SCIENTIFIC ARTICLE

The Effects of Generally Administered Anti—Nerve Growth Factor Receptor (p75NTR) Antibody on Pain-Related Behavior, Dorsal Root Ganglia, and Spinal Glia Activation in a Rat Model of Brachial Plexus Avulsion

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Purpose To investigate the effect of intraperitoneal administration of an anti-p75 neurotrophin receptor (p75NTR) antibody on reducing neuropathic pain in a rat model of brachial plexus avulsion (BPA).

Methods We randomly assigned 40 male Wistar rats to 4 groups. In the BPA group, the C8-T1 roots were avulsed from the spinal cord at the lower trunk level, and saline was administered intraperitoneally. In the anti-p75NTR groups, 1 μL or 50 μL anti-p75NTR antibody was administered intraperitoneally after avulsion. In the sham-operated group, the lower trunk level was exposed, and saline was administered intraperitoneally. Mechanical hyperalgesia and pain-induced walking patterns were measured using von Frey filaments and CatWalk gait analysis at various time points until 15 days after administration.

At 3 and 15 days after administration, sensory neurons involved in pain perception and satellite glial cells in the ipsilateral C7 dorsal root ganglia were immunolabeled with antibodies against calcitonin gene-related peptide and glial fibrillary acidic protein (GFAP), respectively. At both time points, microglial and astrocyte activation, indicative of spinal pain transmission, were immunohistochemically examined in the ipsilateral dorsal horn of the spinal cord (C7) using anti-ionized calcium-binding adaptor molecule 1 and anti-GFAP antibodies, respectively.

Results The gait pattern was significantly improved in both anti-p75NTR groups compared with the BPA group. There were significantly fewer calcitonin gene-related peptide-immunoreactive (IR) neurons, neurons encircled by GFAP-IR satellite glial cells, and GFAP-IR astrocytes in both anti-p75NTR groups compared with the BPA group at both time points. Fewer ionized calcium-binding adaptor molecule 1-IR microglia were quantified in both anti-p75NTR groups compared with the BPA group, but this was only significant at 15 days after administration.

Conclusions Systemic application of the p75NTR inhibitory antibody suppressed neuropathic pain after BPA.

Clinical relevance p75NTR may be a potential therapeutic target for the clinical treatment of neuropathic pain in BPA injury. *(J Hand Surg Am. 2015;40(10):2017—2025.*

Key words Brachial plexus avulsion, DRG, glial cell, neuropathic pain, p75NTR.

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Previous studies have reported a high incidence of severe neuropathic pain in patients with brachial plexus avulsion (BPA).[1] This type of pain is rapid in onset, long-lasting, and often described as a constant crushing sensation with a superimposed lightning-like shooting pain that can be difficult to treat.[1,2] In clinical practice, patients with neuropathic pain receive nonsteroidal anti-inflammatory drugs, opioids, anticonvulsants, antidepressants, surgery, or a combination of these options.[1,3,4] However, these treatments are modestly effective and palliative rather than curative.

Neurotrophic factors (NTFs) have recently been reported to contribute to neuropathic pain and are now recognized as potential therapeutic targets.[5] Among NTFs, nerve growth factor (NGF) plays a crucial role in pain. Thus, antibodies targeting NGF or its cognate receptors would be viable treatment options for pain.[5−7] In clinical studies using an antibody against NGF, patients with osteoarthritis reported significant and clinically meaningful pain reduction; however, certain adverse effects were also described.[8−10]

NGF has 2 structurally distinct receptors: tropomyosin-related kinase A receptor and p75 neurotrophin receptor (p75NTR); p75NTR binds to all NTFs with equal affinity, whereas tropomyosin-related kinase A receptor is specific in its binding.[11−13] We previously showed that local application of the anti-p75NTR antibody directly onto the avulsed brachial plexus at the time of the brachial plexus avulsion surgery could reduce neuropathic pain and suppress glial cell activation in a BPA rat model.[14] However, in clinical practice, it is difficult to directly apply drugs onto the avulsed brachial plexus at the time of injury. We hypothesized that systemic application of the anti-p75NTR antibody 1 week after BPA injury could reduce neuropathic pain. We selected this time point based on the assumption that it would take at least 1 week for patients to be referred to hand specialists after BPA injury.

In the current study, we investigated the effect of different systemic doses of the anti-p75NTR antibody on nociceptive behavior, neuronal calcitonin gene-related peptide (CGRP) expression, satellite glial cell (SGC) activation in the dorsal root ganglia (DRG), and spinal glial cell activation in a rat model of BPA.

MATERIALS AND METHODS

Experimental animals

The ethics committee of our institutions approved all experiments for animal procedures, which were in accordance with the 1996 revision of the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Forty 6-week-old male Wistar rats (170−200 g) were used.

Experimental groups and surgical procedure

Wistar rats were assigned to 4 groups: (a) sham, (b) BPA, (c) 1.0 μL anti-p75NTR, or (d) 50 μL anti-p75NTR. We used 20 rats (5 per group) for immunohistochemical analyses 3 days after anti-p75NTR antibody or saline administration. We used the other 20 rats (5 per group) for behavioral tests and immunohistochemical analyses 15 days after administration. After anesthesia with sodium pentobarbital (40 mg/kg intraperitoneally), the brachial plexus was approached through a horizontal incision parallel to the clavicle that ran from the sternum to the axillary region. The pectoralis major muscle was displaced, and the lower trunk of the brachial plexus was isolated from the surrounding tissues.

In the sham group, the right brachial plexus was exposed without any injury to the nerves, and 1 mL saline was administered intraperitoneally 1 week after surgery. In the BPA group, the right lower trunk was extracted from the spinal cord by traction using forceps, and the skin was closed with 4-0 nylon sutures; 1 mL saline was administered intraperitoneally 1 week after surgery. BPA model rats were generated as previously described by Rodrigues-Filho et al.[15] In the anti-p75NTR groups (1.0 and 50 μL), the right lower trunk was avulsed and either 1.0 μL anti-p75NTR antibody (Millipore, Temecula, CA) + 999 μL saline or 50 μL anti-p75NTR antibody + 950 μL saline was administered intraperitoneally 1 week after surgery.

Behavioral tests

Von Frey withdrawal thresholds: Tactile allodynia was assessed using von Frey filaments (Stoelting, Wood Dale, IL) ranging from 0.008 to 180 g. Allodynia was assessed before and 3 days after surgery, and at 1, 6, 12, and 48 hours and 3, 6, 9, 12, and 15 days after the administration of the anti-p75NTR antibody or saline. Rats were placed in plastic cages with a raised wire mesh floor. The area tested was the plantar aspect of the right front paw from the first digit to the third digit in the C6 and C7 dermatome distribution.[16] Von Frey monofilaments were applied vertically to the paw through the mesh floor until it just bent, and was kept in this position for 6 to 8 seconds.[17] The filaments were applied in ascending order, and the smallest filament that elicited a paw withdrawal response was considered the threshold stimulus. Each rat was tested 3 times at 5-minute intervals in a blinded fashion, and the average threshold stimulus was calculated.