

## 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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the simulation operation, biochemistry tests and enzyme-linked immunosorbent assay were used to measure NOS and  $\beta$ -EP in the hypothalamus, spinal cord, and peripheral blood.

**RESULTS:** Rats with the simulation operation showed significantly higher levels of NOS and  $\beta$ -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  $\beta$ -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  $\beta$ -EP levels in the hypothalamus, spinal cord, and peripheral blood and alleviated L<sub>3</sub> transverse process syndrome.

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**Key words:** Acupotomy; Small needle knife; Electroacupuncture; L<sub>3</sub> transverse process syndrome; Nitric oxide synthase; Beta-endorphin

**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 ( $\beta$ -EP) in the hypothalamus, spinal cord, and peripheral blood of rats with third lumbar vertebrae (L<sub>3</sub>) 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<sub>3</sub> transverse process syndrome. Fourteen days after the simulation operation, EA and acupotomy treatments were applied to the respective groups. Fifty-six days after

**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.<sup>1</sup> Prof. Hanzhang Zhu widely reviewed the Chinese and Western medical literature to develop acupotomy. The "Nine Needles" recorded in the *Ling Shu Jing*,<sup>2</sup> 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<sub>3</sub> transverse process syndrome and promote long-term effects.<sup>3,4</sup> Our earlier stage experiment showed that acupotomy treatment has short-term effects and adjusts analgesic substances.<sup>5,6</sup> 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 ( $\beta$ -EP) in Sprague-Dawley (SD) rats with L<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> transverse process syndrome rat model*

L<sub>3</sub> transverse process syndrome was simulated by modifying the method developed by Wang *et al.*,<sup>7</sup> 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<sub>3</sub>-L<sub>4</sub> 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<sub>3</sub> 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,<sup>8</sup> 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,

including fascia and muscle, around the operation incision. According to standard procedures of pathological tissue preparation, each sample tissue was fixed with 10% formalin for 1 week, hematoxylin-eosin (HE) stained, and sliced for microscopic evaluation. Each blood sample was mixed with 90  $\mu\text{L}$  EDTA- $\text{Na}^2$  and 120  $\mu\text{L}$  aprotinin, and centrifuged ( $4^\circ\text{C}$ , 2000 rpm) for 15 min to separate the serum for biochemistry and enzyme linked immunosorbent assay (ELISA). Tissues of the hypothalamus and lumbar spinal cord were put into preservation liquid (Tris 10 mmol/L, NaCl 137 mol/L, KCl 5 mol/L,  $\text{CaCl}_2$  2 mmol/L,  $\text{MgCl}_2$  2.5 mmol/L, sucrose 0.25 mmol/L, PMSF 0.1 mmol/L, and bovine serum albumen 0.1%) to prepare for ELISA tests.

### Statistical analysis

All data are expressed as mean  $\pm$  standard deviation (SD). SAS 9.0 statistical software (SAS Institute Inc., Cary, NC, USA) was used to analyze the data. Groups were compared using a two-way analysis of variance (ANOVA). The least significant difference (LSD) post-hoc test was used for data with homogeneity of variance, and Tamhane's  $t$ -test was used for those with heterogeneity of variance.  $P < 0.05$  was considered statistically significant.

## RESULTS

### Pathological observations

The normal group showed fine structure of muscle tissue with aligned muscle fibers and uniform thickness of the epimysium (Figure 1A). Fifty-six days after simulation operation, the model group showed foreign body embedded granulomas surrounded by proliferated connective tissue and chaotic-arranged collagen fibers. Visible chronic muscle tissue inflammation with fiber ruptures, chaotic arrangements, shrinkages, widened gaps, and edema were apparent (Figure 1B). The EA group showed glial scars surrounded by inflammatory tissue, but the extent was significantly less than that of the model group (Figure 1C). The acupotomy group showed fewer glial scars, less inflamed muscle tissues in the vicinity of the operation, and reduced leukocyte infiltration than that of the model group (Figure 1D). There were no significant differences between the EA and acupotomy groups.

### Changes in NOS in the hypothalamus, lumbar spinal cord, and peripheral blood

Fifty-six days after the operation, compared with the normal group, the model group showed significantly higher NOS levels in the hypothalamus, lumbar spinal cord, and peripheral blood. The NOS level in the hypothalamus increased more ( $P < 0.01$ ) than that in the spinal cord and peripheral blood ( $P < 0.05$ ). Compared with the normal group, the EA and acupotomy groups had significantly higher NOS levels in the hypothalamus ( $P < 0.05$ ), but no differences in the spinal cord and the peripheral blood.

