Neuroscience Letters 589 (2015) 52–56

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

A new rat model of neuropathic pain: Complete brachial plexus avulsion

Le Wang[a,b,c], Liu Yuzhou[a,b,c], Zhou Yingjie[a,b,c], Lao Jie[a,b,c,*], Zhao Xin[a,b,c]

[a] Department of Hand Surgery, Huashan Hospital, Fudan University, Shanghai, PR China
[b] Key Laboratory of Hand Reconstruction, Ministry of Health, Shanghai, PR China
[c] Shanghai Key Laboratory of Peripheral Nerve and Microsurgery, Shanghai, PR China

HIGHLIGHTS

- We extented the neuropathic pain model of brachial plexus avulsion (BPA).
- We demonstrated that 37.5% of rats had the cold allodynic and mechanical allodynia (up to 6 months) following BPA .
- This neuropathic pain model mimiced human nerve traction injury following traffic accidents.

ARTICLE INFO

Article history:
Received 8 September 2014
Received in revised form 12 January 2015
Accepted 13 January 2015
Available online 14 January 2015

Keywords:
Brachial plexus avulsion
Neuropathic pain
Animal models

ABSTRACT

Brachial plexus avulsion (BPA) is one of the major injuries in motor vehicle accidents and may result in neuropathic pain. Accumulating evidence suggests that 30–80% of BPA developed neuropathic pain in human. In our study, complete brachial plexus avulsion (C5–T1) rats model leads to the results that 37.5% of rats had long-lasting (up to 6 months) mechanical allodynia and cold allodynia. We observed the activation of astrocyte and microglial in cervical spinal cord after BPA. Complete brachial plexus avulsion mimics human nerve root traction injury following traffic accidents. The complete BPA rat model approach human injuries and can be used for further investigations.

Brachial plexus avulsion (BPA) is a severe injury in human caused by traction of spinal cord roots following traffic accidents. Neuropathic pain is the unpleasant sensory as result of neuronal and glial activity which is caused by nerve system injuries. 30–80% of BPA developed neuropathic pain in human [11]. Although some literature reported that the BPA model produces long lasting mechanical and cold allodynia in all rats [10]. However, in accordance with previous studies, not all the BPA patients developed neuropathic pain [2,12,14]. The study reported that the incidence of neuropathic pain after complete BPA was 83.3% [14]. Neuropathic pain resulted from BPA is characterized as burning, tingling and electric shock [6]. Allodynia is a noticeable symptom in patients with neuropathic pain. Allodynia is elicited by a stimulus that normally does not cause pain. A series of animal studies has shown that glia cells were activated at the injured level of spinal cord and the uninjured levels after BPA [5]. Therefore, neuropathic pain is generated by non-avulsed roots rather than avulsed roots [7]. The anatomy of the rat brachial plexus is similar to human. The difference between human and rat is that rat's brachial plexus is entirely located infraclavicularly [8].

The neuropathic pain related to BPA results in mechanical allodynia (pain sensitivity increased) and cold allodynia. The activation of microglia and astrocytes created and maintained pain states [4]. The activation of microglial and astrocytes in dorsal horns of spinal cord lasted 3 months following BPA [7]. Astrocyte and microglia were activated in the injured and uninjured dorsal horn of the spinal cord after brachial plexus injury [9]. Therefore, we could observe the activation of microglia and astrocytes to prove whether the neuropathic pain exists. Our objective was to demonstrate that the complete BPA rat model approach human's injuries following traffic accidents. We hypothesized that only part of the rat produce neuropathic pain following complete BPA.

* Corresponding author at: Department of Hand Surgery, Huashan Hospital, Fudan University, No.12 Wulumuqizhong Road, Shanghai 200032, PR China.
Tel.: +86 13701770978.
E-mail address: laojie27@sina.com (L. Jie).

http://dx.doi.org/10.1016/j.neulet.2015.01.033
0304-3940/

1. Surgical procedure

Adult male Sprague–Dawley rats (250–300 g; Fudan University, China) ($n = 32$) were maintained on a 12/12 h light/dark cycle and allowed free access to food and water. All surgery and experimental procedures were performed during the light cycle and approved by the Animal Ethics Committee of The Fudan University. All efforts were made to reduce animal numbers. All surgical procedures were performed after anesthesia induced by a 1% sodium pentobarbital solution (40 mg/kg body weight). Place the rat prone on a sterilized pad with the head oriented away from the surgeon and the right fore paw abducted and extended. Use the finger-tips to locate the clavicle. With scalpel, make a 1.5 cm horizontal incision in the skin under the clavicle 2 mm. Use micro-dissecting scissors to separate the skin from the superficial fascia, exposing the pectoralis major muscle. The pectoralis major muscle was cut paralleled with the muscle fibers to expose brachial plexus, leaving the cephalic vein intact. The subclavian vessels were located and the upper, middle and lower trunk dissected. In the complete brachial plexus avulsion (BPA) group ($n = 16$), the upper, middle and lower trunk were grasped with forceps and extracted from the spinal cord by traction. The tissues layers were then brought together and the skin was closed with 4–0 silk sutures (ethicon). In the sham-operated group ($n = 8$), the brachial plexus was exposed and dissected without any lesion to the nerves. The control group ($n = 8$) had no surgery. The procedure of BPA surgery (Fig. 1)

