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EXPERIMENTAL STUDY

Effect of acupotomy on nitric oxide synthase and beta-endorphin in third lumbar vertebrae transverse process syndrome model rats

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Abstract

OBJECTIVE: To explore the long-term effects and pain relief mechanism of acupotomy by observing changes in nitric oxide synthase (NOS) and beta-endorphin (β-EP) in the hypothalamus, spinal cord, and peripheral blood of rats with third lumbar vertebrae (L_3) transverse process syndrome.

METHODS: Twenty-eight SD rats were randomly assigned to normal, model, electroacupuncture (EA), and acupotomy group. The last three groups were put through an operation to emulate L_3 transverse process syndrome. Fourteen days after the simulation operation, EA and acupotomy treatments were applied to the respective groups. Fifty-six days after the simulation operation, biochemistry tests and enzyme-linked immunosorbent assay were used to measure NOS and β-EP in the hypothalamus, spinal cord, and peripheral blood.

RESULTS: Rats with the simulation operation showed significantly higher levels of NOS and β-EP in the hypothalamus, spinal cord, and peripheral blood than those in the normal group. The EA and acupotomy groups had significantly lower levels of NOS and β-EP than those in the model group. There was no statistical difference between the EA and acupotomy groups.

CONCLUSION: EA and acupotomy treatments significantly lowered NOS and β-EP levels in the hypothalamus, spinal cord, and peripheral blood and alleviated L_3 transverse process syndrome.

Key words: Acupotomy; Small needle knife; Electroacupuncture; L_3 transverse process syndrome; Nitric oxide synthase; Beta-endorphin

INTRODUCTION

Acupotomy is a new Traditional Chinese Medicine (TCM) treatment procedure invented by Prof. Hanzhang Zhu. Acupotomy combines TCM meridian theory and modern surgical principles, and uses a needle knife as the main treatment tool. It is used to treat chronic soft tissue injury and bone hyperplasia. Acupotomy converts open surgery to closed surgery to reduce side-effects and complications using 14 categories and 33 kinds of patented acupotomy instruments. In

clinic, acupotomy has a set of comprehensive procedures for diagnosis, treatment, and care. There are currently more than one hundred thousand acupotomy practitioners in Western Medicine and TCM in China.[1] Prof. Hanzhang Zhu widely reviewed the Chinese and Western medical literature to develop acupotomy. The "Nine Needles" recorded in the *Ling Shu Jing*,[2] were combined with the modern surgical scalpel to create a bladed needle that is able to reach lesions deep inside the body and perform proper procedures like cutting and peeling. The treatment effects have positive clinical application to chronic soft tissue injury, bone, and joint diseases. Acupotomy has not only achieved the target treatment effects, but also avoided the side effects of open surgery. Because the tool is a combination of acupuncture needle and surgical scalpel, it was named a "small needle knife." Acupotomy strips adhesions, releases contractures, clears blockages, and is characterized by smaller wounds, fewer complications, higher safety, lower cost, and significant treatment efficiency.

Acupotomy theory hypothesizes that disorders of dynamic balance, including adhesion, contracture, scar formation, and blockage, are the fundamental risk factors of chronic soft tissue injury. By stripping adhesions, releasing contractures, and clearing blockages to restore dynamic balance, acupotomy will often achieve higher efficiency than other concurrent medical procedures with L_3 transverse process syndrome and promote long-term effects.[3,4] Our earlier stage experiment showed that acupotomy treatment has short-term effects and adjusts analgesic substances.[5,6] However, there are not animal experiments exploring the long-term effects of acupotomy. Therefore, we prolonged animal survival time in this experiment and aimed to study the long-term effects of acupotomy treatment on nitric oxide synthase (NOS) and beta-endorphin (β-EP) in Sprague-Dawley (SD) rats with L_3 transverse process syndrome.

MATERIALS AND METHODS

Experimental animals and grouping

Twenty-eight 3-month-old male SD rats provided by Victoria-Lihua Animal Laboratory Center with batch number SCXK (Beijing, China) 2007-0001, weighing 250-270 g were randomly assigned into a normal, model, electroacupuncture (EA), and acupotomy group by a random number table method (n=7). No intervention was performed in the normal group. Rats in the remaining groups were prepared with an operation to simulate L_3 transverse process syndrome. No intervention or treatment was given to rats in the model group after model establishment. Fourteen days after the operation, acupotomy was given to the acupotomy group, and EA treatment to the EA group. The experimental procedures were performed under the requirements of the Provision and General Recommendations of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Research Ethics Board of Beijing University of Chinese Medicine.

Establishment of L_3 transverse process syndrome rat model

L_3 transverse process syndrome was simulated by modifying the method developed by Wang *et al*,[7] with 10% chloral hydrate (Beijing Factory of Chemical Reagents, Beijing, China) used for abdominal anesthetization (0.4 g/kg). SD rats were sterile-prepared in the prone position. Hairs were removed around the lumbar area and a 1 cm vertical incision was made 0.5-0.8 cm to the left of the L_3-L_4 spinal transverse process. The deep myofascia were separated and the left lumbar paraspinal muscle was exposed 0.5-0.8 cm left of the central line. The paraspinal muscle was separated to the posterior of L_3 transverse process, and a piece of 0.5 cm×0.5 cm absorbent gelatin sponge was implanted (Nanjing Jinling pharmaceutical Co., Ltd., Nanjing, China). When the operation was done the lumbar paraspinal muscle was sutured with plain gut suture (3-0), the cutaneous incision was sutured with silk (4-0), and the wound was sanitized with gentamicin (2 mL, 80 000 U, Tianjin pharmaceutical Co., Ltd., Tianjin, China) to avoid infection.

Electroacupuncture treatment

According to animal meridian theory,[8] rats were fixed in the prone position with rag strips. Yaoyangguan (GV 3) and left Shenshu (BL 23) were selected acupoints and were electroacupunctured with a 2 and 100 Hz dense-disperse wave, 20 min for each treatment, one treatment every other day. There were six treatments in 2 consecutive weeks.

Acupotomy treatment

Fourteen days after the simulated operation, acupotomy treatment was performed in the rats every seven days, with two acupotomy treatments in total. SD rats in the acupotomy group were slightly anaesthetized with diethyl ether for about 1 min until no resistance was shown. A trigger point or sclerosis in the local soft tissue close to the cutaneous incision left by simulation operation was located. The acupotomy instrument was used to make three cuts parallel to the spine, and then the instrument handle was rotated 90° to make another cut. The acupotomy instrument was removed and the wound was compressed with gauze to avoid excessive bleeding.

When all of the treatments were finished, all rats were fed for another 28 days. There were no further treatments or interventions during the final 28 days.

Blood and tissue sampling

Fifty-six days after the simulated operation, SD rats were decapitated to harvest 6 mL of blood and tissue,