



## Effects of acupotomy on basic fibroblast growth factor and CD34 levels in rabbits with third lumbar vertebral transverse foramen syndrome

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**ABSTRACT.** This study observed the local tissue homogenates in rabbits with third lumbar vertebral transverse foramen syndrome and explored the mechanism of acupotomy in local tissue revascularization. Thirty Japanese white rabbits were randomly divided into the following 5 groups of 6 rabbits each: normal, model, acupotomy, electroacupuncture (EA), and acupotomy-EA groups. All except the normal group were comprised of animal models of third lumbar vertebral transverse foramen syndrome prepared by embedding sponge in the left third lumbar transverse process. The rabbits in the acupotomy and EA groups underwent bilateral acupotomy intervention; those in the acupotomy-EA group underwent acupotomy and EA interventions.

On the 28th day after modeling, the double-antibody ELISA was used to detect b-FGF and CD34 levels in the serum and homogenates of a muscle tissue sample from the left side of the third lumbar transverse process. The b-FGF levels in local muscle homogenates were significantly higher in the modeled rabbits than in the normal rabbits ( $P < 0.01$ ), and the CD34 levels in the modeled group were significantly lower than in the normal group ( $P < 0.01$ ). The b-FGF and CD34 levels in the EA, acutotomy, and acutotomy-EA groups were significantly lower than those in the modeled group ( $P < 0.01$ ); the CD34 levels were significantly higher in the acutotomy-EA group than in the model group ( $P < 0.05$ ); and the differences among the EA, acutotomy, and acutotomy-EA groups were not significant ( $P > 0.05$ ). In conclusion, acutotomy regulates the levels of b-FGF and CD34 levels in serum and muscle tissue as well as local tissue revascularization.

**Key words:** Third lumbar vertebral transverse foramen syndrome; CD34; Acutotomy; Revascularization; Basic fibroblast growth factor

## INTRODUCTION

Revascularization, one of the key processes in the repair of injured tissue (Fan et al., 2006), increases local blood circulation and promotes nutrition and metabolism as well as tissue repair. In this study, the third lumbar transverse process syndrome rabbit model was observed for changes in basic fibroblast growth factor (bFGF) and CD34, which are associated with local muscle tissue angiogenesis, and the possible intervention mechanism of acutotomy in this model was explored.

## MATERIAL AND METHODS

### Material

#### *Experimental animals*

Thirty clean adult Japanese white rabbits (weight, 2.1-2.4 kg) provided by the Beijing Vital River Laboratory Animal Center [Batch No. SCXK (Beijing) 2006-0006] and kept in the animal feeding chamber of the Beijing Chinese Medicine School of Basic Medical Experimental Animal Center were included in this study.

#### *Laboratory equipment*

The following equipment was used for this study: Electro-acupuncture device: LH202H-type Han's acupoint nerve stimulator (Beijing Huayunante Technology Co., Ltd.); Acupuncture (Guangzhou SuiXin Medical Devices Co., Ltd.): 0.25 x 25-mm Global brand sterile disposable acupuncture; Acupuncture: 0.60 x 40-mm Han Zhuang brand disposable acupuncture; Orthopedic surgical instruments (scalpels, scissors, pliers, tweezers), medical suture acupuncture, No. 4 sewing string, and No. 3-0 catgut.

## **Reagents**

The following reagents were used: gelatin sponge: Nanjing Jinling Pharmaceutical Company; chloral hydrate: Beijing Chemical Reagent; gentamicin injection: Tianjin Pharmaceutical Jiaozuo Co., Ltd. b-FGF and CD34 reagent cases: Beijing Shangbo Biological Technology Co., Ltd.

## **Animal grouping**

After 4 days of adaptive feeding, the 30 rabbits were divided into normal, model, acupotomy, EA, and acupotomy-EA groups, with 6 animals in each group.

