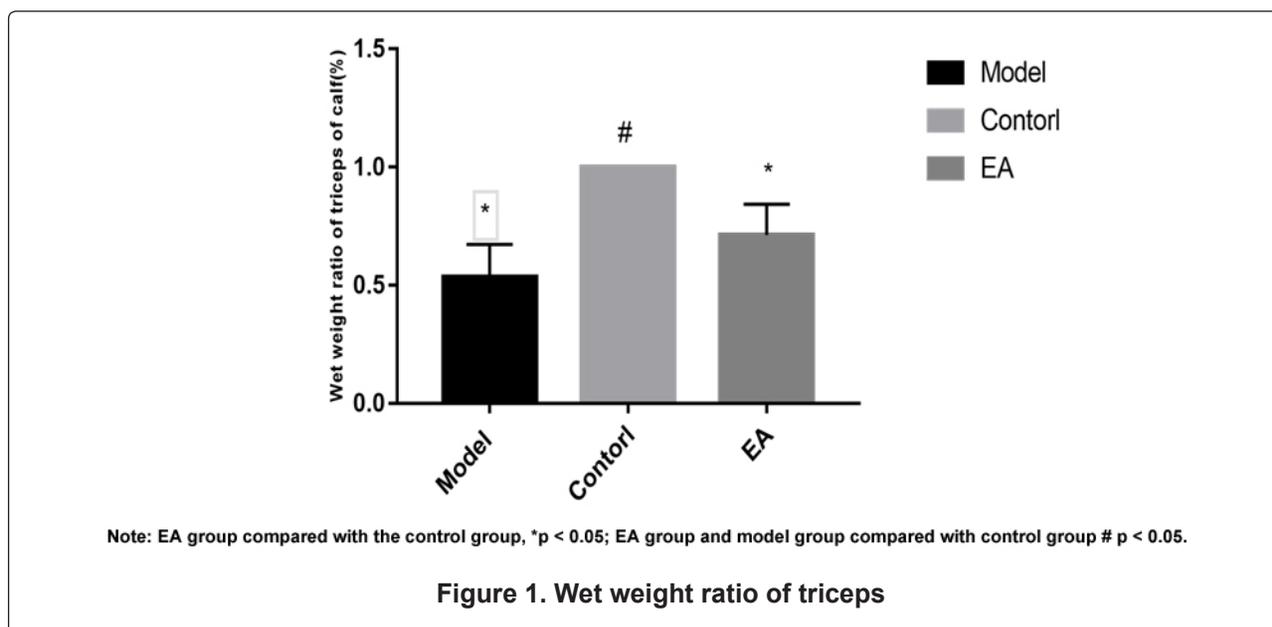
Time	1w	2w	3w	4w	6w
Control	0±0	0±0	0±0	0±0	0±0
Model	87.67±1.45*	73.33±1.67*	62.67±1.45*#	56..3±1.70*#	43.33±0.88*#
EA	83.34±3.35*	73.26±0.57*	42.67±1.45*#	33.35±1.53*#	24.33±2.33*#

Note: Gait analysis, compared with the control group, * $P < 0.05$; EA group compared with model group, # $P < 0.05$.

Wet weight ratio of triceps of calf

The control group and the EA group were higher than the model group and the EA group, with a significant

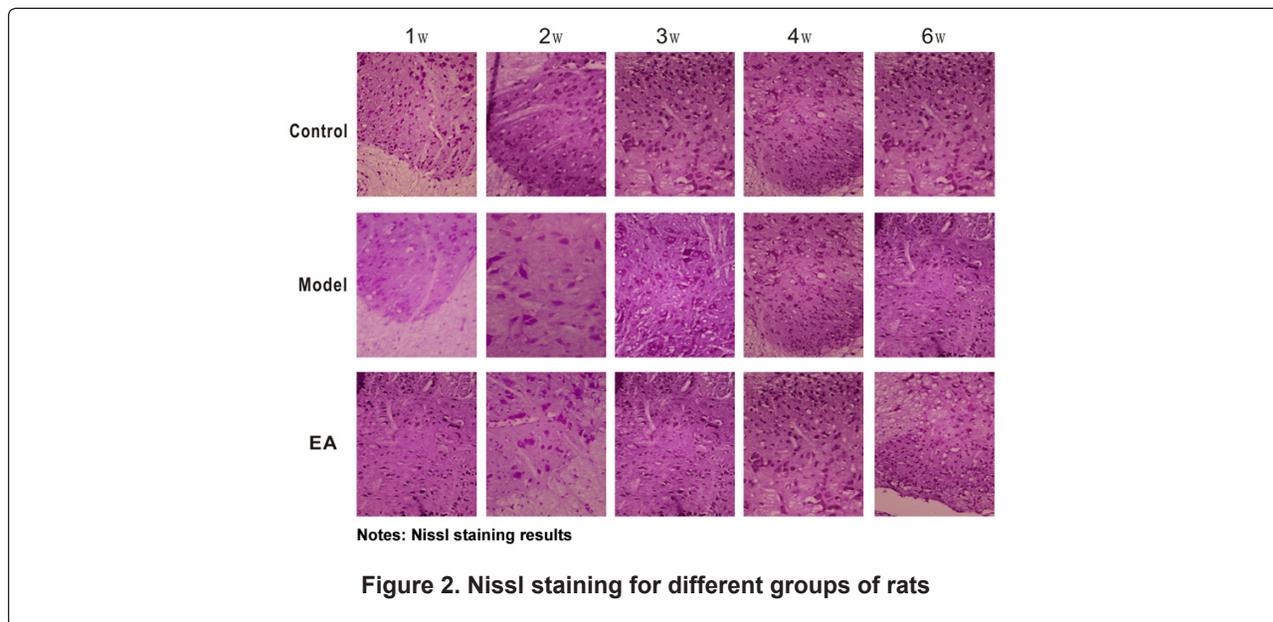
difference ($P < 0.05$); the EA group was higher than the model group, with a significant difference ($P < 0.05$). The results are shown in Figure 1.



