Gabapentin relieves neuropathic pain and inhibits brain and spinal neuroinflammatory responses in rats after L5 spinal nerve transection

Xing-Ming Wang, Ming-Qiang Zhang, Jin-Chun Shen, Jian-Jun Yang*

Background: Gabapentin exerts an antinociceptive effect in the pain management, however the underlying mechanisms are not fully elucidated. The present study aimed to investigate the effect of gabapentin on the hyperalgesia and the brain and spinal neuroinflammatory responses in rats after L5 spinal nerve transection (SNT).

Methods: Thirty male Sprague-Dawley rats were equally randomized into 5 groups: sham operation with saline (Sham group), SNT with saline (SNT group), SNT with gabapentin 100, 200, and 400 mg/kg (G-100, G-200, and G-400 groups), respectively. Paw withdrawal threshold (PWT) and paw withdrawal latency (PWL) of the left hind paws were measured 1 d before operation, 1 h before medication, and 2, 4, 6 and 8 h after medication. The levels of tumor necrosis factor (TNF-α), interleukin (IL)-6, and IL-10 and the activities of nuclear factor-kappa B (NF-κB) were determined 10 h after medication in the right cerebral cortex and lumbar spinal cord.

Results: The results displayed L5 SNT induced a significant reduction in PWT and PWL (P < 0.01), which was reversed by gabapentin medication with a dose-relation manner in rats (P < 0.05). Moreover, gabapentin inhibited the increases in TNF-α, IL-6 levels, NF-κB activities and the decreases in IL-10 levels in the right cerebral cortex and spinal cord in rats after L5 SNT (P < 0.05).

Conclusions: L5 SNT-induced neuropathic pain may be attenuated by gabapentin medication via inhibiting brain and spinal neuroinflammation in rats.

Keywords: L5 SNT-induced neuropathic pain may be attenuated by gabapentin medication via inhibiting brain and spinal neuroinflammation in rats.

( J Perioper Sci 2014, 1:2 )

Introduction

Neuropathic pain is a common clinical disease affecting a number of people, characterized by hyperalgesia and allodynia [1,2]. The development of neuropathic pain involves many mechanisms including the alterations of central neuroglia function, ion channels, and intracellular signaling transduction pathways [3,4]. Recent studies have demonstrated the activation of neuroinflammation contributes to the development of neuropathic pain[5]. Moreover, nuclear factor-kappa B (NF-κB), a transcription factor, regulated the levels of cytokines. Several lines of evidences suggest that the inhibition of NF-κB ameliorates the hyperalgesia and allodynia induced by spinal ventral root transection in rats [6,7].

Gabapentin, a structural analogue of γ-amino butyric acid (GABA), targeting alpha 2 delta 1 subunit of voltage-sensitive calcium channels, has therapeutic effect for neurological and psychiatric disorders such as epilepsy, neuropathic pain, and anxiety [8,9]. Seaval lines of evidences state gabapentin may inhibit inflammatory responses and oxidative stress in brain damage [10-12]. Furthermore, Lee and his colleagues suggest...
gabapentin suppresses the release of cytokines in nerve injury rats [13].

However, the exact antinociceptive mechanisms of gabapentin have not been well established [8,14,15]. The present study was to examine the effect of different doses of gabapentin on neuropathic pain and the brain and spinal neuroinflammatory responses in rats after L5 spinal nerve transection (SNT).

**Materials and methods**

**Ethics and animals**

The experimental protocol was approved by the Animal Care and Use Committee of Nanjing University and in accordance with the university’s guidelines for the care and use of laboratory animals. Thirty adult male Sprague-Dawley rats, weighting 230 ± 20 g, were provided by the Experimental Animal Center of Jinling Hospital. The animals were kept under controlled laboratory conditions with a light/dark cycle 12:12 h in the room temperature of 23 °C or so, and allowed free access to rat pellets and tap water. All animals were acclimated to this condition for one week.

**SNT model**

The SNT model was constructed as previously described [16]. In brief, the rats were deeply anesthetized with intraperitoneal injection of 50 mg/kg sodium pentobarbital, conventional sterilized, prone bundled, and incised along the L4-6 spinal midline. The left vertebral muscle tissue was bluntly separated. Then the L5 spinal nerve was identified, lifted slightly, ligated tightly with a 3-0 silk thread, and then transected. Finally we sutured the incision in layers to avoid postoperative infection. Sham surgery was performed by exposing the L5 spinal nerve without ligation or transection.

**Study groups**

The rats were randomized into 5 groups: sham operation with saline (Sham group), SNT with saline (SNT group), SNT with gabapentin 100, 200, and 400 mg/kg (G-100, G-200, and G-400 groups), respectively. On 5 d after the operation, gabapentin dissolved in 4 ml/kg saline was intragastrically administrated in G-100, G-200, and G-400 groups, whereas the rats in Sham group and SNT group received the same volume of saline in the same way.

**Behavioral tests**

The paw withdrawal threshold (PWT) and paw withdrawal latency (PWL) of the left hind paws were measured 1 d before operation, 1 h before medication and 2, 4, 6 and 8 h after medication.

