Effect of Thalidomide on Neuropathic Pain Induced by Lumbar 5 Ventral Rhizotomy in Rats

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Abstract: Selective injury to motor fiber but keeping primary sensory neuron intact also induced behavioral signs of neuropathic pain. The underlying mechanism, however, is still unclear. Accumulating evidence showed that TNF-α plays an important role in the process of injured nerve degeneration and the production of abnormal pain behaviours after nerve injury. The present study was to examine the role of TNF-α in the development of neuropathic pain induced by lumbar 5 ventral rhizotomy (L5 VR), a model of selective injury to motor fibre in sciatic nerve, with intraperitoneal injection of thalidomide, an inhibitor of TNF-α synthesis, started before and after the surgery. The results showed that L5 VR induced robust and long-lasting abnormal pain behaviours in bilateral hind paws of rats. Compared with sham operated group and the pre-operated baseline, the significantly decrease of paw withdrawal threshold and paw withdrawal latency started 1 day after the surgery and persisted more than 4 weeks. Intraperitoneal injection of thalidomide, started 2 hours before surgery and once per day thereafter, significantly reduced the mechanical allodynia and thermal hyperalgesia in bilateral hind paws induced by L5 VR. Whereas, post-treatment with thalidomide as above started at the 7th day after operation, the established neuropathic pain induced by L5 VR was not affected. Taken together, the above data suggested that TNF-α might be playing an important role in the initiation, rather than maintenance, of the neuropathic pain induced by L5 VT. [Life Science Journal. 2006;3(4): 12-16] (ISSN: 1097-8135).

Keywords: ventral rhizotomy; neuropathic pain; TNF-α; thalidomide

Abbreviations: CCI: chronic constraint injury; DRG: dorsal root ganglia; L5 VR: lumbar 5 ventral rhizotomy; SIN: sciatic inflammatory neuropathy

1 Introduction

Peripheral nerve injury often results in neuropathic pain associated with hyperalgesia and allodynia. Although vast studies have been performed in the past decade, the underlying mechanisms are still remained largely unknown[1]. In clinic, the injured peripheral nerve, such as the lesion of sciatic nerve by accident, often includes afferent and efferent fibers. Therefore, it is important that verify the role of selective injury to efferent fiber in the development of neuropathic pain. Recently, several groups reported that selective injury to motor fiber with primary sensory neuron intact by Lumbar 5 ventral rhizotomy (L5 VR) also induce abnormal pain behaviors which last for several weeks after the surgery[2,3]. And the Wallerian degeneration of injured motor fiber contributes to the production of neuropathic pain after L5 VR[4-6]. Several lines of evidence demonstrated that cytokines and nerve growth factors play an important role in the process of injured fibre degeneration as well as the development of neuropathic pain subsequently[6-8]. Among them, TNF-α appears to play a key role for the initiation of injured nerve degeneration after sciatic nerve or spinal nerve injury[9-11]. Whereas, whether the TNF-α plays a role in the neuropathic pain induced by L5 VR is still unclear. So the present study was to examine the role of TNF-α in the induction and maintenance of the neuropathic pain induced by L5 VR with intraperitoneal injection of thalidomide, an inhibitor of TNF-α synthesis, started 2 hours before and the 7th day after L5 VR, respectively.

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 24 ± 1℃ 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 on animal care and the ethical guidelines for investigation of experimental pain in conscious animal[12].

2.2 Surgical procedures of L5 VR

All experimental procedures were done on rats that were deeply anesthetized with sodium pentobarbital (50 mg/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*[2]. 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 dorsal root ganglia (DRG). Great care was taken to avoid any damage to the nearby L5 dorsal root and its 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.

2.3 Behavioural tests and drug delivery

The rats were accommodated to the testing environment by exposed 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 described previously[13]. 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 2 – 3 seconds application of the von Frey hair to the middle of the plantar surface of the foot with 5 minutes 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 the plantar test (7370, UgoBasile, Comeria, Italy) according to the method described by Hargreaves *et al*[14]. Briefly, a radiant heat source beneath a glass floor was aimed at the plantar surface of the hind paw. Three measurement of latency were taken for each hind paw in each test session. The hind paw was tested alternately with greater than 5 minutes intervals between consecutive tests. The three measurements of latency per side were averaged as the result of per test. Two persons performed the behavioral tests and only one knew the design of the study.

