Inhibitory effect of SB203580 on neuropathic pain behaviors induced by lumbar 5 ventral rhizotomy in rats☆

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Abstract

Previous studies have shown that selective injury to motor fiber, but keeps primary sensory afferents intact, induced abnormal pain behaviors to a similar extent as the rat received lumbar 5 spinal nerve ligation (L5 SNL). Several lines of evidence suggest that the p38 activation in the nociceptive pathway contributes to the development of inflammatory and nerve injury induced neuropathic pain. Whereas, the role of p38 activation in the pain facilitation induced by the motor fiber injury is still unclear. In the present study, the lumbar 5 ventral rhizotomy (L5 VR) was performed in rats, and the animals were treated with SB203580, an inhibitor of activated p38, intrathecal injection started at before and after the surgery. The pain-related behaviors were tested to elucidate the effect of SB203580. The results showed that L5 VR induced a robust and long-lasting mechanical allodynia and thermal hyprogesia in bilateral hind paws in rats started on day 1 and persisted for more than 4 weeks. Intrathecal injection of SB203580 10 minutes before L5 VR and once daily thereafter until 14th day significantly reduced the mechanical allodynia and blocked the development of thermal hyperalgesia in bilateral hind paws. Post-treatment with SB203580 performed at the first day and 8th day also clearly alleviated the established neuropathic pain following L5 VR. Taken together, the above data indicate that p38 activation might be playing an important role in the induction and maintenance of the neuropathic pain induced by the selective motor fiber injury. [Life Science Journal. 2007;4(1):32 - 36] (ISSN: 1097 - 8135).

Keywords: ventral rhizotomy; neuropathic pain; p38; mitogen-activated protein kinase; SB203580

1 Introduction

Damage to peripheral nerve very often results in neuropathic pain. Current treatments for this pain are only partially effective, and additional development is hindered by our incomplete knowledge of how neuropathic pain is induced and maintained[1]. Increasing evidence shows that mitogen-activated protein kinases (MAPKs) play important roles in the induction and maintenance of chronic pain[2,3]. The MAPK family has three major members, extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK), representing three different signal transduction pathways. It has been reported that p38 activation in dorsal root ganglia (DRG) and spinal cord plays an important role in the pain hypersensitivity state induced by L5 spinal nerve ligation (L5 SNL)[4-6]. Recently, several groups show that selective lesion to motor fibers by L5 ventral rhizotomy (L5 VR), but keeps sensory neurons intact, produces behavioral signs of neuropathic pain to a similar extent as in rats with L5 SNL[7-9]. While, the underlying mechanisms of L5 VR induced neuropathic pain is still remained largely unknown. Therefore, it was hypothesized that p38 activation may be also play a role in the generation of pain-related behaviors induced by the model of selective motor fiber injury. To testify this hypothesis, in present study the L5 VR was performed in rats and the role of p38 activation in the production of mechanical allodynia and thermal hyperalgesia was evaluated by intrathecal injection of p38 inhibitor SB203580.

2 Materials and Methods

2.1 Animals

Male Sprague-Dawley rats weighing 180 - 250 g were used. The rats were housed in separated cages with free access to food and water. The room temperature was kept at 23 ± 2 ℃ under a 12∶12 light-dark cycles. All animal experimental procedures were approved by the local animal care committee and were carried out in accordance with the guideline of the National Institutes of Health of America on animal care and the ethical guidelines for investigation of experimental pain in con-

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scious animal[10].