Mechanical nociceptive threshold was measured with PWT using Electro von Frey Anesthesiometer (Model 2390CE, IITC Life Science, Inc.), united g. The rats, placed on a wire mesh platform and covered with a transparent Plexiglas container, were allowed to acclimatize for 20 min before the test. Then the filaments were presented, in ascending order of strength, perpendicular to the plantar surface with sufficient force to cause slight bending against the paw. Brisk withdrawal or paw flinching was considered as a positive response. The test was performed 5 times with a time interval of 10 min. The maximum and the minimum values were discarded and the average of the left 3 measures represented PWT.

PWL to noxious heat stimuli was determined using a paw stimulator analgesia stimuli meter (Model 390, IITC Life Science, Inc.). The animals, placed under an inverted transparent plexiglas dome on an elevated glass plate, were allowed to acclimatize for 20 min before the test. PWL was measured with 390-type radiant heat tester. A light beam with stimulus intensity set at 40% was focused onto the plantar surface of the left hind paw through the glass plate. Nociceptive endpoints in the radiant heat test were apparently withdrawal of the hind paw, while the time to the endpoint was considered as PWL. The test was performed 5 times with a time interval of 10 min and a 20 s cut-off was used to avoid tissue damage. The maximum and the minimum values were discarded and the average of the left 3 measures represented PWL.

**Tissue harvest**

The right cerebral cortex and lumbar spinal cord of rats were quickly removed 10 h after medication, the levels of TNF-α, IL-6, IL-10 and the activities of NF-κB were determined using enzyme-linked immunosorbent assay (ELISA) and electrophoretic mobility shift assay (EMSA), respectively.

**ELISA for TNF-α, IL-6 and IL-10 levels**

The levels of the spinal cord cytokines, TNF-α, IL-6, and IL-10, were measured according to manufacturer’s kit instructions ( Biosource International, USA ). The photodensity was analyzed using a microplate reader (Bio-Rad USA).
Co) at a wavelength of 450 nm and a reference wavelength of 620 nm, and then the levels of the cytokines were analyzed according to their standard curves.

**EMSA for NF-κB activities**

EMSA was performed for the determination of NF-κB activities using a commercial kit (Promega, Madison, WI, USA). Briefly, equal amounts of nuclear extract (10 μg) were added to 9 μl of gel shift binding buffer for 15 min at 23 °C or so. Then the mixture was incubated for 30 min with 1μl of 32P-labelled oligonucleotide probe. At last, 1 μl of loading buffer was added and the sample was electrophoresed in a 4% polyacrylamide gel. The gel was vacuum-dried exposed to X-ray film at -70 °C for 48 h. The activity of NF-κB was measured by densitometry. The density of the NF-κB band was normalized for the background density of the X-ray film.

**Statistical analysis**

Statistical analysis was performed using the Version 17.0 of Statistical Product for the Social Sciences (SPSS, Inc., Chicago, Illinois, USA). Data are presented as the mean ± SEM. For PWT and PWL, comparisons were performed using repeated measures of analysis of variance (ANOVA). For cytokine levels and NF-κB activities, comparisons were performed using ANOVA followed by Bonferroni tests. Difference was defined as significant at P < 0.05.

**Results**

**PWT and PWL**

No significant difference was observed in PWT and PWL during all groups on 1 d before operation (P > 0.05). L5 SNT resulted in the reduction in PWT and PWL of rats compared with sham operation. After gabapentin medication, PWT and PWL increased at 2 h in group G-100, at 2 and 4 h in G-200 group, at 2, 4, 6, and 8 h in G-400 group compared with SNT group (P < 0.05). PWT and PWL are higher in G-400 group than in the other 4 groups 4, 6, and 8 h after medication (P < 0.05), however 4 rats (66.7%) presented somnolence in G-400 group. (Fig 1)

![Graph showing changes in PWT and PWL](#)

Fig.1 The changes of PWT and PWL in different groups. The PWT and PWL significantly decreased after L5 SNT compared with after sham operation in rats (**p < 0.01**). The alterations of PWT and PWL were attenuated at 2 h in group G-100, at 2 and 4 h in G-200 group, at 2, 4, 6, and 8 h in G-400 group compared with SNT group (#p < 0.05). Data are presented as mean ± SEM. 1 d pre: 1 day before operation; 1 h pre: 1 h before medication; 2 h post: 2 h after medication; 4 h post: 4 h after medication; 6 h post: 6 h after medication; and 8 h post: 8 h after medication;

**Levels of cytokines**

Compared with Sham group, the levels of TNF-α and IL-6 increased, but the levels of IL-10 decreased, in the right cerebral cortex and lumbar spinal cord in SNT group 10 h after medication (P <0.01). Gabapentin administration attenuated the increases in TNF-α, IL-6 levels and the decreases in IL-10 levels in the right cerebral cortex and spinal cord.
compared with saline administration (P < 0.05). Compared with G-100 group, IL-10 levels in lumbar spinal cord (P < 0.05) increased, and TNF-α levels in the right cerebral cortex (P < 0.01) decreased in G-400 group. Moreover, the levels of TNF-α were lower in G-400 group than in G-200 group in the right cerebral cortex (P < 0.05). (Fig 2)