To investigate the role of TNF-α in neuropathic pain induced by L5 VR, drug delivery was performed as follows. Thalidomide (Sigma, St. Louis, USA) was dissolved in dimethyl sulfoxide (DMSO) and then diluted in saline to a final concentration of 20 mg/ml (the concentration of DMSO was 10%, v/v). In one group of rats thalidomide was injected (50 mg/kg) intraperitoneally (*i. p.*) 2 hours before surgery and once per day thereafter until the 7th day after surgery. In another group of rats the same dose of thalidomide as above was injected at the 7th day after surgery, and once per day thereafter for 7 days. The control group received vehicle injection.

2.4 Statistical analysis

Differences in changes of values over time were tested using Friedman ANOVA followed by Wilcoxon matched pairs. The data between groups on a given testing day were analyzed with Mann-Whitney U test. Statistical test 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 and heat hypersensitivity in bilateral hind paws of rats

In consistence with a previous work[2], we found that selective transection of L5 ventral root produced robust and prolonged bilateral mechanical allodynia and thermal hyperalgesia. After L5 VR, the paw withdrawal thresholds on the ipsilateral side were significantly decreased compared with pre-operative baseline ($P < 0.001$) and with those in sham operated group ($P < 0.001$, Figure 1A). The paw withdrawal thresholds of contralateral hind paw were also significantly decreased after L5 VR. In the same group of rats paw withdrawal latencies to radiant heat on both ipsi- and contralateral sides were significantly lower compared with baseline ($P < 0.05$, Figure 1B) and with those in sham operated group ($P < 0.01$, Figure 1B). The behavioural signs of neuropathic pain persisted more than 4 weeks after L5 VR.

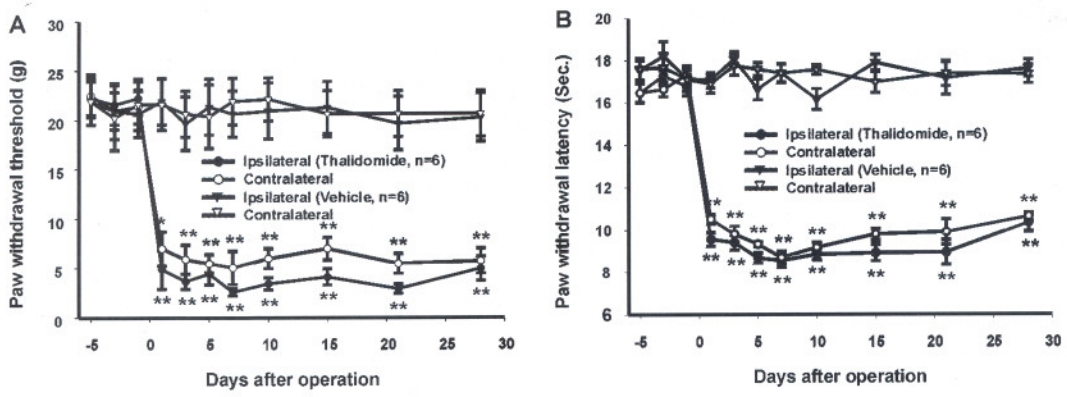

Figure 1. L5 VR induced pain-related behaviors in bilateral hind paws

A: Show the changes of paw withdrawal threshold in bilateral hind paws following L5 VR. B: Show 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 exhibited a significantly decrease compared with pre-operative baseline as well as sham operated group starting on day 1 and persistent for more than 4 weeks after L5 VR. *: $P<0.05$, **: $P<0.01$ *vs*. the sham operated group, respectively.

