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Aloperine attenuated neuropathic pain induced by chronic constriction injury via anti-oxidation activity and suppression of the nuclear factor kappa B pathway

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ABSTRACT

Objective: To investigate whether aloperine (ALO) has antinociceptive effects on neuropathic pain induced by chronic constriction injury, whether ALO reduces ROS against neuropathic pain, and what are the mechanisms involved in ALO attenuated neuropathic pain.
Methods: Mechanical and cold allodynia, thermal and mechanical hyperalgesia and spinal thermal hyperalgesia were estimated by behavior methods such as Von Frey filaments, cold-plate, radiant heat, paw pressure and tail immersion on one day before surgery and days 7, 8, 10, 12 and 14 after surgery, respectively. In addition, T-AOC, GSH-PX, T-AOC and MDA in the spinal cord (L4/5) were measured to evaluate anti-oxidation activity of ALO on neuropathic pain. Expressions of NF-κB and pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) in the spinal cord (L4/5) were analyzed by using Western blot.
Results: Administration of ALO (80 mg/kg and 40 mg/kg, i.p.) significantly increased paw withdrawal threshold, paw pressure, paw withdrawal latencies, tail-curling latencies, T-AOC, GSH-PX and T-SOD concentration, reduced the numbers of paw lifts and MDA concentration compared to CCI group. ALO attenuated CCI induced up-regulation of expressions of NF-κB, TNF-α, IL-6, IL-1β at the dose of 80 mg/kg (i.p.). Pregabalin produced similar effects serving as positive control at the dose of 10 mg/kg (i.p.).
Conclusion: ALO has antinociceptive effects on neuropathic pain induced by CCI. The antinociceptive effects of ALO against neuropathic pain is related to reduction of ROS, via suppression of NF-κB pathway.

1. Introduction

Neuropathic pain (NP) is defined by the International Association for the Study of Pain (IASP) as "Pain caused by a lesion or disease of the somatosensory nervous system" [1]. Neuropathic pain is a major chronic pain condition that remains difficult to treat and a common condition with an overall prevalence between 0.9% and 8.0% [2–4]. Also, it can be categorized as central and peripheral neuropathic pain. Neuropathic pain associated with peripheral nerve injury is clinically well characterized by various sensory abnormalities such as spontaneous pain, hyperalgesia (an increased response to painful stimuli) and allodynia (pain in response to a stimulus that does not normally provoke pain) [5]. Previous studies suggested that individuals with NP were known to experience more severe pain compared to non-NP sufferers [3]. Neuropathic pain which afflicted people worldwide severely affected the quality of life, reduced individual productivity and increased patient and healthcare resource expenditure [6,7]. This serious phenomenon indicates that innovative treatment strategies are needed to control this disease.

Sophora alopecuroides L. (Leguminosae) is a commonly used traditional Chinese herbal, which widely distributed in the northwestern region of china and commonly used as antipyretic, antipyretic, anti-inflammatory and analgesic [8]. Aloperine (ALO), one of alkaloids isolated from *S. alopecuroides* L, possess a variety of pharmacological activities. Recently, studies have found that aloperine

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possessed anti-inflammatory, anticancer, anti-microbial, antiviral and anti-allergic effects [9–11]. However, its analgesic potential on neuropathic pain has been no reported. Therefore, the current studies were undertaken to explore the antinociceptive effects of ALO on neuropathic pain.

To study the mechanisms of neuropathic pain, a large of animal nerve injury models have been developed [12–17]. But chronic constriction injury (CCI) model was a widely employed for induction of neuropathic pain in experimental animals [12]. Extensive studies have demonstrated that ROS played an important role in neuropathic pain [18,19]. At the same time, NF-κB and its downstream pro-inflammatory cytokines which included TNF-α, IL-6, IL-1β also played a vital role in neuropathic pain [20]. Along this line, we speculated that anti-oxidation activity and NF-κB pathway may be involved in ALO attenuated neuropathic pain. In addition, the novel compounds pregabalin (Lyrica) which is a selective Cav 2.2 (a2-δsubunit) channel antagonist have been proven clinical efficacy in neuropathic pain [21,22] and served as positive control in this study. Therefore, the present studies were undertaken to determine the above speculations.

2. Materials and methods

2.1. Experiment animals

Male ICR mice weighing 18–22 g were obtained from the Experimental Animal Center of Ningxia Medical University (Certificate number was SYXK Ningxia 20050001). The animal house temperature was controlled at 22–24 °C and the relatively humidity of the house was kept at 45–65% under a 12 h light and dark cycles. The experimental protocol was duly approved by the institutional animal ethics committee of Ningxia Medical University, Yinchuan city, Ningxia. This study complied with the internationally accredited guidelines and ethical regulations on animal research.