![Fig.2 The levels of cytokines in different groups 10 h after medication. Gabapentin reduced the increased levels of TNF-α (A, B) , IL-6 (C, D), and the decreased levels of IL-10 (E, F) in the lumbar spinal cord and right cerebral cortex in rats after L5 SNT (# p < 0.05). The levels of TNF-α decreased (△△ p < 0.01) in the right cerebral cortex and the levels of IL-10 increased (* p < 0.05) in the lumbar spinal cord in G-400 group compared with G-100 group. The levels of TNF-α were lower in G-400 group than in G-200 group in the right cerebral cortex (* p < 0.05).](image1)

**Activities of NF-κB**

SNT resulted in the up-regulation of NF-κB activity in lumbar spinal cord and right cerebral cortex 10 h after medication compared with sham operation (P < 0.01), which was reversed by gabapentin medication (P < 0.05). The activities of NF-κB in lumbar spinal cord and the right cerebral cortex were lower in G-200 and G-400 groups than in G-100 group 10 h after medication (P < 0.01). The activities of NF-κB in G-400 group were lower than in G-200 group 10 h after medication in the lumbar spinal cord and right cerebral cortex (P < 0.05). (Fig 3)

![Fig.3 The activities of NF-κB in different groups 10 h after medication. The activities of NF-κB increased in rats after SNT compared with after sham operation 10 h after medication in the lumbar spinal cord (A) and right cerebral cortex (B) (** p < 0.01). Gabapentin administration attenuated the increases in NF-κB activities 10 h after](image2)
medication in the lumbar spinal cord (A) and right cerebral cortex (B) compared with saline administration (#p < 0.05). The activities of NF-κB were lower in group G-400 than in G-200 group 10 h after medication in the lumbar spinal cord (A) and right cerebral cortex (B) (*p < 0.05). The NF-κB activities decreased in G-200 group compared with that in G-100 group 10 h after medication in the lumbar spinal cord (A) and right cerebral cortex (B) (△p < 0.05).

**Discussion**

In the present study, L5 SNT resulted in the mechanical and heat hyperalgesia, which was dose-related attenuated by gabapentin medication in rats. Moreover, the increased TNF-α and IL-6 levels, and NF-κB activities, as well as the decreased IL-10 levels in L5 SNT rats were reversed by gabapentin.

The antinociceptive effect of 400 mg/kg gabapentin is better than 100 or 200 mg/kg gabapentin. Though such a single dose could not ameliorate the hyperalgesia completely, somnolence, occurred in some rats, which was consistent with the result of previous study [17,18] and suggested that gabapentin could not completely relieve the neuropathic pain even in a single dose that might induce somnolence.

The release of proinflammatory cytokines such as TNF-α and IL-6 play an important role in the neuroinflammation [1]. Recent studies have suggested that peripheral nerve injury leads to the hyperalgesia concomitant with the increases in TNF-α and IL-6 levels in the spinal cord and cerebral cortex, which was consistent with the present study [19-21]. The enhanced TNF-α and IL-6 production contributes to the ongoing inflammatory cascade, synaptic dysfunction, neuron death, thus reduces the nociceptive thresholds [1]. In the present study, the hyperalgesia induced by L5 SNT were ameliorated by gabapentin, which is associated with the inhibition of inflammatory responses.

IL-10 may inhibit the release of TNF-α and IL-6 [22]. Exogenous administration of IL-10 impeded the development of hyperalgesia through inhibiting spinal glial cell activation induced by the nerve injury in the central nervous system (CNS) [13]. In the present study, IL-10 decreased significantly in the spinal cord and right cerebral cortex after L5 SNT, which was reversed after gabapentin administration.

Complicated mechanisms are involved in the neuroprotection of gabapentin such as strengthening the activity of GABAergic neurons, binding with N-methyl-D-aspartate receptor, and suppressing inflammatory responses [13,23,24]. NF-κB, involved in diverse biological processes including immune responses, inflammatory reactions, cell growth, and spinal hyperexcitability, can be activated by cytokines and in turn regulated the expression of cytokines [25,26]. Park and coworkers [27] have demonstrated that substance P-induced NF-κB activation is inhibited by gabapentin in rats. In the present study, gabapentin restrains neuroinflammatory responses in a dose-related manner probably via suppressing NF-κB activity in the spinal cord and cerebral cortex.

In conclusion, SNT induces hyperalgesia associated with increased cytokines, which may be ameliorated via gabapentin-suppressed activation of NF-κB in the spinal cord and cerebral cortex in rats, suggesting the involvement of CNS inflammation in the pathogenesis of neuropathic pain.

**Acknowledgements**

Each author has contributed to the design and conduct of the work; the manuscript has been written, read, and approved by all the authors.

**References**


