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Short communication

Effect of cyclooxygenase and nitric oxide synthase inhibitors on vincristine induced hyperalgesia in rats

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Abstract:
The purpose of this study was to investigate the effect of cyclooxygenase (COX) inhibitors and nitric oxide synthase (NOS) inhibitors on the development of vincristine (VIN)-induced hyperalgesia. Indomethacin (IND) and celecoxib (CEX) were used as relatively selective inhibitors of COX-1 and COX-2, respectively. NOS inhibitors included the nonspecific inhibitor N^G-nitro-L-arginine (L-NOArg) and L-N6-(1-iminoethyl)lysine (L-NIL), which preferentially acts on inducible NOS, as well as 7-nitroindazole (7-NI), which is a relatively specific neuronal NO synthase inhibitor. Both IND and CEX markedly suppressed hyperalgesia, whereas all three NOS inhibitors prevented the development of hyperalgesia due to VIN administration. The results of this study suggest participation of COX-1 and COX-2 as well as iNOS and nNOS in the transmission of pain stimuli in VIN-induced hyperalgesia.

Key words:
hyperalgesia, vincristine, indomethacin, celecoxib, cyclooxygenase, nitric oxide inhibitors, rats

Abbreviations: CEX – celecoxib, COX-1 – cyclooxygenase-1, COX-2 –cyclooxygenase-2, IND – indomethacin, iNOS – inducible nitric oxide synthase, L-NIL – L-N6-(1-iminoethyl)lysine, L-NOArg – N^G-nitro-L-arginine, nNOS – neuronal nitric oxide synthase, NO – nitric oxide, 7-NI – 7-nitroindazole, VIN – vincristine

Introduction

Neuropathic pain during anticancer therapy constitutes a difficult clinical problem [1]. The mechanisms underlying the development of neuropathic pain are poorly understood [30, 31].

As previously reported, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), as well as inducible nitric oxide synthase (iNOS), but not neuronal nitric oxide synthase (nNOS), participate in streptozotocin-induced hyperalgesia [3]. The experimental model of neuropathic pain caused by administration of vincristine (VIN) (known as the VIN-induced neuropathy/hyperalgesia model) is commonly used to investigate pain in a toxic neuropathy animal model.

Therefore, it was of interest to investigate the possible involvement of both cyclooxygenases and NO synthases in hyperalgesia produced by VIN.

Materials and Methods

Laboratory animals

The experiments were performed on male Wistar rats (240–290 g) according to the guidelines of the Ethical Committee for Experiments on Small Animals, Medical University of Warsaw.

Chemotherapy (VIN)-induced painful neuropathy

VIN neuropathy was induced as described by Aley et al. [2]. VIN sulphate was dissolved in distilled water to a stock concentration of 1 mg/ml and then stored at 4°C. Immediately before administration, the stock was diluted in distilled water to a concentration of 100 μg/ml. This solution was administered into the tail vein at a dose of 100 μg/kg. Administrations of VIN were performed daily Monday through Friday for 10 days (this phase of the experiment lasted 12 days; no drug doses were given on Saturdays or Sundays). The dosage calculations were based on daily body weight. Weight-matched control rats received injections of distilled water [2]. No weight gain was observed in rats receiving intravenous VIN at a dose of 100 μg/kg.

Chemicals

N^G-nitro-L-arginine (L-NOArg) and 7-nitroindazole (7-NI) were purchased from Research Biochemicals International; L-N6-(1-iminoethyl) lysine (L-NIL) was purchased from Bachem Switzerland. Indomethacin (IND) was purchased from Polfa Kutno, Poland; celecoxib (COX) was purchased from Searle, and vincristine sulfate was purchased from Sigma Chemical Co., USA.

Drug administration

Preparation of drugs

VIN was administered as described above. CEX, IND, L-NOArg and 7-NI were suspended in a 0.1% solution of methylcellulose immediately before injection. L-NIL was dissolved in 0.9% saline.

Application of drugs

CEX and IND were administered subcutaneously (*sc*) in a dose of 1 mg/kg. L-NOArg and L-NIL were applied intraperitoneally (*ip*) in a dose of 10 mg/kg, and 7-NI was applied *ip* in a dose of 1 mg/kg.

Time schedule

IND and CEX were given every day 30 min before VIN for 10 days (2 × 5 – see above). L-NOArg, L-NIL and 7-NI were administered every day 10 min before VIN for 10 days (2 × 5 – see above).

Controls

Control animals were injected according to the same time schedule: (1) *ip* with 0.1% solution of methylcellulose (control to L-NOArg and 7-NI); (2) *ip* with 0.9% saline (control to L-NIL), or (3) *sc* with 0.1% solution of methylcellulose (control to IND and CEX).

Measurement of the nociceptive threshold

Measurements of nociceptive thresholds were performed as reported elsewhere [4]. The changes in nociceptive thresholds were determined using mechanical stimuli (the modification of the classic paw withdrawal test described by Randall and Selitto) [24]. In order to perform a mechanical stimulation, progressively increasing pressure was applied to the dorsal surface of the rat's paw using an analgesimeter. The instrument increased the force on the paw at a rate of 32 g per second. The nociceptive threshold was defined as the force, in grams, at which the rat attempted to withdraw its right hind paw. The values of that pressure were recorded instantaneously. Three threshold measurements were performed per rat every day, and the mean was used for further calculations.

