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Basic Medicine

Effects of continuous peripheral nerve block by tetrodotoxin on growth associated protein-43 expression during neuropathic pain development*☆

Chen Wang[1], Xiaoyu Huang[2]

1*Department of Anesthesiology, Second Hospital of Xiamen City, Xiamen 361021, Fujian Province, China*
2*Department of Anesthesiology, Second Affiliated Hospital of Shantou University Medical College, Shantou 515041, Guangdong Province, China*

Abstract

BACKGROUND: Peripheral nerve injury may lead to neuropathic pain and cause a markedly increase expression of growth associated protein-43 (GAP-43) in the spinal cord and dorsal root ganglion, local anesthetics blocking electrical impulse propagation of nerve fibers may also affect the expression of GAP-43 in the spinal cord and dorsal root ganglion.

OBJECTIVE: To determine the effects of continuous peripheral nerve block by tetrodotoxin before and after nerve injury on GAP-43 expression in the dorsal root ganglion during the development of neuropathic pain.

DESIGN: A randomized controlled animal experiment.

SETTINGS: Department of Anesthesiology, the Second Hospital of Xiamen City; Department of Anesthesiology, the Second Affiliated Hospital of Shantou University Medical College.

MATERIALS: Thirty-five Sprague Dawley (SD) rats, weighing 200–250 g, were randomly divided into four groups: control group (n =5), simple sciatic nerve transection group (n =10), peripheral nerve block before and after sciatic nerve transection groups (n =10). All the sciatic nerve transection groups were divided into two subgroups according to the different postoperative survival periods: 3 and 7 days (n =5) respectively. Mouse anti-GAP-43 monoclonal antibody (Sigma Co., Ltd.), supervision TM anti-mouse reagent (HRP, Changdao antibody diagnosis reagent Co., Ltd., Shanghai), and HMIAS-100 image analysis system (Qianping Image Engineering Company, Tongji Medical University) were employed in this study.

METHODS: This experiment was carried out in the Department of Surgery and Pathological Laboratory, the Second Affiliated Hospital of Shantou University Medical College from April 2005 to April 2006. ①The animals were anesthetized and the right sciatic nerve was exposed and transected at 1 cm distal to sciatic notch. ② Tetrodotoxin 10 μg/kg was injected percutaneously between the greater trochanter and the posterior superior iliac spine of right hind limb to block the sciatic nerve proximally at 1 hour before or 4 hours after nerve injury respectively, the injection was repeated in all the rats every 12 hours. ③ At 3 or 7 days after nerve injury, immunohistochemistry and image analysis were used to evaluate the expression of GAP-43 in the dorsal root ganglions of L_5 to the transected sciatic nerve, and quantitative analysis was also performed. ④ Statistical analysis was performed using one way analysis of variance followed by t test.

MAIN OUTCOME MEASURE: Expression of GAP-43 in the right dorsal root ganglions of L_5.

RESULTS: All the 35 SD rats were involved in the final analysis of results. In normal rats, there were very low expressions of GAP-43 in the dorsal root ganglions. In simple sciatic nerve transection rats 3 and 7 days after sciatic nerve transection, the average absorbance value of GAP-43 immunopositive neurons were significantly different from that in normal rats (t =8.806, 6.771, $P < 0.01$). Whereas 3 and 7 days after sciatic nerve transection in rats with peripheral nerve block before and after nerve injury, the average absorbance value of GAP-43 immunopositive neurons were not significantly different from that in normal rats ($P > 0.05$).

CONCLUSION: Local anesthetic continuous peripheral nerve block before or after nerve injury can suppress nerve injury induced high expression of GAP-43 during the development of neuropathic pain.

Key Words: growth associated protein-43 (GAP-43); neuropathic pain; sciatic nerve; tetrodotoxin

Chen Wang ☆ , Doctor, Professor, Department of Anesthesiology, Second Hospital of Xiamen City, Xiamen 361021, Fujian Province, China

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Corresponding author: Chen Wang, Doctor, Professor, Department of Anesthesiology, Second Hospital of Xiamen City, Xiamen 361021, Fujian Province, China **E-mail:** wcmzk@126.com

INTRODUCTION

Neuropathic pain is resulted from central and/or peripheral nervous system diseases or injury. Previous studies have showed that neuropathic pain is closely related to the plasticity of central nervous system[1-4]. It was also found that the expression of Growth associated protein 43 (GAP-43) was increased during the development of neuropathic pain [5-7]. Now it is believed that the regulation of GAP-43 expression is associated with the retrograde transporting signs from the target tissue. During treatment for the neuropathic pain, long period of peripheral nerve block is often used to inhibit the conduction of electrical impulses in the nerve fibers, and this could result in the denervation from target tissue that may affect the expression of GAP-43 in the dorsal root ganglion and neuroplasticity. This experiment was designed using immunohistochemical techniques to study the effects of continuous peripheral nerve block by tetrodotoxin on GAP-43 expression during the development of neuropathic pain, to investigate the effects of continuous peripheral nerve block before and after nerve injury on the neuroplasticity and the mechanisms for regulating the GAP-43 expression.

