

Pre-emptive Effect of Methylprednisolone on the Mechanical Allodynia Development after Peripheral Nerve Injuries in Rats

Department of Dental Anesthesiology and Dental Research Institute, Seoul National University College of Dentistry, Seoul, Korea

*Department of Anesthesiology, Seoul National University School of Medicine, Seoul, Korea, †Department of Neurology, Seoul Municipal Boramae Hospital and Seoul National University College of Medicine, Seoul, Korea

Min Young Lee, D.D.S., Tae Gyoon Yoon, M.D.*, Jung-Joon Sung, M.D.†,
Hyun Jeong Kim, M.D., and Kwang Won Yum, M.D.

Background: Glucocorticoids have anti-inflammatory effects and have been used to treat many types of nerve injury-associated chronic pain conditions. A randomized double-blind study was performed to determine if methylprednisolone could prevent the development of neuropathic pain after a peripheral nerve injury in rats.

Methods: Two groups of rats, one group (n = 50) injected intraperitoneally with methylprednisolone (100 mg/kg/day, for 7 days starting from 3 days prior to the nerve injury) and the other (n = 58) treated with saline with same manner, were compared in terms of the incidence and intensity of allodynia after a superior caudal trunk transection at the level between the 3rd and 4th sacral spinal nerves. The tail-flick responses to normally innocuous mechanical and thermal stimuli applied to the tail were observed as the behavioral signs of neuropathic pain.

Results: The proportions of rats exhibiting tail-flick responses to the mechanical (but not thermal) stimuli 7, 14 and 21 days after the nerve injury were significantly smaller in the methylprednisolone-treated group (2, 3 and 4 of 50 rats, respectively) than in the saline-treated, control group (11, 14 and 15 of 58 rats, respectively) (P = 0.009). However, the pain intensity was similar in mechanical allodynia developed rats of the two groups (P > 0.05), which was estimated based on the frequency and latency of the tail-flick responses after applying mechanical and thermal stimuli, respectively.

Conclusions: These results suggest that a pre-emptive treatment with high methylprednisolone doses may be used to prevent the development of mechanical allodynia following peripheral nerve injuries. (Korean J Anesthesiol 2004; 46: S 17~S 21)

Key Words: allodynia, axotomy, methylprednisolone, neuropathic pain, steroid.

INTRODUCTION

Neuropathic pain syndromes are caused by damage, disease, or a nervous system dysfunction, usually in the peripheral nervous system. Normally, neuropathic pain sufferers experience spontaneous burning pain in and radiating from the area innervated by the damaged nerves. In addition, they show an exquisite sensitivity to the light touch stimuli (allodynia), and hyperalgesia. The neuropathic pain is usually very difficult to

treat once it occurs,¹⁾ and the patient suffers from severe intractable pain. However, it is unclear why some individuals develop pain after losing their afferent input while others with the same degree of deafferentation do not.²⁾ Furthermore, there are no predictors to indicate which patients will develop neuropathic pain. So, its prevention is very important.

Glucocorticoids have anti-inflammatory effects and have been used to treat many types of nerve injury-associated symptoms and complex regional pain syndromes. Among steroids, methylprednisolone is a synthetic steroid with strong glucocorticoid activity. It is commonly used, along with other synthetic steroids, for its anti-inflammatory effects because it is distinguished by the absence of any significant salt-retaining activity.

To date, there are few reports concerning the pre-emptive effect on the development of neuropathic pain after nerve

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Corresponding to : Hyun Jeong Kim, Department of Dental Anesthesiology and Dental Research Institute, Seoul National University College of Dentistry, 28 Yeongseon-dong, Jongno-gu, Seoul 110-744, Korea. Tel: +82-2-760-3042, Fax: +82-2-766-9427, E-mail: dentane@plaza.snu.ac.kr

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injury.³⁻⁵⁾ However, there are no reports concerning the pre-emptive effects of glucocorticoids on neuropathic pain. Therefore, we performed a randomized double-blind study to determine whether methylprednisolone could prevent neuropathic pain after peripheral nerve injury in rats.

MATERIALS AND METHODS

The experiments were performed on male Sprague-Dawley rats (initial weight; 150–200 g). All the animals were cared for and handled in accordance with the guidelines specified in the NIH Guide for the Care and Use of Laboratory Animals (NIH publication No. 86–23, revised 1985) as well as the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals.⁶⁾ The animals were housed in a temperature-controlled room (20–24°C) in groups of four or five per cage. They were allowed access to water and food *ad libitum* and maintained under a 12/12 h light/dark cycle.

Behavioral tests

The animals were acclimatized for at least 3 days prior to the experiment. After acclimatization, the pre-injury mechanical and thermal allodynia behavioral tests were performed three times in 230 rats. Approximately half of them, which were then enrolled into this study, showed initially allodynia free behavior patterns. Rats showing tail-flick responses to the mechanical or thermal stimuli during three sets of pretests were excluded from this study.

