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## Effects of Electro-Acupuncture and Acupotomy Dissolution on mRNA Expression of Center Pro-Opiomelanocortin Detected by In Situ Hybridization in Rats with Non-Specific Low Back Pain

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### Abstract

**Background:** Pro-opiomelanocortin (POMC) mainly exists in the pituitary gland, hypothalamus, and peripheral tissues and can relieve pain through its degradation product  $\beta$ -endorphin. Its mRNA expression quantity represents the level of gene expression of endorphin system. We aimed to determine the effects of electro-acupuncture and acupotomy dissolution on the mRNA expression of center POMC in rats with non-specific low back pain. **Materials and Methods:** This study was performed on 42 Sprague-Dawley rats in four groups of normal, model, electro-acupuncture, and acupotomy. The normal group did not receive any intervention, while non-specific low back pain was established in other groups. Then, the model group did not receive any treatment, electro-acupuncture and acupotomy groups were treated with electro-acupuncture therapy and acupotomy, respectively. Microscopic images of the slices, prepared from spinal dorsal horn and hypothalamus, were analyzed to evaluate the mRNA expression of center POMC. **Results:** Under light microscopy examination, the positive POMC mRNA cells of electro-acupuncture and acupotomy groups increased more than the model group, while its expression in the hypothalamus and spinal dorsal horn was less than the model group, but the difference was not significant ( $P < 0.01$ ). **Conclusion:** Electro-acupuncture and acupotomy could reduce POMC mRNA expression in spinal cord and increase it in the hypothalamus of rats with non-specific low back pain. [GMJ.2016;5(4):194-99]

**Keywords:** Acupotomy; Electro-acupuncture; Non-specific Lower Back Pain; Pro-opiomelanocortin; mRNA Expression

### Introduction

Non-specific low back pain is a clinically common disorder of the legs and lumbar region of the back. Workers doing particularly heavy physical labor and young adults account for a considerable proportion of patients sustaining this disorder [1]. Its pathogenesis is related to the anatomical and physiological

characteristics of the third lumbar vertebra, which is in the middle of the physiological lordosis of the lumbar vertebrae, serving as an axis of movement of the lumbar region. Therefore, the traction stresses borne by both of its transverse processes are the strongest among the lumbar vertebrae.

Needle-scalpel therapy (acupotomy) is based on the meridian theory of traditional Chinese

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medicine and under the guidance of the principle of modern surgery. On theoretical innovation, it combines modern medical science with traditional Chinese medicine to gain a new therapeutic insight into chronic soft tissue injury and bone hyperplasia [2]. In treatment technology, acupotomy is an innovation based on modern surgical techniques to convert open surgery to a close one, and to reduce the side effects and complications of open surgery. Acupotomy by decompression of the compartment syndrome improves the micro-circulation, decreases ischemia, and promotes the absorption of inflammatory products releasing pain. It can also release the adhesion and reduce proliferation of connective tissue between muscle fibers and precipitate the recovery of damaged muscles [3].

Pro-opiomelanocortin (POMC) is the precursor of  $\beta$ -endorphin and several kinds of well-defined non-opioid peptides including adrenocorticotrophic hormone (ACTH) mainly exists in the pituitary gland, hypothalamus, and peripheral tissues. There are no POMC immunopositive cells in the normal tissue of spinal cord [4]. The POMC is synthesized by neurons in hypothalamus arcuate nucleus and regulates the release of  $\beta$ -endorphin and 5-HT, which have analgesic effects [5]. The POMC can relieve pain through its  $\beta$ -endorphin [6] and ACTH. The hydrolysis of POMC can directly affect the concentration of  $\beta$ -EP, and the quantity of its mRNA expression indicates the level of gene expression in the endorphin system.  $\beta$ -EP, known as the main transmitter, plays a powerful analgesic role in the brain [7,8].

$\beta$ -EP inhibits release of substance P effect for pain transmission. The POMC can evaluate the effectiveness of analgesia treatment to non-specific low back pain, so we aimed to determine the effects of electro-acupuncture and acupotomy dissolution on the mRNA expression of center POMC in rats with non-specific low back pain.