3.2 The effects of pre-treatment with thalidomide intrapertoneal injection on L5 VR induced neuropathic pain in rats

To evaluate the role of TNF-α in the neuropathic pain induced by L5 VR, thalidomide, a specific inhibitor of TNF-α synthesis, was injected intraperitoneally (50 mg/kg) before L5 VR. In eight rats treated with the drug, started at 2 hours before L5 VR and once daily thereafter until 7 days after surgery, the paw withdrawal thresholds of bilateral hind paws displayed an initial decrease at the 1st day and 3rd day after operation, but returned to normal at the 7th day, and then maintained at the level up to 13 days after operation (Figure 2A). The paw withdrawal thresholds between thalidomide treated group and vehicle treated group were not significantly different until the 5th day after operation ($P>0.05$, Figure 2A). In the same group of rats the decrease of paw withdrawal latencies to radiant heat was completely abolished. In contrast, in vehicle treated group the paw withdrawal latency dropped significantly after L5 VR (Figure 2B). The difference of bilateral paw withdrawal latencies between thalidomide treated group and vehicle treated group was significant from the 1st day to the 13th day after surgery ($P<0.01$, Figure 2B).

3.3 The effects of post-treatment with thalidomide on the established neuropathic pain following L5 VR in rats

To evaluate the effect of thalidomide on the established neuropathic pain, administration of thalidomide was designed at the 7th day after surgery. Although the animals received injection of thalidomide once daily for 7 days, the paw withdrawal threshold as well as paw withdrawal latency between the thalidomide treated group and vehicle treated group was not different ($P>0.05$, Figure 3A－B). These results suggest that the abnormal pain behaviours can be abolished or alleviated by inhibition of the TNF-α synthesis at the early stage of the neuropathic pain rather than when it has been established.

4 Discussion

In the present study, we found that L5 VR induced long-lasting abnormal pain behaviours in bilateral hind paws in rats. Intraperitoneal injection of thalidomide started before surgery significantly reduced the mechanical allodynia and completely blocked the thermal hyperalgesia after L5 VR. Whereas, post-treatment with thalidomide started at the 7th day there is no effect on the established neuropathic pain. It suggests that the TNF-α might be playing an important role in the initiation, but not maintenance, of the neuropathic pain induced by L5 VR.

4.1 L5 VR induced neuropathic pain in rats

As reported by several groups previously[2,3], L5 VR in the present study also induced abnormal pain behaviours to a similar extent as in rats received lumbar 5 spinal nerve ligation. Ventral root consist of myelinated efferent fibers mainly, and the unmyelinated afferent fibers less than 4%. Furthermore, very recent study shows that selective transected L5 dorsal root failed induced pain-related behaviors[15]. Therefore, the motor fiber injury produced by L5 VR induced the pain related behaviors in the present study. It indicates that not only primary sensory afferents but also motor fibre injury responsible for the development of neuropathic pain after peripheral nerve injury.

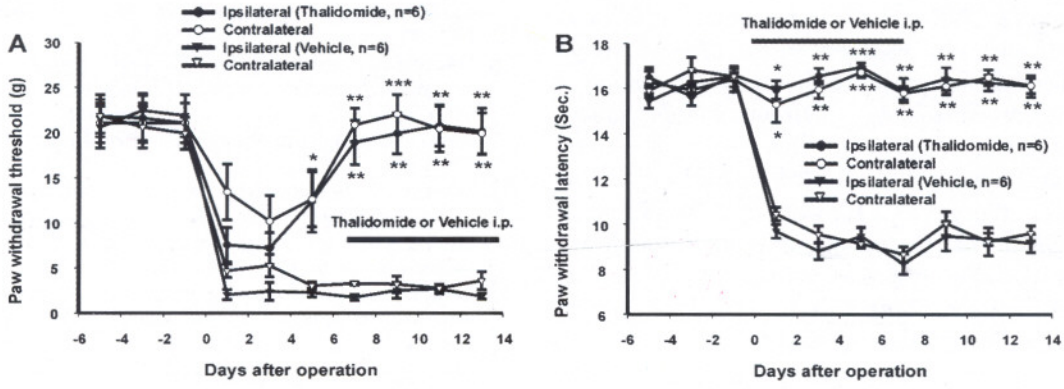

Figure 2. Pre-treatment with thalidomide reduced the pain-related behaviors produced by L5 VR

A－B: Intraperitoneal injection of thalidomide (50 mg/kg), applied 2 hours before L5 VRT and once daily thereafter until 7 days after surgery, attenuated mechanical allodynia (A) and thermal hyperalgesia (B). $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ *vs*. vehicle group (thalidomide treated group, $n=8$; vehicle treated group, $n=6$).