2.2 Surgical procedures and drug delivery

All experimental procedures were done on rats that were deeply anesthetized with sodium pentobarbital (50 mgl/kg body weight, *i. p.*). Special care was paid to prevent infection and to minimize the influence of inflammation. The L5 VR was done following the procedures described by Li *et al*[7]. Briefly, after a midline skin incision in the L4-S1 region, the left L5 vertebra was freed of its muscular attachment. An L5 hemilaminectomy was performed, and the dura matter and arachnoid membrane were incised. The L5 ventral root was identified as it lays at the most lateral side of spinal canal and just beneath the dorsal root. The ventral root was gently pulled out and carefully transected 2 – 3 mm proximal to the DRG. Great care was taken to avoid any damage to the nearby L5 dorsal root and it's DRG. In the sham group, all procedures of operation were identical with the experimental group except that the exposed ventral root was not transected. After surgery, the wound was washed with saline and closed in layers with 3 – 0 silk thread. At the end of each study, animals in L5 VR groups were deeply anesthetized with intra-peritoneal 20% urethane and were dissected to verify that the lesions were done at the correct level. Animals that had a lesion at wrong level were excluded from the study.

For intrathecal injection, a method described by Jin *et al*[5] was followed. In brief, a polyethylene-10 (PE-10) catheter was inserted into the rat's subarachnoid space through the incision of L5 VR, and the tip of the catheter was implanted at the L5 spinal segmental level. In one group of the rats, the p38 inhibitor SB203580 (Sigma-Aldrich Co., St. Louis, MO, USA) was injected intrathecally (10 μg/10 μl) and flushed with 10 μl of saline started 10 minutes before L5 VR and once daily thereafter for 14 days. In another group of the rats, a single injection of SB203580 (10 μg/10 μl) was performed on day 1 and day 8 after surgery. The control group received same volume of vehicle (saline contained 2% DMSO) injection at same time as above. The SB203580 was dissolved in 100% DMSO and diluted by saline. The final concentration of DMSO was 2%. Behavioral test was done after the injection of SB203580 according to the experimental design.

2.3 Behavioral test

The rats were accommodated to the testing environment by exposing the rats to the testing chambers for a period of 15 – 20 minutes on three separate days just prior to the pre-operative testing. Mechanical sensitivity was assessed using von Frey hairs and the up-down method following the procedure as described previously[11]. Briefly, three rats were placed under separate transparent plexiglas chambers positioned on a wire mesh floor. Five minutes were allowed for habituation. Each stimulus consisted of applicating for 2 – 3 seconds of the von Frey hair to the middle of the plantar surface of the foot with 5-minute interval between stimuli. Quick withdrawal or licking of the paw in response to the stimulus was considered a positive response.

Heat hypersensitivity was tested using a plantar test (7370, UgoBasile, Comeria, Italy) according to the method described by Hargreaves *et al*[12]. Briefly, a radiant heat source beneath a glass floor was aimed at the plantar surface of the hind paw. Three measurements of latency were taken for each hind paw in each test session. The hind paw was tested alternately with greater than 5-minute intervals between consecutive tests. The three measurements of latency per side were averaged as the result per test. Two persons performed the behavioral tests and only one knew the design of the study.

2.4 Statistical analysis

Differences in changes of values over time were tested using Friedman ANOVA followed by Wilcoxon matched pairs test. The data between groups on a given testing day were analyzed with Mann-Whitney *U* test. Statistical tests were performed with SPSS 10.0 (SPSS Inc, USA). All data are expressed as mean ± SE. $P < 0.05$ was considered significant.

3 Results

3.1 L5 VR induced mechanical allodynia and thermal hyperalgesia in bilateral hind paws in rats

In consistent with a previous work[7], we found that selective transection of L5 ventral root produced robust and prolonged bilateral mechanical allodynia and thermal hyperalgesia. Compared with sham group and pre-operative baseline, the 50% paw withdrawal threshold (Figure 1A) and paw withdrawal latency (Figure 1B) significantly decreased 1 day after L5 VR ($P < 0.05$), and persisted more than 4 weeks after surgery in bilateral hind paws.