## Materials and Methods

### *Animals*

The experiments were performed on 42 healthy male Sprague-Dawley rats (mean

weight:  $250 \pm 10$  g, Grade SPF), aged three months old, obtained from the Experimental Animal Center of Beijing Weitonglihua Company, China. The animals were kept in the experimental animal rooms, at School of Basic Sciences, Beijing University of Chinese Medicine, for three days. The subjects were maintained at  $20^\circ\text{C}$ , 50% air humidity, 12 hours of light, and with free access to water and food all day.

### *Study Design*

Six rats were selected randomly as the normal group that survived for 28 days; the remaining 36 rats were randomly divided into three groups of model, electro-acupuncture (EA), and acupotomy. They survived for 28 days and 56 days, respectively. The normal group did not receive any intervention, the model group rats with no treatment, the electro-acupuncture group underwent EA therapy, and acupotomy group received acupotomy therapy, both from the 15th day after simulation of lower back pain and establishing the model. The animals survived for 28 days and 56 days from the day that beginning to establish model, and there were six rats in each survival time group.

### *Surgical Procedure*

For establishing the model, we used modifying Jian-Ruei Wang's method to simulate non-specific low back pain. The experimental animals were injected with 10% chloral hydrate; 0.4 g/kg of rats' weigh into the peritoneum for abdominal anesthesia. SD rats were sterile-prepared and put in prone position, the hair around the lumbar area was removed, and a 1 cm vertical incision close to the 0.5-0.8 cm of the left side of L3-L4 spinal transverse process was performed. Then, the deep myofascial was separated, and the left lumbar paraspinal muscle was exposed from the left 0.5-0.8 cm to the central line.

The paraspinal muscle was separated from the posterior of the third lumbar transverse process, and a piece of  $0.5\text{cm} \times 0.5\text{cm}$  spongy gelatin absorbent was implanted. When simulation operation was performed, the lumbar paraspinal muscle was sutured with #3-0 plain gut and also the cutaneous incision was

sutured with #4-0 silk, and finally, the incision site was sanitized with gentamicin (2 ml, 80000 units) to prevent infection.

Except for the normal group, the other groups were treated from the 15th day after establishing the model. In the model group, after preparation by a simulated operation for the non-specific low back pain, no treatment was administered. In the EA group, 15 days after received stimulated operation, fixed the rats in prone position with rag strips, selected the Yao-Yang-Guang and left Shen-Shu, and EA was performed on the rats in these points with 2 Hz and 100 Hz dense-disperse wave. Twenty minutes of EA for each treatment, and one treatment every other days was administered. There were six treatments in two consecutive weeks (days 8, 18, 20, 23, 25, 28).

In the acupotomy group, 15 days after the rats were prepared by the simulated operation, acupotomy treatment was performed every seven days. There were two acupotomy treatments in total (days 15 and 22). The rats in the acupotomy group were slightly anesthetized with diethyl ether for about one minute until showing no sign of strong resistance. Palpate for trigger point or sclerosis in the local soft tissue close to the cutaneous incision left was performed by simulation operation.

Acupotomy instrument was used to have three cuts parallel to the spine, and then rotated the instrument handle 90 degrees to make another cut. The acupotomy instrument was pulled out, and the wound was pressed with a tampon to avoid excessive bleeding. When all of the treatments were finished, the rats in each group were fed for twenty-eight days. There was no further treatment or intervention during the twenty-eight days. Fifty-six days after the simulated operation, rats in the 56-day groups were prepared for decapitation and frozen section. The tissue preparation of the brain and spinal cord were respectively on the 28th and 56th day after establishing the model.

#### *Histopathological Study*

The slices with ideal detection site were chosen according to the anatomic site with no gas bubble to study the mRNA expression of POMC by in situ hybridization (detected by

Boshide Company, China).

In situ hybridization is a type of hybridization that uses a labeled complementary DNA, RNA or modified nucleic acids strand to localize a specific DNA or RNA sequence in a portion or section of tissue. The slices with in situ hybridization were observed by optical microscope (XSZ-107BN, Olympus, Japan), and taking photographs. One coronal plane slice of the brain arcuate nucleus was chosen and one cross section slice of lumbar segments [1-5] of the spinal cord of each animal in each survival time. Six slices in each group of animals were chosen with each locum in each survival time group. For the brain slices, the position of habenular nucleus and hypothalamus were considered. For the spinal cord slices, the position of the posterior horn was considered. Images were captured by the Nikon Eclipse 50i camera (10 fields for each case). Images were symmetrically chosen in each visual field for every slice, and the locum was in one regulation as far as possible. The images were analyzed by Image-Pro Plus 5.0, and the data were obtained. The intensity of positive granules was shown with optical density.