2. Mechanical withdrawal thresholds

We evaluated 32 rats for mechanical allodynia (BPA group, $n = 16$; sham-operation group $n = 8$; control group $n = 8$). Mechanical allodynia was assessed by using the von Frey filaments (Stoelting, USA; bending force: 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, and 26.0 g) according to the method described by Chaplan et al. [13]. Rats were placed in individual transparent plastic chambers ($25 \times 40 \times 18$ cm) with wire mesh bottoms and allowed to acclimatize to the environment for 30 min. Filaments were applied to the middle surface of the left forepaw. The threshold was the lowest force that evoked a consistent, brisk and withdrawal response. Each von Frey filament was applied five times. When rats showed at least two withdrawal responses to a filament, the bending force of the filament was defined as the withdrawal threshold. For control, only rats with basal paw withdrawal threshold equal to 15 g were recruited in the further experiments. In order to determine the basal mechanical thresholds, all the groups were submitted to pre-surgical evaluation and re-evaluated at several intervals of time after surgery. After surgery, the rats with no pain were excluded from the study (the mean baseline normal mechanical was 16.2 ± 4.2 g, 32 rats forepaws mechanical withdrawal thresholds mean $\pm$ SD). The testing time point was 0 days, 2 days, 7 days, 14 days, 21 days, 28 days, 2 months, 3 months, 4 months, 5 months and 6 months.

3. Assessment of cold allodynia

For the assessment of cold allodynia, the rats were placed on a metal mesh covered with a plastic dome, and were allowed to habituate until exploratory behavior diminished. Cold allodynia was measured by an acetone spray test as described by Choi et al. [15]. Where 250 μl of acetone was squirted onto the middle surface of the left forepaw. Control rats (saline treated) either ignore the stimulus or occasionally respond with a very small and brief withdrawal. Neuropathy rats frequently responded with a withdrawal that was clearly exaggerated in amplitude and duration. The withdrawal responses were evaluated on a scale of 0–3 points, after application of acetone was made to each paw: (a) no response, 0 points- the paw was not moved; (b) mild response, 1 point- a response in which the paw had little or no weight on it; (c) moderate response, 2 points-a response in which the paw was elevated and was not in contact with any surface; (d) robust response, 3 points-a vigorous response in which the rat licked, bit or shock the paw. The testing time point were 0 days, 2 days, 7 days, 14 days, 21 days, 28 days, 2 months, 3 months, 4 months, 5 months and 6 months.

4. Immunohistochemistry for GFAP(astrocyte) and Iba1 (microglial) (a marker of glial cells activation)

21 days after the operation, the rats were anesthetized by 1% sodium pentobarbital solution (40 mg/kg, body weight) and transcardially perfused with 0.9% saline, followed by 500 ml of 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4). Next, spinal cord from C7 levels were resected and the specimens immersed in the same fixative solution overnight at 4 °C. After storage in 0.01 M phosphate-buffered saline (PBS) containing 20% sucrose for 20 h at 4 °C, the specimens were then treated for 90 min at room temperature in blocking solution consisting of 0.01 M PBS containing 0.3% Triton X-100 and 3% skim milk. The immunohistochemical procedure was performed as described previously [9]. For staining of dorsal horn, the sections were processed for GFAP immunohistochemistry using a rabbit antibody to GFAP (1:200; Abcam, USA) and for Iba1 immunohistochemistry using a rabbit antibody to Iba1(1:250; Abcam, USA) diluted in blocking solution and incubated for 20 h at 4 °C. Sections were incubated with DAB Envision anti rabbit reagents (Dako, K5007, Denmark). After each step, we rinsed the sections 3 times in 0.01 M PBS. We examined the sections using

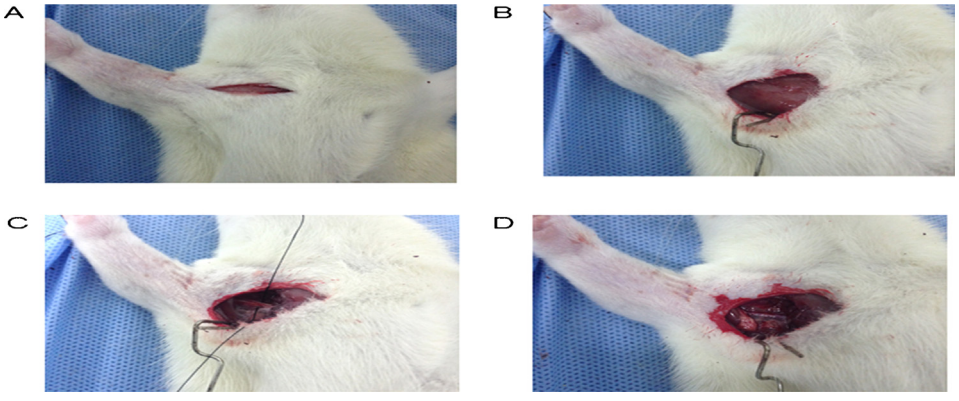

Fig. 1. The procedures of complete brachial plexus avulsion: (A) The incision was under the clavicle 2 mm. (B) The exposure of pectoralis major muscle. (C) Cut the pectoralis major muscle and exposed the brachial plexus. (D) Complete brachial plexus root avulsion.