## **Model preparation**

The third lumbar transverse process syndrome models were prepared according to the description provided by Wang et al. (2003) and other model-preparing methods as follows:

The animals were anesthetized using an ear vein 10% chloral hydrate (2 mL/kg weight) injection. They were then placed in the prone position and the wool sheared for skin preparation. Using a sterile technique, a 1-cm longitudinal skin incision was made in the left lower back region (approximately 1.5 cm from the third lumbar spinous process). A 0.5 x 0.5-cm gelatin sponge was inserted into the posterior segment in the third lumbar transverse process. Care must be taken to prevent injury to the spinal nerves. The cut myofascia was sutured with No. 3-0 catgut and the skin with the No. 4 sewing string. The surgically incised wound was rinsed with 2 mL gentamicin to prevent infection.

All animals in the 4 groups other than the normal group were modeled according to the method described above.

## **Treatment**

Following is the description of the treatment received by the different groups.

Normal group: Received normal feeding without any treatment.

Model group: Received normal feeding after model preparation.

Acupotomy group: Acupotomy treatment was administered 2 weeks after modeling. The skin in the modeled region was palpated and an incision was made. After locating the induration and cords following local sterilization procedures, the 4 steps of acupotomy were carried out based on the principles of acupotomy, and dredge it in cross shape. The lines of the knife blade were parallel to the direction of compression and muscle fiber separation and piercing. After the acupuncture touched induration or cords, first with the muscle fibers parallel to the direction of the longitudinal three knives, 90° rotary handle and then cut another time. Then, debond the induration and cords pull out the acupuncture and press with cotton balls for a moment. The treatment was performed once a week for a total of 2 sessions.

EA group: Acupuncture treatment was performed 2 weeks post-modeling. The rabbits' ears were fixed and operated upon gently to reduce the irritation caused to the animals. According to the "Experimental Acupuncture" method (Li, 2003), bilateral "Weizhong" was selected, and was pierced to the depth of 10 mm using the HANS LH202H electro-acupuncture device. The frequency was set at 2/100 Hz wave per second, and the current was set at a

level not higher than 2 mA. The treatment was considered to be appropriate if trembling of the lower limbs with no screams or signs of struggle was observed. The treatment was administered once a day for 20 min, 3 times/week, for a total of 6 sessions.

Acupotomy-EA group: Acupuncture and acupotomylysis interventions were administered simultaneously.

### **Survival period and sample collection**

We obtained the sample 28 days after modeling. First, the animals were anesthetized with 10% chloral hydrate (2 mL/kg weight) ear vein injection; they were then decapitated, and the left third lumbar transverse muscle tissue was exposed. Along the middle and lower transverse bone surface, we clipped about 1 x 1 x 1-cm tissue blocks, which were weighed and stored at -20°C in a refrigerator for testing.

### **Outcome measurements**

#### ***General observation of local tissues***

We observed the color of the third lumbar transverse local muscle tissue and assessed the area for congestion, adhesions, and scar nodules.

#### ***Muscle tissue cytokine detection***

We used the double antibody sandwich ELISA to detect the b-FGF and CD34 levels in the local muscle tissue homogenates according to the instructions provided in the kit.

### **Statistical processing**

We used the SAS statistical software (Chicago, IL, USA) for statistical analysis. All data are reported as means  $\pm$  standard deviation and the groups were compared using ANOVA. The groups were compared with ANOVA using LSD *post hoc* analyses;  $P < 0.05$  was considered to be statistically significant.

## **RESULTS**

### ***Local tissue general observation***

We exposed the third lumbar transverse muscle tissues of the experimental animals and observed with naked eye if the color was normal red, the muscle fibers were tidy, and if adhesions, scars, and nodules were present. In the model group, the local muscle tissue had darkened and local congestion and capillary bleeding was seen along with soft tissue scar nodules. Compared with the model group, the muscles of the rabbits in the acupuncture and acupuncture-EA group were red but still slightly darker than normal; the muscle fibers were tidy with no obvious signs of local congestion or bleeding. The scars and nodules were smaller compared to that in the model group.

***Effect of acupotomy on b-FGF and CD34 levels in the local muscle***

After modeling, the b-FGF levels in local muscle tissue homogenates were higher than normal ( $P < 0.01$ ), the CD34 levels were significantly lower than normal ( $P < 0.01$ ); the levels in the acupotomy, EA, and acupotomy-EA groups were not significantly different ( $P > 0.05$ ); there was no significant difference between the acupotomy and EA groups as well ( $P > 0.05$ ). CD34 levels in the acupotomy-EA group rose significantly ( $P < 0.05$ ); there was no significant difference among the EA, acupotomy group, and acupotomy-EA groups ( $P > 0.05$ ; Table 1).