#### *Statistical Analysis*

All the data were shown as mean  $\pm$  standard deviation (SD). Determination of the inter-class difference was done by homogeneity test of variance at first, or by Mann-Whitney U test if the variance was heterogeneous. Then, they were compared with each other. A P-value less than 0.05 was considered statistically significant.

## **Results**

#### *Effects of Acupotomy Treatment on mRNA Expression of POMC in the Spinal Dorsal Horn*

The iodine (IOD) value of POMC mRNA-positive cells in the spinal dorsal horn is shown in Table-1.

In each group, the IOD value of positive cells between left and right sides was not different; however, the IOD value of the model group was higher than the normal group ( $P < 0.01$  and  $P < 0.05$ , respectively). The IOD value of the acupotomy and EA groups was lower than the

model group with a significance difference ( $P < 0.01$  and  $P < 0.05$ , respectively). However, compared to the normal group, there was no difference between the acupotomy and normal groups and between the EA and normal groups. In addition, there was no significant difference between the EA and acupotomy groups.

#### *Effects of Acupotomy Treatment on mRNA Expression of POMC in Hypothalamus*

The IOD value of the POMC mRNA positive cells in the hypothalamus is shown in Table-2. The IOD value of each group's positive cells between left and right sides was not significantly different, but the IOD value of the model group was significantly higher than the normal group ( $P < 0.01$  and  $P < 0.05$ , respectively). The IOD values of the acupotomy and EA groups were significantly higher than the model and normal groups ( $P < 0.01$  and  $P < 0.05$ , respectively).

#### **Discussion**

mRNA expression of POMC plays an important role on electro-analgesia. While studying

the effects of electro-analgesia, it was found that electro-acupuncture could accelerate mRNA expression of POMC and preproenkephalin in CNS and at the time of analgesia [9]. Meanwhile, some scholars believe that gene expression of opioid peptide increased the release of opioid peptides in the electro-acupuncture process, and this accelerated expression may regulate the long-term effect of acupuncture [10, 11].

Our results showed that POMC was significantly higher after the electro-acupuncture and acupotomy treatments. In the central nervous system (CNS), POMC is the congener precursor of  $\beta$ -endorphin, ACTH, and  $\alpha$ -MSH, and  $\beta$ -endorphin mainly precipitates the body's modulation to analgesia, and pain will be relieved if its expression increases. On day 28, when the intervention was completed, the POMC level in the EA and acupotomy groups increased more than the normal and model groups with significant statistical difference. Although there was no significant difference between the electro-acupuncture and acupotomy groups, suggesting that the acupotomy group, similar to the electro-acupuncture group, could significantly decrease

**Table 1.** IOD of POMC mRNA Positive Cells in Spinal Dorsal Horn. Data Were Presented as mean $\pm$ SD

Group	28 days	
	Right-side	Left-side
Normal	0	0
Model	700.61 $\pm$ 85.02 <sup>†</sup>	760.15 $\pm$ 87.17*
Acupotomy	514.07 $\pm$ 79.49 <sup>▲▲</sup>	581.16 $\pm$ 72.41 <sup>▲</sup>
Electroacupuncture	457.73 $\pm$ 105.99 <sup>▲</sup>	454.00 $\pm$ 130.68 <sup>▲</sup>

\*  $P < 0.05$ , <sup>†</sup>  $P < 0.01$  vs normal group; <sup>▲</sup>  $P < 0.05$ , <sup>▲▲</sup>  $P < 0.01$  vs model group

**Table 2.** IOD of POMC mRNA Positive Cells in Hypothalamus. Data Were Presented as mean $\pm$ SD

Group	28 days	
	Right-side	Left-side
Normal	367.58 $\pm$ 55.88	384.65 $\pm$ 64.63
Model	846.16 $\pm$ 181.11 <sup>†</sup>	833.28 $\pm$ 66.67 <sup>†</sup>
Acupotomy	1618.44 $\pm$ 105.41 <sup>†▲▲</sup>	1555.76 $\pm$ 114.38 <sup>†▲▲</sup>
Electroacupuncture	1562.74 $\pm$ 273.69 <sup>†▲▲</sup>	1647.02 $\pm$ 190.04 <sup>†▲▲</sup>

\*  $P < 0.05$ , <sup>†</sup>  $P < 0.01$  vs normal group; <sup>▲</sup>  $P < 0.05$ , <sup>▲▲</sup>  $P < 0.01$  vs model group

the elevated POMC level.