The mechanical allodynia tests were performed using a von Frey hair (19.6 mN, i.e. 2.0 g). The most sensitive spot, which was first located by poking various areas of the tail systematically with the von Frey hair, was stimulated ten times at 5–10 s intervals, and the resulting tail-flick responses were counted to calculate the percent response frequency. For the cold and warm allodynia tests, the tail was immersed into cold (4°C) and warm (40°C) water, and the latency of the tail-flick response was measured with a cut-off time of 15 sec. The measurement was repeated five times at a 5-min interval.

Grouping and drug treatment

A total of 108 rats, which did not show even one chance of allodynia behavior in all three sets of pretests, underwent surgery. The animals were randomly allocated into two groups, the methylprednisolone (n = 50) and control groups (n = 58). The rats in the methylprednisolone group were treated with 100

mg/kg/day methylprednisolone (SOLU-MEDROL™ Sterile Power, Pharmacia & Upjohn Korea, Korea) intraperitoneally for 7 days (starting from 3 days before nerve injury), and the rats in the control group were treated with physiological saline with the same manner.

Surgical preparation

The nerve injury was inflicted as previously described.⁷⁾ Briefly, under enflurane anesthesia (0.5–2%), the superior caudal trunk was transected at the level between the S3 and S4 spinal nerves. The behavioral tests for the mechanical, cold and warm allodynia in the tail were conducted 1 day prior to and 1, 4, 7, 14 and 21 day (s) after the nerve injury. The investigator performing the behavior tests was blinded to the treatment and was instructed to classify the rat as mechanical (or thermal) allodynia-positive if it showed an obvious tail-flick response to the mechanical or thermal stimuli.

Statistics

The data is expressed as a mean \pm SD. A Chi-square test and Mann-Whitney U test were used to compare the differences of the allodynia development and pain intensity between the groups, respectively. The differences were considered to be statistically significant at the level of $\alpha = 0.05$.

RESULTS

The number of rats exhibiting behavioral signs of mechanical or thermal allodynia on post-injury day (s) 1–21 are given in Table 1. As shown in the table 1, no rats showed obvious signs of either mechanical or thermal allodynia on the first day after nerve injury. The mechanical allodynia signs began to appear from the fourth day, and the thermal allodynia signs began to appear from the seventh day. The proportions of rats with mechanical allodynia 7, 14 and 21 days after the nerve injury were significantly smaller in the methylprednisolone-treated group than in the saline-treated, control group ($P = 0.009$). Only 2, 3 and 4 out of 50 rats, respectively, were positive for the mechanical allodynia signs in the methylprednisolone-treated group, whereas 11, 14 and 15 out of 58 rats, respectively, were positive in the control group.

However, the pain intensity of rats showing tail-flick responses to the mechanical stimulus after the nerve injury in control and methylprednisolone group, was similar at 1, 4, 7, 14 and 21 day (s) after the nerve injury in the two groups (Fig.

Table 1. The Incidence of Neuropathic Pain in the Methylprednisolone Treatment Group and the Control Group after Peripheral Nerve Injury in Rats

Post-nerve injury day (s)	Control group (n = 58)					Methylprednisolone group (n = 50)				
	1	4	7	14	21	1	4	7	14	21
Mechanical allodynia (n)	0	7	11	14	15	0	3	2*	3*	4*
Thermal allodynia (n)	0	0	3	6	12	0	0	2	6	8
Both allodynia (n)	0	0	2	0	3	0	0	0	2	3
Total allodynia (n)	0	7	12	20	24	0	3	4*	7*	9*
Total allodynia (%)	0	12.1	20.7	34.5	41.4	0	6.0	8.0*	14.0*	18.0*

* $P < 0.05$ as compared to the control group value.

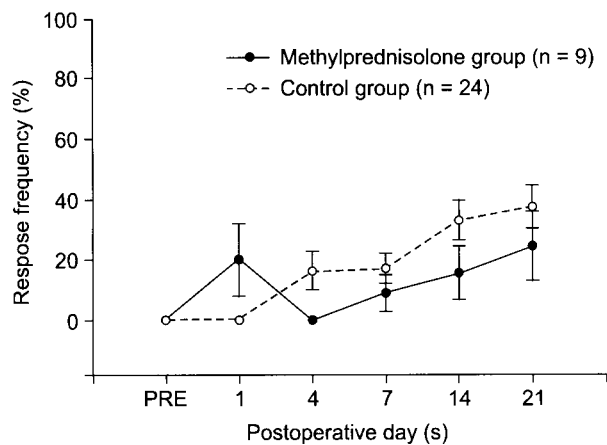


Fig. 1. After transection between the 3rd and 4th sacral nerve root, the mechanical allodynia intensities were measured over 21 days. From 3 days before nerve injury to 3 days after, all the initially allodynia-free rats were blindly injected intraperitoneally with normal saline (control group, $n = 58$) and 100 mg/kg/day methylprednisolone ($n = 50$). Considering the pain intensity of rats showing tail-flick responses to the mechanical stimulus at 21 days after the nerve injury in control ($n = 24$) and methylprednisolone group ($n = 9$), there were no significant pain intensity differences at any time points between groups ($P > 0.05$).