Compared with the model group, the EA and acupotomy groups had significantly lower NOS levels in the hypothalamus, lumbar spinal cord, and peripheral blood. The EA and acupotomy groups had significantly lower hypothalamic NOS levels than those in the model group ( $P < 0.01$ ). The EA and acupotomy groups also had significantly lower NOS levels in spinal cord and peripheral blood than those in the model group ( $P < 0.05$ ).

There was no difference in NOS levels between the EA and acupotomy groups on NOS level in the hypothalamus, spinal cord, and peripheral blood ( $P > 0.05$ ) (Table 1).

Table 1 NOS levels in the four groups ( $\bar{x} \pm s$ )

Group	n	Hypothalamus	Spinal cord	Peripheral blood
Normal	7	3.2 $\pm$ 1.0	4.3 $\pm$ 1.1	34.7 $\pm$ 3.8
Model	7	6.6 $\pm$ 1.2 <sup>a</sup>	5.7 $\pm$ 2.0 <sup>a</sup>	38.7 $\pm$ 1.9 <sup>b</sup>
EA	7	4.6 $\pm$ 0.7 <sup>bc</sup>	4.0 $\pm$ 0.6 <sup>d</sup>	33.7 $\pm$ 3.4 <sup>d</sup>
Acupotomy	7	4.6 $\pm$ 0.9 <sup>bc</sup>	3.7 $\pm$ 0.4 <sup>d</sup>	33.6 $\pm$ 3.1 <sup>d</sup>

Notes: rats in the normal group were used as controls. In the model group rats underwent a simulation operation to emulate  $L_3$  transverse process syndrome and did not receive treatment. In the electroacupuncture group, rats were treated with electroacupuncture. In the acupotomy group, rats were treated with acupotomy. NOS: nitric oxide synthase; EA: electroacupuncture. <sup>a</sup> $P < 0.01$ , compared with the normal group; <sup>b</sup> $P < 0.05$ , compared with the normal group; <sup>c</sup> $P < 0.01$ , compared with the model group; <sup>d</sup> $P < 0.05$ , compared with the model group. There are no significant differences between the EA and acupotomy groups.

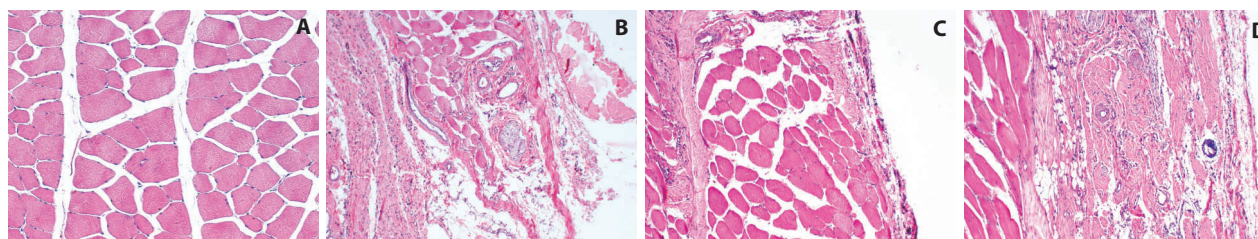


Figure 1 Biopsy around the operation incision in four groups under light microscope (HE stain,  $\times 126$ )

A: biopsy from the normal group; B: 56-day biopsy from the model group; C: 56-day biopsy from the electroacupuncture group; D: 56-day biopsy from the acupotomy group. Rats in the normal group were used as controls. In the model group, rats underwent an operation to emulate  $L_3$  transverse process syndrome and did not receive treatment. In the electroacupuncture group, rats were treated with electroacupuncture. In the acupotomy group, rats were treated with acupotomy. HE: hematoxylin-eosin.

### Changes in $\beta$ -EP in the hypothalamus, lumbar spinal cord, and peripheral blood

Fifty-six days after the operation, compared with the normal group, the model group had significantly higher  $\beta$ -EP levels in the hypothalamus, lumbar spinal cord, and peripheral blood ( $P < 0.01$ ). Compared with the normal group, both the EA and acupotomy groups had significantly higher  $\beta$ -EP levels in the hypothalamus and peripheral blood ( $P < 0.01$ ). The  $\beta$ -EP levels in the spinal cord were not significantly different among the EA, acupotomy, and normal groups.

