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Nuclear factor erythroid 2-related factor 2 antibody attenuates thermal hyperalgesia in the dorsal root ganglion: Neurochemical changes and behavioral studies after sciatic nerve-pinch injury

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ABSTRACT

Oxidative stress is generated in several peripheral nerve injury models.Nuclear factor erythroid 2-related factor 2 (Nrf2) is activated to have a role in antioxidant effect. After nerve injury, the severely painful behavior is also performed. However, little has been explored regarding the function of Nrf2 in this painful process. Therefore, in this study, we compared the effects of Nrf2 antibody administration following sciatic nerve-pinch injury on painful behavior induced in young mice and neurochemical changes in dorsal root ganglion neurons. After pinch nerve injury, we found that the magnitude of the thermal allodynia was significantly decreased after application of Nrf2 antibody (5ul, 1 mg/ml) in such injured animals and phosphorylated ERK(*p*-ERK) as well as the apoptotic protein (i.e., Bcl-6) in DRG neurons were also down-regulated in the anti-Nrf2-treated injured groups compared to the saline-treated groups. Taken collectively, these data suggested that the Nrf2 antibody reduced thermal hyperalgesia via ERK pathway and the down regulation of Bcl-6 protein from the apoptosis pathway might be protecting against the protein deletions caused by anti-Nrf2 effect and suggested the new therapeutic strategy with Nrf2 inhibitor following nerve injury.

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Introduction

Neuropathic pain is severe pain originating from peripheral nerve injury. Several rodent models for nerve-injured pain have been reported, such as chronic constriction of the sciatic nerve [1], partial nerve injury [2], spinal segmental nerve injury and discherniation as so on [3,4]. These models are widely used to study neuropathic pain hypersensitivity and show prolonged mechanical and thermal hyperalgesia, and up or down regulation of cytokines and neuropeptides in dorsal root ganglion (DRG) neurons and spinal dorsal horn. However, the methods for producing these models are complicated.

A simple sciatic nerve-pinch model has been widely used for its easy to produce. However, the reliability of this model for evaluating pain-related behavior and changes in neuropeptides and signaling transduction pathway in DRG neurons has not been fully examined. In recent years, some studies suggested that rats showed pain-related behavior in a sciatic nerve-pinch model. Kato group reported that a sharply increased mechanical hyperalgesia was evident at one week after Pinch in rats [5]. George group suggested that nerve-pinch injury induced a transient period of hypoalgesia to heat followed by the development of hyperalgesia to heat and mechanical stimulation [6]. Moreover,Bester group also found that sciatic nerve pinch resulted in inconsistent, but marked tactile allodynia manifesting first at 3 weeks and persisting for up to 52 weeks in rats [7].

Oxidative stress has an important significance in several peripheral nerve injury models. After nerve injury, a large amount of free radicals generated by tissues have a severe injury effects on neurons, inhibiting oxidative stress reaction becomes an important means to prevent neuronal degeneration. Nuclear factor-erythroid 2-related factor 2 (Nrf2) is an important transcription factor regulating oxidative stress [8,9]. Study showed that the activation of Nrf2/anti-oxidant response elements (ARE) pathway could increase nuclear localization of Nrf2, and induce the expression of the Nrf2/ARE-dependent genes such as heme oxygenase-1 (HO-1), lessen painful states [10,11]. Hence, it is presumed that oxidative stress generated by nerve injury enhanced the painful behavior may be associated with the activity of Nrf2 as well as its downstream effectors. Thus, the work studied the effect and function of Nrf2 on painful behavior after Pinch nerve injury and

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the mechanisms through the across talking of apoptosis and oxidative stress signaling pathway [12–15].

In the present study, we have analyzed the evaluation of painful behavior and expression of neuropathic pain markers in this simple sciatic nerve-pinch model; In parallel, we have examined the effect of the application of Nrf2 after nerve pinch injured in mouse. Even more, changes of some key proteins in signal transduction pathway were detected to elucidate the mechanisms of Nrf2 on pinch nerve injured pain.

Materials and methods

Animals experiments

The experiments were performed on male ICR mice(25–30 g), were kept under standard conditions on a 12-h day/night cycle with free access to food and water. The animal protocols were approved by Animal Ethics Committee of School of medicine, Jishou University and disposal of animals during the experiment accorded with "Guidance Suggestions for the Care and Use of Laboratory Animals" from the Ministry of Science and Technology of the People's Republic of China.

Pinch nerve-injury model and drug application

The male ICR mice were anesthetized with sodium pentobarbital (4 mg/kg, intraperitoneally (i.p.). Their left sciatic nerves were exposed and pinched gradually, and pinching was stopped if the mice showed cramps in its hind paw (pinch-operated group, n = 5). In sham-operated mouse, the left sciatic nerves were exposed; however, the sciatic nerves were not pinched from this group (sham-operated group, n = 5). After pinching, 5ul of anti-Nrf2 polyclonal antibody (abcam ab31163, 1 mg/ml) (pinch + anti-Nrf2 group, n = 5) or 5ul of saline (pinch +saline group, n = 5) was applied onto the sciatic nerve in the pinched group. In the sham-operated group, a similar volume of saline was applied onto the sciatic nerves. The nerve were not pinched in this group (sham-operated group, n = 5).