2.2. Compounds

Aloperine (purity ⩾ 98.0%), Sodium pentobarbital and pregabalin were purchased from Ningxia Zi Jing Hua Pharmacy, Yinchuan Ningxia, Sigma-Aldrich, Steinheim, Germany and Pfizer Manufacturing Deutschland GmbH, Betriebsstatte Freiburg, respectively. All compounds were dissolved in saline solution (0.9% NaCl), but Aloperine also were dissolved in hydrochloric acid (5%). All compounds were injected intraperitoneally (i.p.) in an application volume of 0.1 ml/10 g body weight and were administered 15 min prior to testing for seven consecutive days from the 8th day.

2.3. CCI model surgery

Neuropathic pain was induced in experimental animals by CCI of the sciatic nerve which was performed as described method of Bennett and Xie [12]. Briefly, mice were anesthetized with sodium pentobarbital. Four ligatures (silk4-0) were tied loosely around proximal bifurcation part of the nerve with 1 mm spacing each ligature until a brisk twitch of the right hind limb was observed, respectively. In sham groups, an identical surgical procedure was performed, except that the sciatic nerve was not ligated [23].

2.4. Behavioral test

2.4.1. Von Frey filaments test

Mice were divided into seven groups: sham, CCI, CCI + Pregabalin (10 mg/kg), CCI + ALO 80 mg/kg, CCI + ALO 40 mg/kg, CCI + ALO 20 mg/kg, ALO 80 mg/kg group. Mechanical sensation of the hind paw as an index of mechano-allodynia was assessed as described method of Chaplan et al. [24]. Briefly, mice were placed in a Plexiglas box with a wire mesh grid that allowed their paws access to the von frey filaments. Von Frey filaments were applied to vertically stimulate the mid plantar surface of right hind paw until it bowed slightly. A modified version of the up–down paradigm was used. The 4.0 g filament was used as a cut-off. When clear a brisk withdrawal of the right hind paw were considered a positive reaction. Then, the force of the next filament was decreased or increased according to the response [23].

2.4.2. Cold-plate test

Mice were divided into seven groups: as Von Frey filaments test. Cold allodynia of the right hind paw was assessed using the cold plate as described method of Jasmin et al. [25]. Briefly, mice were placed in a Plexiglas box with metal plate that allowed access to the hind paws. The temperature of the metal plate was maintained at 4 ± 0.5 °C. Cold allodynia was sensitive to the reaction concerning either paw withdrawal. The total numbers of observation of hind paw withdrawal, licking or shocking on the operated side were recorded during the period of 5 min.

2.4.3. Radiant heat test

Mice were divided into seven groups: as Von Frey filaments test. Radiant heat hyperalgesia of the right hind paw was assessed by using PL-200 thermal sting apparatus as described method of Hargreaves et al. [26], for assessing the reactivity to noxious thermal stimuli. Briefly, mice were placed in a Plexiglas box and allowed to adapt themselves to the tested environment and temperature by 30 min. Then a radiant heat source was applied to vertically position under the plantar surface of the operated side hind paw. The cut-off time was 20 s to prevent tissue damage. The paw withdrawal latencies were recorded as the time interval between the start application of the heat beam and the first overt withdrawal appeared.

2.4.4. Paw pressure test

Mice were divided into seven groups: as Von Frey filaments test. Mechanical hyperalgesia of the hind paw was assessed by using YLS-3E electronic pressure apparatus as described by Randall and Selitto [27], for assessing sensitization to pressure stimulation. Briefly, mice were placed in a Plexiglas holder that allowed the right hind paw of mice to access to the pressure, until mice appeared nociceptive behavior reaction. Withdrawal of right hind paw was used to assess the mechanical nociceptive threshold that expressed in grams. The cut-off pressure was 450 g to prevent tissue damage.

2.4.5. Tail immersion test

Mice were divided into seven groups: as Von Frey filaments test. Spinal thermal hyperalgesia was assessed by the tail immersion test as described by Goyal et al. [28]. Briefly, mice were placed in a Plexiglas holder that allowed the mice tail to expose. The terminal part 3 cm of the mice tail was immersed in heat-noxious water (50 ± 0.5 °C), until the tail withdrawn above the water. The cut-off time was 15 s to prevent tissue damage.

2.4.6. T-AOC, T-SOD, GSH-PX and MDA estimation

All the mice were sacrificed by spinal dislocation on the 14th day after behavioral measurements. Then, the spinal cord (L4/5) was isolated from the body immediately. The spinal cord was homogenated with 0.9% saline using glass homogenate and centrifuged at 2500 r/min for 10 min. Supernatant of homogenate (10%,w/v) was employed for this test. Tissue protein concentration, T-AOC, GSH-PX concentration, T-SOD concentration and MDA levels were estimated by using protein quantitative kits, T-AOC kits,