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A new model for the study of neuropathic pain after brachial plexus injury

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ABSTRACT

The study was to introduce a new and reliable behavioral model of upper trunk of brachial plexus avulsion for the study of persistent neuropathic pain. 60 rats were divided into three groups randomly: upper trunk of brachial plexus avulsion (UTBPA) group (20), global brachial plexus avulsion (GBPA) group (20), and sham- operated group (20). The animals were tested for behavioral responsiveness before surgeries and 3, 7, 14, 21, 28, 56, 84 days after surgeries. The injured level of spinal cord was resected and the sections were processed for GFAP (astrocyte) and Iba1 (microglia) immunohistochemistry 3 weeks after surgeries. The UTBPA group developed significant signs both of mechanical and cold hypersensitivity, which matched the immunohistochemistry result, as well as the nature of avulsion was close to the clinical type of injury, the UTBPA group could be used as a suitable and effective persistent neuropathic pain model following brachial plexus injury.

Introduction

Brachial plexus injury (BPI) is usually very complex, because of the involvement of both spinal nerve and spinal root ruptures, with associated avulsion of one or several roots from the spinal cord [1]. Adult traumatic brachial plexus injuries can have devastating effects on the upper extremity function [2]. In addition to motor and sensory deficits, pain can be equally debilitating [3,4,5,6]. The main characteristics of BPI pain are the rapid onset of pain (an effect which occurs immediately after the trauma) and the long-lasting development of neuropathy, which may be evidenced distant from the site of the lesion [7,8]. The neuropathic pain would lead to mechanical allodynia and cold allodynia.

Brachial plexus avulsion (BPA) is the frequent type of human nerve root traction injury following traffic accidents [9], which is the most of the reasons for brachial plexus injuries. In Hua Shan hospital, there were about 1000 brachial plexus avulsion patients per year [10], in which the percentages of lower trunk avulsion, upper trunk avulsion and complete BPA were about 5%, 35%, 60% relatively. According to previous studies, not all the BPA patients developed neuropathic pain [11,12,13]. 30–80% of BPA developed neuropathic pain in human [14].

It is now widely accepted that alterations in both the peripheral and central nervous systems are involved in the genesis of neuropathic pain [7]. A series of studies showed that glia cells were activated at the injured level of spinal cord after BPA [15]. The activation of microglia and astrocytes created and maintained pain states [16], which lasted 3 months following BPA [17]. Therefore, the activation of microglia and astrocytes could prove the existence of neuropathic pain.

The aim of the present study was to demonstrate the upper trunk avulsion rat model was a suitable and effective persistent neuropathic pain model, which approached human's brachial plexus injuries following traffic accidents.

Materials and methods

All procedures were performed on adult (200–250 g) male Sprague Dawley rats. All animals were kept in cages with free access to food and water. They were raised in a temperature-controlled room (20 ± 2 °C). Rats were normally housed in groups of up to 4 per cage. All surgery and experimental procedures were performed during the light cycle and approved by the Animal Ethics Committee of the Fudan University.

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Surgery

All surgical procedures were performed at right side of the rats after anaesthesia induced by pentobarbital sodium injected intraperitoneally.

1. Upper trunk of brachial plexus avulsion (UTBPA)

The brachial plexus was approached through a horizontal supraclavicular incision. The sternocleidomastoid muscle was cut off and the omohyoid muscle was pulled aside, leaving the transverse cervical vessels intact. The brachial plexus was located in the scalene fissure, including upper, middle and lower trunks. The upper trunk was grasped with forceps and extracted from the spinal cord by traction, leaving middle and lower trunks intact (Fig. 1). The tissue layers were then brought together and the skin was closed with 4–0 silk sutures.

2. Global brachial plexus avulsion (GBPA)

Accoding to the procedure above, the brachial plexus was exposed. All the 3 trunks were grasped with forceps and extracted from the spinal cord by traction (Fig. 2). The tissue layers were then brought together and the skin was closed with 4–0 silk sutures.