**Table 1.** Rabbit b-FGF and CD34 level comparison according to groups.

Group	Case number	b-FGF (means $\pm$ SD pg/mg)	CD34 (means $\pm$ SD, pg/mg)
Normal	6	25.49 $\pm$ 4.96	43.33 $\pm$ 3.34
Model	6	38.65 $\pm$ 6.17**	33.84 $\pm$ 4.05**
Needle knife	6	25.97 $\pm$ 4.18 $\blacktriangle\blacktriangle$	39.00 $\pm$ 2.85
Curative	6	23.15 $\pm$ 9.00 $\blacktriangle\blacktriangle$	40.03 $\pm$ 5.87
Needle knife + cupping	6	23.21 $\pm$ 5.80 $\blacktriangle\blacktriangle$	41.80 $\pm$ 5.02 $\blacktriangle$
F		0.001	0.0105
P		6.46	4.13

Compared with normal group: \*\* $P < 0.01$ ; Compared with model group:  $\blacktriangle P < 0.05$ ,  $\blacktriangle\blacktriangle P < 0.01$ .

**DISCUSSION**

Under normal physiological conditions, vascular endothelial cell proliferation and differentiation occurs in a dynamic equilibrium state; however, in ischemia, hypoxia, inflammation, or shear stress, the equilibrium is disturbed and this state promotes angiogenesis (Fam et al., 2003). CD34 is the most sensitive marker of vascular endothelial cell proliferation (Bai and Zhao, 2005) and of neovascularization ironomic antigens (He et al., 2009). Change in the expression of CD34 directly reflects the amount of local vessels. b-FGF is one of the most effective pro-angiogenic factors (Xu and Zuo, 2008). b-FGF acts on vascular endothelial cells by upregulating the  $\beta 1$  integrin to express and enhance adhesion to endothelial cells, identification, migration (Böttcher and Niehrs, 2005), and other functions to stimulate endothelial progenitor cells, including stem cell to mobilize and home to participate in the vascular regeneration (Hirschi et al., 2002) and promote revascularization. Furthermore, b-FGF also plays a role in promoting tissue repair; b-FGF stimulates angiogenesis in early stage of posttraumatic and induces synthesis of vascular endothelial growth factor. It promotes the start of revascularization and has a significant repair effect (Koivisto et al., 2004).

This experimental model in this study was prepared by inserting gelatin sponge in the third lumbar transverse tip to simulate the third lumbar transverse process syndrome pathological state. Because the stimulation of gelatin sponge in rabbits' third lumbar transverse process forms a relatively limited inflammatory mass resulting in a local blood circulation disorder, it is difficult for this inflammation to be dissipated and absorbed. As can be seen from the experimental results, in the 4th post-modeling week, the CD34 levels in the local muscle tissue of rabbits were significantly lower than normal ( $P < 0.01$ ) while the b-FGF levels were significantly higher than normal ( $P < 0.01$ ). In local inflammatory lesions, vascular regeneration reduced the emergence of local blood circulation disorder, and thus stimulate endothelial cells to secrete b-FGF facilitating local vascular regeneration, thereby increasing the b-FGF

levels. Acupotomy-EA intervention can effectively promote local angiogenesis, improve local circulation, promote local inflammation dissipation and absorption, promote tissue repair, and restore local muscle homeostasis.

Acupotomylysis is a new closed, minimally invasive release method, in which a miniature scalpel is employed for the 2 roles of a “needle” and “knife”. This method acts directly on a localized lesion by reducing the inflammation caused by adhesions and scar contracture, and also performs acupuncture, which clears the meridians and reduces tendon pain.

The minimally invasive stimulation provided by this method can also improve micro-circulation (Guo et al., 2007) and repair diseased tissue. This study confirmed that acupotomylysis has a positive regulatory effect on local tissue, and it provides further experimental evidence for the mechanism underlying acupotomylysis.

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