Compared with the model group, both the EA and acupotomy groups had significantly lower  $\beta$ -EP levels in the hypothalamus, lumbar spinal cord, and peripheral blood. The EA and acupotomy groups had significantly lower  $\beta$ -EP levels in the hypothalamus and spinal cord than those in the model group ( $P < 0.01$ ). The EA and the acupotomy groups also had significantly lower peripheral blood  $\beta$ -EP level than that in the model group ( $P < 0.05$ ). (Table 2)

Table 2  $\beta$ -EP levels in the four groups ( $\bar{x} \pm s$ )

Group	n	Hypothalamus	Spinal Cord	Peripheral Blood
Normal	7	4.3 $\pm$ 1.5	2.7 $\pm$ 1.3	16.5 $\pm$ 7.9
Model	7	15.6 $\pm$ 2.6 <sup>a</sup>	15.7 $\pm$ 7.3 <sup>a</sup>	96.8 $\pm$ 23.7 <sup>a</sup>
Electroacupuncture	7	8.7 $\pm$ 1.0 <sup>ab</sup>	7.0 $\pm$ 2.4 <sup>b</sup>	65.0 $\pm$ 24.7 <sup>ac</sup>
Acupotomy	7	8.4 $\pm$ 2.3 <sup>ab</sup>	7.7 $\pm$ 2.5 <sup>b</sup>	64.0 $\pm$ 25.8 <sup>ac</sup>

Notes: rats in the normal group were used as controls. In the model group, rats underwent an operation to emulate L<sub>3</sub> transverse process syndrome and did not receive treatment. In the electroacupuncture group, rats were treated with electroacupuncture. In the acupotomy group, rats were treated with acupotomy.  $\beta$ -EP: beta-endorphin; EA: electroacupuncture. <sup>a</sup> $P < 0.01$ , compared with the normal group; <sup>b</sup> $P < 0.01$ , compared with the model group; <sup>c</sup> $P < 0.05$ , compared with the model group. There were no significant differences between the EA and acupotomy groups.

## DISCUSSION

This animal experiment modeled<sup>7</sup> L<sub>3</sub> transverse process syndrome by implants of absorbent gelatin sponges in the lumbar paraspinal muscle to create soft tissue aseptic inflammation. Smaller local injury and easier to locate operation positions are the major merits of modifications. Local tissue pathological observation and pain threshold study also show that this enhanced simulation method is comparable to traditional animal models of L<sub>3</sub> transverse process syndrome.

Nitric oxide (NO) is a second messenger for neurotransmitters.<sup>9</sup> It plays an important role in signal transduction, and amplifies the strength of injury signal in the spinal cord.<sup>10</sup> Some experiments showed that NO is important in the formation and maintenance of pain sensation and hyperalgesia.<sup>11</sup> NOS is the rate limiting enzyme to synthesizing endogenous NO. There-

fore, NOS is commonly used to reflect the formation of NO.<sup>12</sup>  $\beta$ -EP is one of the major endogenous opioid peptides. It is an agonist of the opioid receptors, and is therefore considered to be the main transmitter for the analgesic system.<sup>13</sup>

Our animal experiment showed that NOS and  $\beta$ -EP levels were higher in the hypothalamus, spinal cord, and peripheral blood in model rats than those in normal rats. This implies that the operation causes chronic inflammation, activates peripheral NOS, and therefore increases NO synthesis. Increased NO synthesis causes pain and central pain hypersensitivity, which results in higher NOS levels in the spinal cord hypothalamus. The stimulation of chronic inflammation and NO-transduced central pain hypersensitivity induce the hypothalamus and spinal cord to produce more  $\beta$ -EP to release into the blood to alleviate pain. After electroacupuncture and acupotomy treatments, rats had significantly lower levels of NOS and  $\beta$ -EP in the peripheral and central nerve system, compared with the model group. This suggests that both the electroacupuncture and acupotomy treatments are capable of regulating NOS and  $\beta$ -EP to alleviate pain because of chronic inflammation in L<sub>3</sub> transverse process syndrome.

This animal model involves embedding of absorbent gelatin sponges to simulate chronic inflammation. The chronic inflammation produces pathological changes like adhesion and scar formation. These pathological changes are difficult to eliminate and will therefore generate chronic inflammation. As the peripheral chronic inflammation continues, NOS and  $\beta$ -EP levels in the peripheral and central nervous system remain elevated. This further suggests that our simulation method for L<sub>3</sub> transverse process syndrome is applicable to building models for long-term studies.

Our experiment only included two acupotomy treatments on the 14th and 21st days after operation. Fifty-six days after the simulation operation were when NOS and  $\beta$ -EP were measured. However, the levels were still significantly lower than those in the model group. This shows that acupotomy treatment on L<sub>3</sub> transverse process syndrome provides short treatment time and long-lasting treatment effects.

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