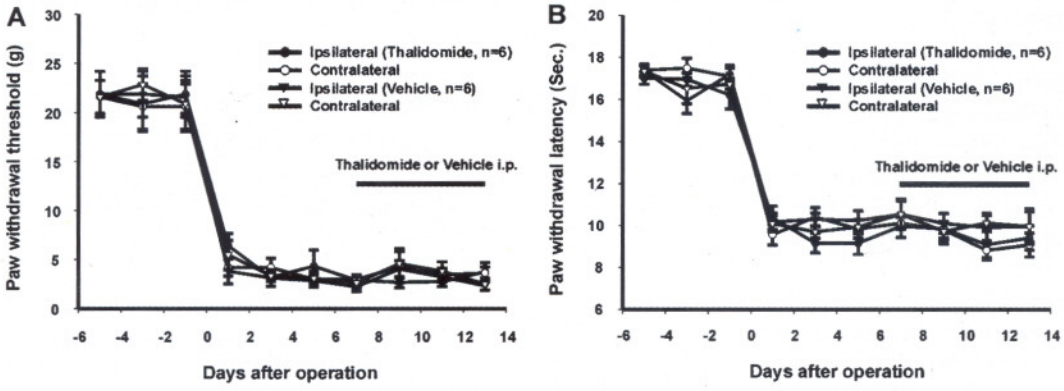

Figure 3. Effect of thalidomide on the established neuropathic pain induced by L5 VR

Compared with vehicle treated group, thalidomide applied at the 7th days after operation and once daily thereafter has no effect on the established mechanical allodynia (A) and thermal hyperalgesia (B).

4.2 The role of TNF-α in the development of neuropathic pain induced by L5 VR

Accumulating evidence shows that Wallerian degeneration contributes to the development of neuropathic pain after nerve injury. 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[8;16－18]. L5 VR created a selective injury to motor fiber but keeping sensory neuron intact and induced neuropathic pain. One possible explanation for the result is that Wallerian degeneration of the injured fibers in the peripheral nerve leads to changes in adjacent, uninjured primary afferents. Therefore, inhibited the synthesis of TNF-α, may be delayed the process of Wallerian degeneration of the injured motor fibers and prevented the development of neuropathic pain. Thalidomide reduces TNF-α production in macrophages by reducing TNF-α mRNA half-life[19]. Previous studies showed that thalidomide also exhibits a potent inhibition to the production of TNF-α in injured nerve[20,21]. In the present study, pre-treatment with thalidomide intraperitoneal injection significantly reduced abnormal pain behaviours following L5 VR. However, thalidomide treatment started at 7 days after L5 VR there is no any effect on the established neuropathic pain. It indicates that TNF-α plays an important role in the initiation of the neuropathic pain induced by the selective motor fibre injury, and therefore our data provides a therapeutic window for treatment the patient of neuropathic pain with the blocker of TNF-α in clinic.

Previous studies have demonstrated that acute injection of zymosan around the sciatic nerve produces bilateral mechanical allodynia[22] and that spinal glia and proinflammatory cytokines, including TNF-α, play 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 blockage of

the action of TNF-α[23,24]. Apparently, it is in agreement with our discovery in the present study. It has been shown 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 constraiut injury (CCI) model[25]. Accordingly, we speculate that the communications through gap junctions between ipsi- and contralateral spinal dorsal horn may contribute to L5 VR induced mirror image pain in contralateral hind paw.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (No. 30570599).

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Received October 5, 2006