In this study, no mRNA expression of POMC in the spinal cord of the normal group was observed. It must be due to the fact that POMC-positive cells in the spinal cord result from the projection of solitary nucleus. The content of POMC mRNA in the brain of rats in the model group increased significantly. The increase in mRNA expression of POMC in the rats' brain is in agreement with most previous studies. This increase may be caused by negative feedback activation in the body's analgesia system after the rats were injured for simulation of the syndrome.

The content of POMC mRNA in the spinal cord of the EA and acupotomy groups was lower than the model group and close to the normal group, but higher than the model group in the hypothalamus. The increase in mRNA expression of POMC in the hypothalamus after electro-acupuncture treatment is in line with most of the reports in the past. Perhaps the increase in mRNA expression of POMC boosts the release of  $\beta$ -EP. Moreover, an increase in mRNA expression of POMC in the acupotomy group can be similar to the EA group. The change in POMC mRNA in the spinal cord was the same as the change of  $\beta$ -EP, which proved that hydrolysis of POMC could directly influence the concentration of  $\beta$ -EP. Nevertheless, this change is not in agreement with the results of several studies in the effect of electro-analgesia on promoting mRNA expression of POMC.

Fu Yi *et al.* demonstrated that electro-acupuncture, acupuncture pin, acupuncture bloodletting, and injectio ad acumen could relieve the pain caused by inflammation. In the evaluation of the analgesic effect of different techniques of needling on hypothalamus of rats with adjuvant arthritis, the injectio ad acumen and acupuncture bloodletting had no significant effect on the mRNA expression of POMC in the hypothalamus [9]. It is believed that other pathways induce an analgesic effect. Variation in POMC mRNA in our study was the same as the change in  $\beta$ -EP, showing that electro-acupuncture and acupotomy could relieve pain through regulating the mRNA expression of  $\beta$ -EP and POMC in CNS; however, further studies are required

to investigate its other effects. Another study on electro-analgesia showed that electro-acupuncture could accelerate mRNA expression of POMC and pre proenkephalin at the time of analgesia [9]. Nevertheless, some scholars assume that one of the function meanings of gene expression of the opioid peptide was increasing in releasing of the opioid peptide in the electro-acupuncture process. The accelerated expression can precipitate the regulation of long-term effect of acupuncture [10, 11].

Some studies demonstrated that electroacupuncture improved POMC mRNA expression in the spinal cord, which is not in agreement with the results of the present study. The possible reasons for this discrepancy are: 1) the empirical procedures of hybridization in situ were different, and it was easily contaminated by mRNA enzyme. This result may be caused by contamination of the electro-acupuncture and acupotomy groups. Therefore, in this case, there was no stain of positive particles on tissue slice, which rules out the possibility of the existence of part positive particles on tissue slice; 2) It was concluded from the previous reports that the longest time effect of electro-acupuncture affecting the mRNA expression of POMC in the brain was after 24 of treatment with electro-acupuncture and sacrificed to obtain samples. It was concluded that effect of electro-acupuncture could last for 24 hours. However, in our experiment, obtaining samples was after 48-72 hours after electro-acupuncture treatment. The mRNA expression of POMC in the electroacupuncture and acupotomy groups was lower than the model group, which might be due to the consumption of POMC caused by metabolism, which the function of release  $\beta$ -EP is stronger than the accelerate the mRNA expression of POMC of electro-acupuncture treatment; 3) mRNA expression of POMC may be related to noxious stimulation. It was possible that after electro-acupuncture and acupotomy treatment, the repair of local soft tissue injury was promoted and the transmission of pain signal was reduced, then the mRNA expression of POMC in the center was increased. POMC is not only the precursor of  $\beta$ -EP, but also it can release ACTH and  $\beta$ -lipotropin; however, further studies are needed on this issue.

## Conclusion

Electro-acupuncture and acupotomy could reduce POMC mRNA expression in the spinal cord and increase it in the hypothalamus of the rats having non-specific lower back pain. There was no difference between the acupotomy and electro-acupuncture groups regarding reduction of POMC mRNA.

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## Conflict of Interest

The authors declare there is no conflicts of interests.

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