Behavioral tests

Evaluation of mechanical hyperalgesia

Mice were evaluated for tactile hyperalgesia (sham-operated group, n = 5; pinch group, n = 5). There were no animal drop outs on any day. Mechanical thresholds of the left hind paw were assessed using von Frey filaments with bending forces of 1.00 g (noxious stimulation) before surgery and tested every day after surgery. The von Frey filaments were applied to left hind paws for five trials at approximately 5 min intervals. The responses to these stimuli were ranked as follows: 0, no response; 1, withdrawal from the von Frey filament; and 2, immediate flinching or licking of the hind paw. Scores of five trials in each animal were added (Total score form 0–10 in each animal). The average nociceptive score was calculated (Takasaki et al., 2000).

Nociceptive score = $\sum$total score in each animal/number of animals n = 5

Evaluation of thermal hyperalgesia

Hargreaves device. The animals were placed under an inverted clear plexiglass cage (23 × 18 × 13 cm) on a 3-mm-thick glass plate to detect changes in response to noxious heat. A radiant heat beam was focused from a projection bulb placed directly under the left hind paw. Following an acclimation period of 30 min in the cage, paw withdrawal latencies (PWLs) to radiant heat were measured. A digital timer automatically read the duration between the start of heat stimulation and paw withdrawal. Temperature of the glass plate was adjusted so that the baseline PWLs of normal rats were 6–10 s, and a cut off time of 15 s was used to avoid any tissue damage. Three trials were performed at each time point and the latencies of paw withdrawal for all trials were averaged.

Evaluation of vido-based parameters

For all animals, rear, left-, and right-side views of walking trials were captured prior to Pinch nerve injury operation and at different time-points after surgery with a video camera. Selected frames in which the animals were seen, which were used for measurements performed with ImageTool 2.0 software. Three to six measurements were performed and averaged for each parameter, animal, and time-point of observation [16].

Western blot and quantifications

The L-4 and L-5 DRGs were removed and lysed in RIPA buffer containing 150 mM NaF, 2 mM sodium orthovanadate and protease inhibitors (protease inhibitor mixture; Roche). Protein of total lysate (30 μg) was loaded and blotted. Primary antibodies against Phospho-ERK (Cell Signaling), and Bcl-6 (Santa Cruz Biotechnology) were used. The proteins were detected using horseradish peroxidase-conjugated anti- rabbit, or anti-mouse secondary antibodies and quantified with Image J 1.48 V (developed at the US National Institutes of Health, available at http://rsb.info.nih.gov/ij/, accessed 6 October 2014.) pain thresholds are presented as means ± SEM. Data were subjected to statistical evaluation using Student T-tests. The criterion for significance in all analyses was defined as $P < 0.05$.

Results

Nerve pinch injury-Induced hyperalgesia

We carried out a pinch injury to the sciatic nerve or exposed the nerve without pinch injury (sham control), and measured painful behavior for 14 days. The following groups of mice (n = 5 animals per group) were used: (1) nerve pinch injury group; (2) Sham pinch group. Following Pinch, mice appeared pain hypersensitivity in the pinch injury groups compared with either the sham group animals or baseline values before surgery, as indicated by a sharp increase in pain scores to mechanical stimuli and a sharp decrease in paw withdrawal latency to thermal stimuli (Fig. 1). On the ipsilateral side of Pinch (left hindpaws), it induced a short-term significant attenuation of both mechanical allodynia (Fig. 1A) and thermal hyperalgesia (Fig. 1B). However, on the contralateral side (right hindpaws), there were no significant differences in either the mechanical or the thermal pain sensitivity. Even more, the video-based parameters evidence also show the painful characters in the pinch group animals compared to the sham groups (Figs. 2 and 3). The stepping deficit can be reliably estimated by measurements of angles between the foot axis and the horizontal plane at toe-off position during walking, the foot-base and the lateral foot-base angle (Fig. 2A–D). The toe spread on the injured side is decreased compared with uninjured side (Fig. 3 A–B).

Effects of Nrf2 antibody

Pain behavior induced by Pinch led to thermal and mechanical hyperalgesia as reflected by a robust decrease in paw withdrawal latency and increase in pain scores. As shown in Fig. 4, the thermal hyperalgesia was significantly attenuated 2–7 d after pretreatment with anti-Nrf2 (5ul, 1 mg/ml). This anti-nociceptive effect of anti-Nrf2 disappeared on 8 day. In contrast to the case of thermal hyperalgesia, pretreatment with anti-Nrf2 (5ul, 1 mg/ml) did not affect the development of mechanical hyperalgesia over the entire period compared with the saline treated groups (n = 5) (Fig. 4).

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