The mean of nociceptive thresholds to mechanical stimuli measured on the first day of the 28-day study immediately before administration of VIN alone or VIN with investigated drugs constituted the baseline pain threshold (A). Consecutive measurements of nociceptive thresholds to mechanical stimuli (B) were conducted every day before administration of the investigated compounds (from experimental day 2 to day 5 and from day 8 until day 12) and then after drug discontinuation (from day 14 until 28 of the experiment). In all experimental sessions until the end of the

study, the values of the thresholds obtained (B) were compared to the baseline (A) defined above.

Changes in pain threshold were calculated as percent of baseline value according to the following formula:

$$\% \text{ of hyperalgesia} = (\frac{B}{A} \cdot 100\%) - 100\%$$

where A represents baseline pressure (in g) measurement on the first day before drug administration (as mentioned above), and B represents pressure (in g) measurement performed daily (excluding day 1) before drug administration.

Percent of hyperalgesia values calculated as above for individual animals were subsequently used to calculate average values in particular experimental groups and for statistical analyses.

Statistical analysis

The results are expressed as the mean values ± standard error of the mean (± SEM). The statistical significance of differences between groups was evaluated by the Student *t*-test and the Newman-Keuls multiple-range test. $p \leq 0.05$ was accepted as statistically significant. Calculations were performed using the computer software described by Tallarida and Murray [29].

Results

Effect of VIN on nociceptive thresholds to mechanical stimuli

Starting from day 2, a statistically significant gradual decrease of the nociceptive threshold was observed in VIN-treated animals. The decrease reached its peak on experimental day 10 and remained approximately stable until day 18. After discontinuation of VIN administration at day 20, nociceptive thresholds to mechanical stimuli gradually increased and, on day 28, returned to baseline values (Fig. 1).

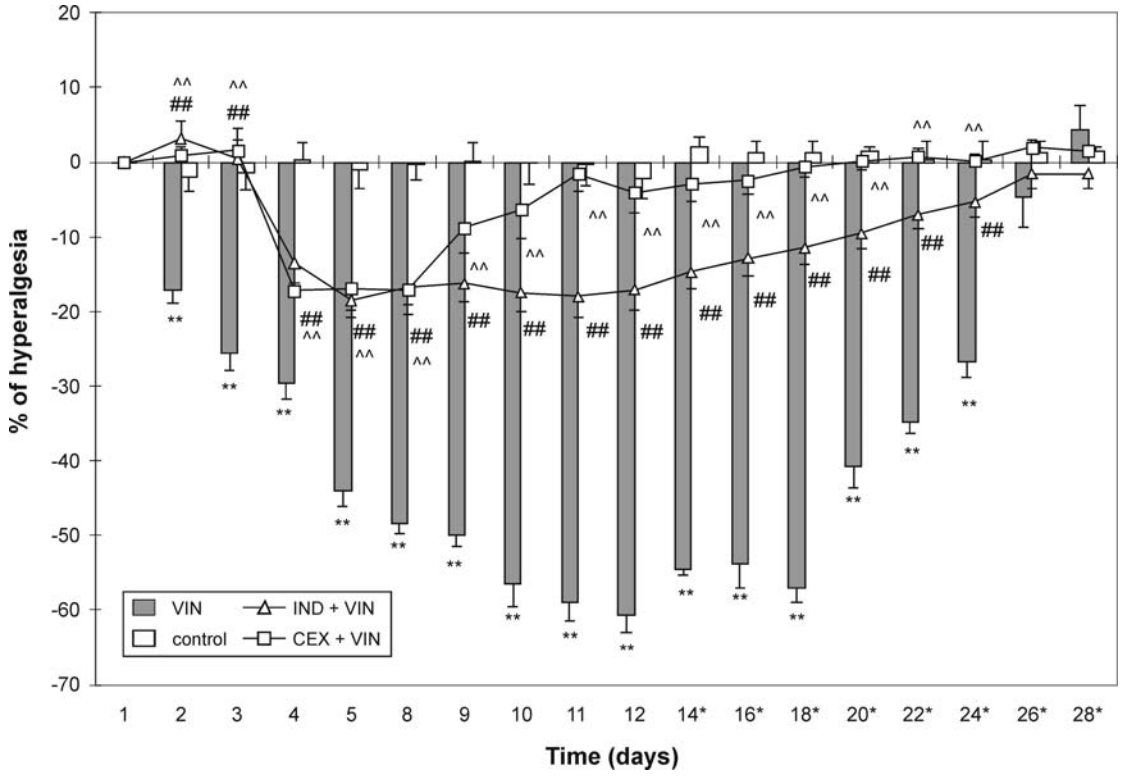

Fig. 1. Effect of indomethacin (IND) at a dose of 1 mg/kg *sc* or celecoxib (CEX) at a dose of 1 mg/kg *sc* on vincristine (VIN) hyperalgesia. Days 1–12 – measurements of prolonged activity of the investigated drugs. Days 14*–28* – after discontinuation of administration. Values are the means ± SEM. VIN *vs.* control ** $p \leq 0.01$; IND + VIN *vs.* VIN ## $p \leq 0.01$; CEX + VIN *vs.* VIN ^^ $p \leq 0.01$