3. Sham-operated group (Sham)

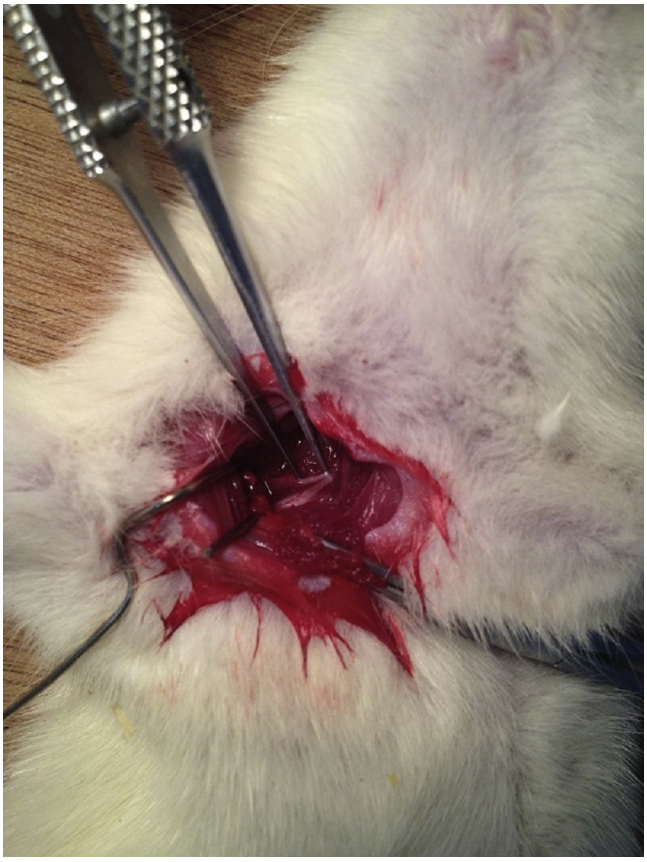

Fig. 1. Upper trunk of brachial plexus avulsion (UTBPA): The upper trunk was exposed and would be extracted from the spinal cord, leaving middle and lower trunks intact.

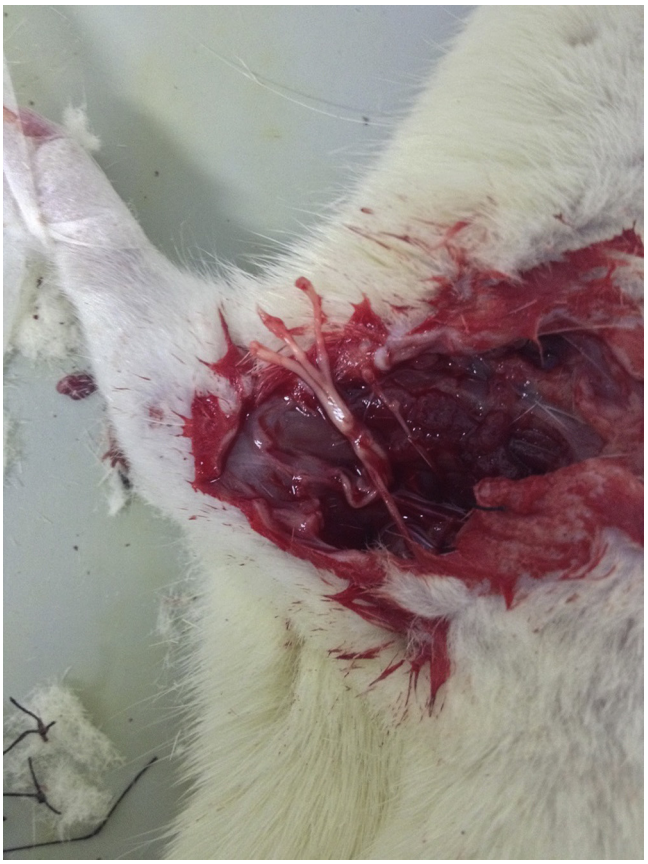

Fig. 2. Global brachial plexus avulsion (GBPA): All the 3 trunks were grasped with forceps and extracted from the spinal cord by traction.

The brachial plexus was exposed and dissected without any lesion to the nerves. Then the wound was closed.

Mechanical allodynia

Animals were placed on a metal mesh floor, covered by a transparent plastic box and raised 30 cm above the floor. The plantar surface of the right forepaw was stimulated with a series of ascending force von Frey monofilaments. The threshold was taken as the lowest force that evoked a brisk withdrawal response to one of five repetitive stimuli [18]. A withdrawal response was considered valid only when the paw was completely removed from the platform [19].

Cold allodynia

Cold allodynia was measured by an acetone spray test as described by Choi et al. [20]. 250 micro ml of acetone was squirted onto the mid-plantar surface of the right forepaw. The withdrawal responses were evaluated on a scale of 0–3 points: 0 points—the paw was not moved; 1 point—a response in which the paw had little or no weight on it; 2 points—a response in which the paw was elevated and was not in contact with any surface; 3 points—a vigorous response in which the rat licked, bit or shook the paw [21].

Immunohistochemistry for iba 1(microglia) and GFAP (astrocyte)

The rats were anesthetized by 1% sodium pentobarbital solution injected intraperitoneally 3 weeks after the operation. They were perfused with 0.9% saline, followed by 500 ml of 4%