3.2 Pre-treatment with SB203580 attenuated the abnormal pain behaviors following L5 VR

To elucidate whether the activation of p38 plays a role in the behavioral signs of neuropathic pain produced by L5 VR, a p38 inhibitor SB203580 was injected intrathecally 10 minutes before L5 VR and once daily thereafter lasting for 14 days after surgery. The results showed that the magnitude of mechanical allodynia in ipsilateral hind paw was significantly reduced and duration was shortened (Figure 2A). While mechanical allodynia in contralateral side and the thermal hyperalgesia in bilateral sides were blocked completely (Figure 2A and B). Intrathecal injection of SB203580 as above had no effect on the basal behavioral test in naive rats (data not shown).

3.3 The effect of post-treatment with SB203580 on

the established neuropathic pain induced by L5 VR

To evaluate the role of p38 activation on the established neuropathic pain, a single injection of SB203580 was designed at the first and 8th day after L5 VR, and the behavioral tests were done at 1, 3, 6 and 12 hours after the treatment. The results showed that the mechanical allodynia and thermal hyperalgesia were significantly reduced at both day 1 and day 8 in treatment groups. But, the effect of SB203580 only lasted for several hours (Figure 3). These results suggest that p38 activation may involve in the development and maintenance of the neuropathic pain induced by L5 VR.

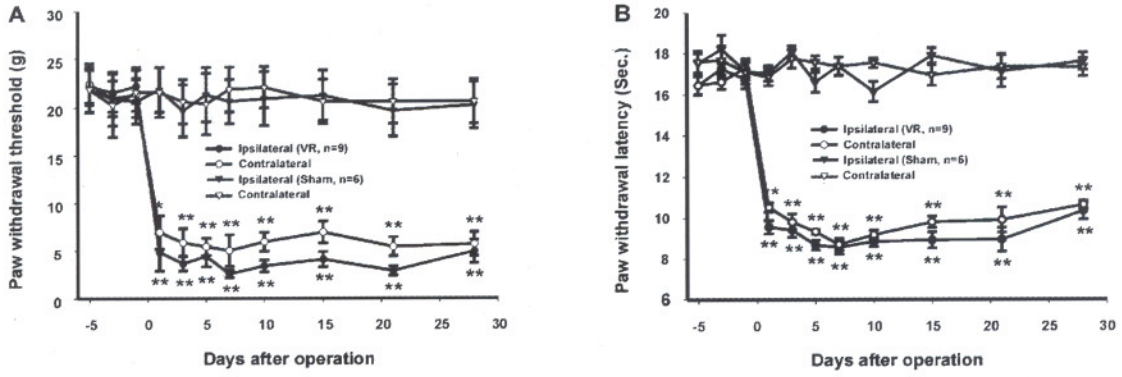

Figure 1. Lumbar 5 ventral rhizotomy (L5 VR) induced pain-related behaviors in bilateral hind paws. A: showed the changes of paw withdrawal threshold in bilateral hind paws following L5 VR. B: showed the changes of paw withdrawal latency in bilateral hind paws following L5 VR. The results revealed that both paw withdrawal threshold and paw withdrawal latency decreased significantly compared with pre-operative baseline as well as sham operation group starting on day 1 and persistent for more than 4 weeks after L5 VR. $^{*}: P < 0.05$; $^{**}: P < 0.01$ compared with the sham operation group, respectively.