MATERIAL AND METHODS

Materials

This experiment was carried out in the Department of Surgery and Pathological Laboratory, the Second Affiliated Hospital of Shantou University Medical College from April 2005 to April 2006. Total 35 adult Sprague Dawley (SD) rats, weighing 200–250 g, were provided by the Experimental Animal Center of Southern Medical University [certificate number: 2004A068]. The rats were randomly divided into 4 groups: normal control group (n =5), simple sciatic nerve transection group (n =10), peripheral nerve block before sciatic nerve transection group (n =10), peripheral nerve block after sciatic nerve transection group (n =10). All the sciatic nerve transection groups were divided into two subgroups according to different postoperative survival periods: 3 and 7 days (n =5) respectively.

Main reagents and apparatus: Tetrodotoxin (Hebei Aquatic Products Research Institute) Mouse anti-GAP-43 monoclonal antibody (Sigma Co., Ltd.), supervision TM anti-mouse reagent (HRP, Changdao antibody diagnosis reagent Co., Ltd., Shanghai), and HMIAS-100 image analysis system (Qianping Image engineering Company, Tongji Medical University) were enployed in this study.

Methods

Preparation of peripheral nerve injury animal model

In the sciatic nerve transection groups, the rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (40 mg/kg), the right sciatic nerve was exposed and transected at thigh around ischial tuberosity under sterilized condition. Then incision was closed and rats were allowed to recovery. Rats in the normal control group did not receive any treatment.

Long-acting local anesthetic and sciatic nerve block

The effect and specificity of tetrodotoxin+adrenaline (1:100 000) had been reported in the previous studies[8,9]. Recent studies showed that tetrodotoxin combined with vasoconstrictors might sufficiently improve the therapeutic indices to permit use as long-acting local anesthetics. Tetrodotoxin solution was injected at 10 μg/kg and 1 mL/kg, preliminary experiments and previous studies showed that the effects of sciatic nerve block lasted for 13 hours after a single injection of tetrodotoxin[8]. The sciatic nerve was blocked using percutaneous injection of local anesthetic according to the methods described by Thalhammer *et al*[10]. Tetrodotoxin was injected between the greater trochanter and the posterior superior iliac spine of right hind limb when the rat was wrapped in a towel leaving its hindlimbs free. In the peripheral nerve block before and after sciatic nerve transection groups, tetrodotoxin was injected 1 hour before or 4 hours after sciatic nerve transection respectively. The injection of tetrodotoxin was repeated every 12 hours in all the rats and lasted throughout whole experimental periods.

Specimen preparation

Three or seven days after sciatic nerve transection, the rats were anesthetized again with intraperitoneal injection of pentobarbital sodium (40 mg/kg), and perfused with 150 mL of normal saline and fixed by perfusion of 300 mL paraformaldehyde (40 g/L) in 0.1 mol/L phosphate buffer solution (PBS, pH 7.4) through the left ventricle of the heart. The rats were anatomized from sciatic nerve upwards to dorsal root ganglions, and then the dorsal root ganglions of L_{5-6} at right side were removed, postfixed in perfusion fixative for 24 hours, rinsed for 24 hours with distilled water, then dehydrated in serially increasing concentration of alcohol, and embedded in parafin. Serial sections at 4 μm thick were cut vertically and mounted on poly-L-lysine coated slides.

Immunohistochemical staining for GAP-43 expression

The paraffin embedded sections were washed for 3 times with 0.02 mol/L PBS for 2 minutes, and treated with 3 mL/L H_2O_2 at 37 ℃ for 15 mintues to block endogenous peroxidase. They were incubated with mouse anti-GAP-43 monoclonal antibody diluted 1∶1 000 for overnight at 4 ℃, then washed for three times with 0.02 mol/L PBS for 2 minutes, and incubated with supervision TM anti-mouse reagent at 4 ℃ for 30 minutes. The immunolabeled structures were visualized with DAB/H_2O_2 according to manufacturer recommendations (Bohegrin Co., Wuhan). The immunostaining was observed under microscope and terminated through water washing. Then the sections were restained with hematoxylin, dehydrated, hyalinized and fixed with resin. Goat serum was used to replace anti-GAP-43