Nissl staining result

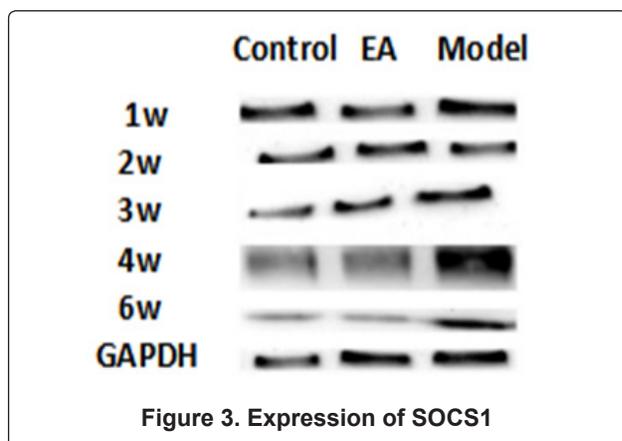
After Nissl staining, under the microscopic observation, the sciatic neurons in each group were vacuolated, the Nissl bodies were dark purple, and the nuclei were light purple (Figure 2). The number of sciatic nerve neurons in the model group was obviously lower than

that in the control group. The number of neurons in the EA group was obviously higher than that in the model group. The number of neurons in the EA group was close to that in the control group at 6 weeks. The results are shown in Figure 2.



Expression of SOCS1 in neurons of sciatic nerve

There was significant difference between the control group and the EA group and the model group ($P < 0.05$), and the inhibition of protein expression in the EA group was significantly higher than that in the model group ($P < 0.05$). The results are shown in Figure 3.



recovery of nerve is still unclear. TCM believes that sciatic nerve injury is mainly caused by Foot Yangming Meridian and foot Shaoyang meridian. Foot Yangming meridian is one of the 12 meridians, and "Zusanli" point is one of the main points of "Foot Yangming stomach meridian". It is located outside the leg, 3 inches below the calf nose. The calf nose is connected with Jiexi [9]. The superficial layer is covered with lateral sural cutaneous nerve. Yang Lingquan, gallbladder belongs to Yang Jing, lateral knee belongs to Yang, fibula small head like mausoleum, the depression below the mausoleum through meteorological water into the deep like spring, so called "Yang Lingquan", also known as Jinhui, Yangling, Yangling Lingquan; is the pulse of foot and Shaoyang into the closure point, for the eight clubs of Jinhui. Skin, subcutaneous tissue, deep leg fascia, peroneal longus and peroneal brevis [10]. The skin is distributed by the lateral cutaneous nerve of the calf. After the common peroneal nerve was separated from the sciatic nerve in the superior horn of the fossa, the long peroneal muscle was penetrated along the lateral wall of the fossa to the posterior inferior part of the fibular capillary, which was divided into the superficial and deep peroneal nerves. The muscular branches of the superficial peroneal nerve govern the long and short muscles of the fibula. Hemiplegia, lower limb paralysis, numbness, knee swelling and pain, beriberi, flank rib pain, bitter mouth, vomiting, jaundice, children's panic, etc. Now

DISCUSSION

At present, clinical studies have confirmed that EA has a reliable effect on the functional recovery of patients with peripheral nerve injury [8]. However, the mechanism of EA stimulation promoting the functional

it is mainly used for sciatica, hepatitis, knee arthritis, chorea in children, etc.

Signal transduction is the process of maintaining the integrity and coordination of the stimulus response of cells in vivo and in vitro, maintaining the normal physiological activities of cells, especially proteins, and is a highly ordered multi-enzyme cascade reaction of regulatory network in animals. Information transmission [9,11].

Information substances mainly rely on information substances, including intercellular information substances and intracellular information molecules, in which cytokines are the most important information substances in animal organisms, and play an important role in cell differentiation and proliferation, anti-infection response, immune regulation and other biological functions [12]. The biological functions of cytokines are regulated by many factors, of which suppressor of Cytokine in E signal transduction inhibitor (SOCS) is an important regulator.

SOCS 1 regulates cell signaling pathways in three ways: 1. Inactivation of the N terminal of JAK kinase by binding to phosphorylated tyrosine acid residues in the SH2 region. Thus inhibiting signal transduction. At the same time, the KIR domain of SOCS 1 is the inhibitory domain of JAK tyrosine kinase activity, which can inhibit the activity of JAK tyrosine kinase. The binding site of SOCS 1 promoter containing STAT rotor can inhibit the activation of STAT to some extent. SOCS 1 box can promote the ubiquitination of signal transduction intermediates. Inhibition of cell induced signal transduction cascades, thereby promoting the degradation of bound proteins [13-16]. In this experiment, the expression of SOCS-1 in sciatic nerve injury treated by EA was detected to explore the possibility of EA treatment of peripheral nerve injury.

To sum up, this experiment found that EA at Zusanli and Yanglingquan had obvious promoting effect on the related function after sciatic nerve injury [17]. One of the mechanisms might be through promoting SOCS 1 expression. In recent years, more and more studies have been carried out on neurological diseases. Therefore, the specific mechanism of EA on promoting peripheral nerve injury needs further study.

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