1, $P > 0.05$).

The proportion of rats with thermal allodynia (either cold or warm) between post-injury days 7, 14 and 21 were not significantly different between the methylprednisolone-treated and control groups (Table 1, $P > 0.05$).

DISCUSSION

This study demonstrated that systemic methylprednisolone treatment during the peraneural injury period decreased the

incidence of mechanical allodynia (not thermal allodynia) in a rat model of peripheral neuropathic pain. In addition, this study also showed that the methylprednisolone treatment did not alter the intensity of the mechanical allodynia, if the neuropathic pain occurred in the nerve-injured rats.

Behaviorally well-defined hyperalgesia and allodynia have been produced in a variety of neuropathic pain animal models,⁷⁻¹¹ which are induced by partial denervation. These animal models are extremely useful for investigating the pain mechanisms and for developing new treatment targets. The advantages of Na's neuropathic model used in this study⁷ are that the accurate nerve injury is possible and behavioral tests are easy, comfortable and definite, and so, it is suitable to apply for large amount of rats.

Both peripheral and central mechanisms have been implicated in the pathogenesis of neuropathic pain.¹² The important peripheral mechanism is hyperexcitability and an ectopic action potential discharge in the axon and cell body of the injured sensory neurons. Glutamate is related to central sensitization, which plays an important role in the development of neuropathic pain.^{13,14}

Like neuropathic pain, inflammatory pain is associated with a local cutaneous hypersensitivity in the form of hyperalgesia and allodynia. Although the exact mechanisms of neuropathic and inflammatory pain are unknown, there is some evidence showing that the two types of pain conditions involve similar pathophysiological mechanisms. After the peripheral nerve injury, infiltrating inflammatory cells and their secretory products, cytokines and arachidonic acid metabolites, participate in the nerve regeneration process. Therefore, it has been suggested that an inflammatory reaction is partly responsible for the generation and maintenance of the chronic ongoing neuropathic

pain.¹⁵⁾

The mechanisms by which steroids modify pain include a reduction in neurogenic extravasation,¹⁶⁾ reduced ectopic discharges from the neuromas,¹⁷⁾ and rapid and massive invasion of the site by macrophages,¹⁸⁾ which is known as a glutamate releaser.¹⁹⁾ It should be noted that methylprednisolone enhances the long-term recovery from spinal cord injury in human patients when administered in a 24-hr high-dose regimen beginning within 8 h after the injury.²⁰⁾ This article suggests that methylprednisolone has potential clinical applications in the nerve-injured patients to prevent the development of neuropathic pain.

Pre-emptive analgesia and the administration of drugs prior to the painful stimuli aim to reduce both the postoperative pain and the subsequent development of chronic pain after the tissue injury. This is believed to occur because the medications prevent or blunt the establishment of the spinal facilitation evoked by the nociceptive input to spinal cord.²¹⁾

Systemic methylprednisolone treatment, which began immediately after the bilateral sciatic and saphenous nerve transection in rats, led to a dose-dependent reduction in the autotomy behavior and neurogenic extravasation mediated by substance P.¹⁶⁾ Recently, Kingery et al.²²⁾ also reported the effects of continuously infused methylprednisone, which was begun after the development of neuropathic hyperalgesia, reversed both the thermal and mechanical hyperalgesia over 2 weeks in sciatic nerve-transected rats.

However, the pre-emptive methylprednisolone treatment had no effect on the development of thermal allodynia in this study. The causes of the differences between our results and those reported by Kingery et al.²²⁾ might be due to the use of another type of neuropathic pain animal model. In addition, in this study, a pain behavior test was performed three times prior to surgery in order to exclude the rats, which showed the initial pain behavior.

There are some reports on the preventive effects on neuropathic pain. Yamamoto et al.³⁾ investigated the pre-emptive effects of intrathecally-administered nociceptin on the development of thermal hyperalgesia using two neuropathic pain models involving sciatic nerve injury. Nociceptin significantly delayed the development of thermal hyperalgesia in the chronic constriction injury model, but not in the partial sciatic nerve injury model.

In summary, this study suggests that high-dose methylprednisolone has a preventive effect against the development

of mechanical allodynia, but not thermal allodynia, this anti-inflammatory steroid is also ineffective in reducing the intensity of neuropathic pain.

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