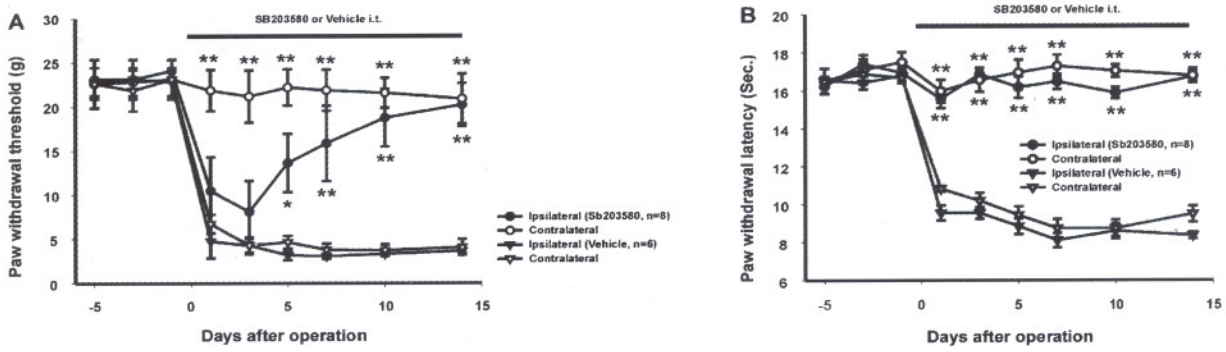

Figure 2. Intrathecal injection of p38 inhibitor SB203580 started before surgery significantly reduced the abnormal pain behaviors induced by L5 VR. A-B: Intrathecal injection of SB203580 applied 10 minutes before L5 VR and once daily thereafter until day 14 after surgery significantly attenuated mechanical allodynia (A) and thermal hyperalgesia (B). $^{*}: P < 0.05$; $^{**}: P < 0.01$ versus vehicle treated group (SB203580 treated group, $n = 8$; vehicle treated group, $n = 6$).

4 Discussion

In the present study, we found that selective injury to myelinated efferent fibers by L5 VR induced abnormal pain behaviors in bilateral hind paws of rats. Intrathecal injection of SB203580, started before L5 VR, prevented the development of the mechanical allodynia and thermal hyperalgesia. Post-treatment with SB203580 also significantly reduced the pain related behaviors. It suggests that p38 activation may play an important role in the induction and maintenance of the neuropathic pain induced by L5 VR.

4.1 Neuropathic pain induced by L5 VR

As reported by several groups previously[7,8,13,14], L5 VR, performed in the present study, created a selective injury to motor fibers in L5 spinal nerve and induced a robust and long-lasting neuropathic pain in animals. Ventral root is predominantly consisting of myelinated efferent fiber, and the unmyelinated afferent fiber is less than 4%[15-17]. Recent study shows that selective transected L5 dorsal root failed to induce abnormal pain behaviors[14]. Whereas, selective injury to myelinated efferent fibers after ventral rhizotomy induced an increased spontaneous activity in uninjured C-fiber nociceptive afferents and this injury also induced hyperalgesic behav-

ior[13]. It indicates that the motor fiber injury after L5 VR is responsible for the development of neuropathic pain.

4.2 Inhibition of p38 activation reduced the thermal hyperalgesia and mechanical allodynia induced by L5 VR

The p38 inhibitor SB203580 does not inhibit the phosphorylation of p38 but binds to the ATP pocket in the enzyme, thereby inhibiting its activity[18]. There is increasing evidence showing that neuropathic pain induced by peripheral nerve injury can be prevented by block p38 activation[5,6]. In the present study we found that intrathecal injection of SB203580 before L5 VR, the pain related behaviors in bilateral hind paws were clearly alleviated. Post-treatment with SB203580 started on day 1 and day 8 after L5 VR also reduced the neuropathic pain. Several lines of evidence shows that Wallerian degeneration of injured motor fiber contributes to the development of pain hypersensitivity after L5 VR[13,19]. TNF-α is the pioneer cytokine, which released 4 to 6 hours after nerve injury, and play a key role in the initiation of the Wallerian degeneration of injured fibers and neuropathic pain subsequently[20,21]. Our recent works showed that L5 VR induced a significant upregulation of TNF-α and TNF receptor 1 (p55) in ipsilateral L4, L5 DRG and in bilateral L5 spinal dorsal horn[9]. It has been reported that exogenous TNF-α activated p38 activation in cultured DRG neurons[22]. The p38 activation in injured DRG neurons induced by L5 SNL can be blocked by intraperitoneal injection of TNF-α anti-agonist etanercept[23]. Therefore we speculated that p38 activation in the present study might be one of results of TNF-α, which is released in sciatic nerve after L5 VR.

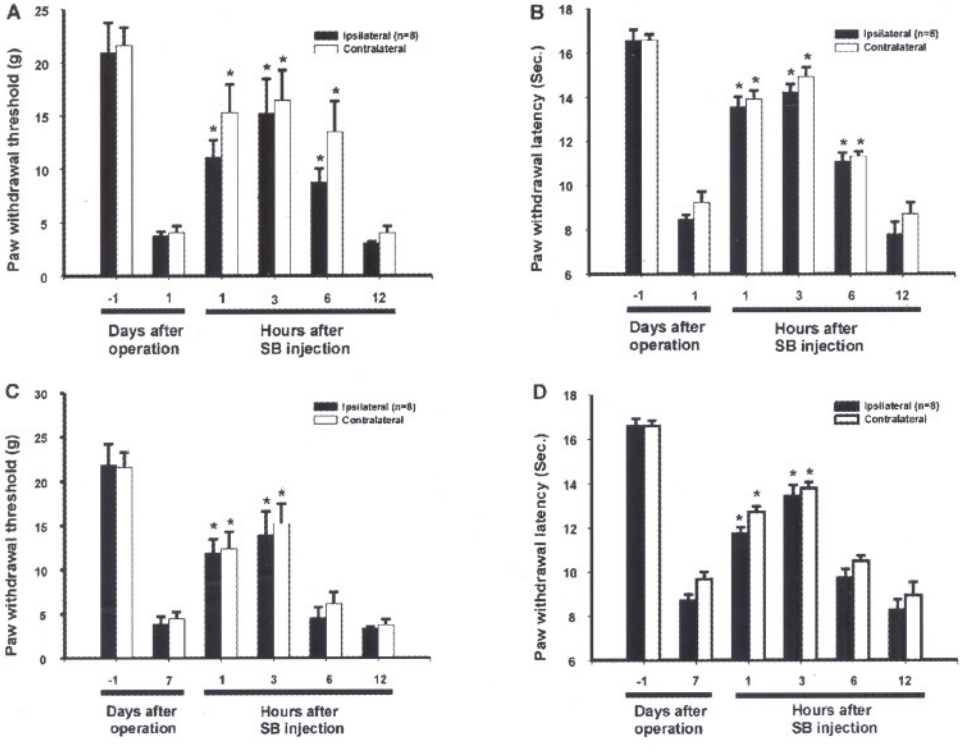

Figure 3. Effect of post-treatment with SB203580 intrathecal injection on the established neuropathic pain induced by L5 VR. A-B: SB203580 applied at 1st day after operation significantly reduced the mechanical allodynia (A) and thermal hyperalgesia (B). C-D: SB203580 applied at 8th day after operation also clearly alleviated the mechanical allodynia (C) and thermal hyperalgesia (D). $P<0.05$; versus L5 VR base (the value of 1st day after L5 VR in Figure A and B; the value of 7th day after L5 VR in Figure C and D) ($n=8$).

Previous study showed that acute injection of zymosan around the sciatic nerve produced bilateral mechanical allodynia[24] and that spinal glia as well as p38 activation played important roles in the so-called sciatic inflammatory neuropathy (SIN), since both ipsilateral and mirror image allodynia can be attenuated by a glial metabolic inhibitor or by CNI-1493, a potent inhibitor of p38[25,26]. Recently, it had been reported that intrathecal administration of low dose carbenoxolone, a gap junction decoupler, reverses mirror image pain, while leaving ipsilateral mechanical allodynia unaffected in SIN or chronic constriction injury model[27]. Therefore, it is likely that the communication between ipsilateral and contralateral spinal cord through glial gap junc-

tion may contribute to L5 VR induced p38 activation in contralateral spinal cord, and to mirror image pain.

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