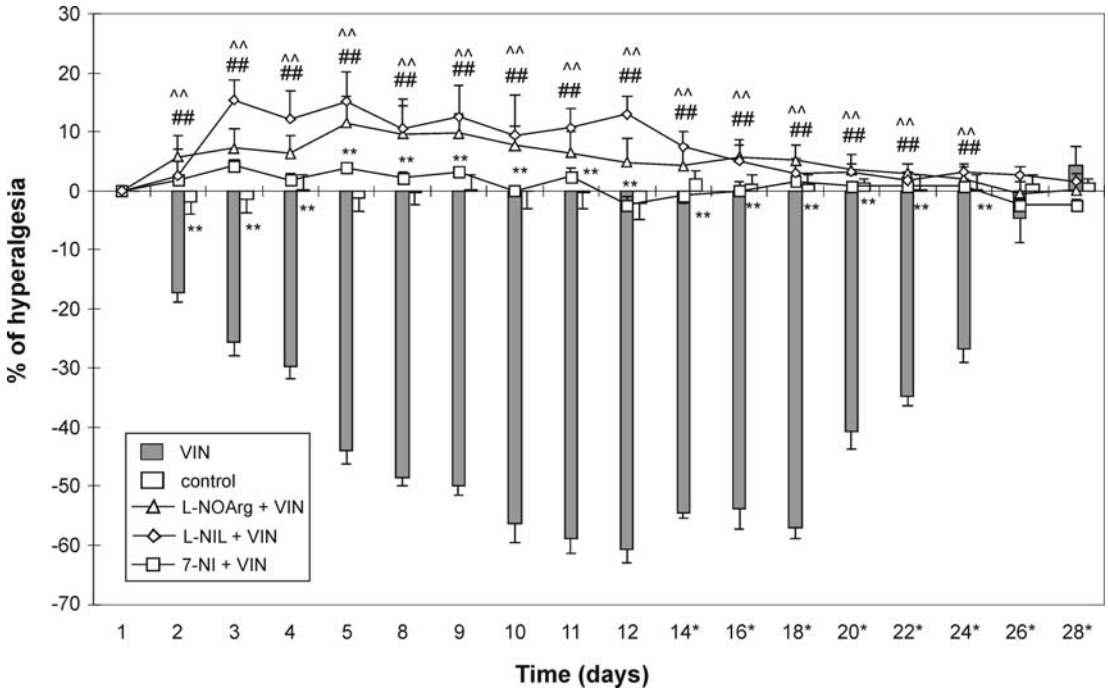

Fig. 2. Effect of N^G-nitro-L-arginine (L-NOArg) at a dose of 10 mg/kg *ip*, L-N(6)-(1-iminoethyl) lysine (L-NIL) at a dose of 10 mg/kg *ip* or 7-nitroindazole (7-NI) at a dose of 1 mg/kg *ip* on vincristine (VIN) hyperalgesia. Values are the means ± SEM. L-NOArg + VIN *vs.* VIN ^^ $p \leq 0.01$; L-NIL + VIN vs. VIN ## $p \leq 0.01$; 7-NI + VIN *vs.* VIN** $p \leq 0.01$

Effect of COX and NOS inhibitors on the development of VIN-induced hyperalgesia

Both IND (1 mg/kg *sc*) as well as CEX (1 mg/kg *sc*) significantly attenuated hyperalgesia produced by VIN administration. This effect occurred on day 4 and was maintained following cessation of drugs. It is interesting to note that, after CEX administration, hyperalgesia was completely abolished starting on day 11 of the experiment (Fig. 1).

L-NOArg and L-NIL (10 mg/kg *ip*) not only prevented VIN-hyperalgesia but also produced weak antinociception, whereas 7-NI completely abolished VIN-hyperalgesia. This effect was prolonged and maintained after a cessation of drug administration (Fig. 2).

Discussion

Vincristine, a vinca alkaloid, is a chemotherapeutic agent used in anti-cancer therapy. In the present study, after administration of VIN, a significant gradual decrease of the nociceptive threshold was observed. Hyperalgesia reached its maximum on day 10 and persisted at stable levels during the remaining 8 days. After withdrawal of VIN, nociceptive thresholds slowly increased and, on day 20 of the experiment, returned to baseline values.

The results of this study are similar to those reported by Aley and co-workers [2], who demonstrated the appearance of hyperalgesia in response to mechanical stimulus after administration of VIN doses of 100 and 200 μg/kg. No significant differences in intensity of hyperalgetic effects between doses were observed in this study. However, unlike animals receiving VIN at a dose of 100 μg/kg, rats given 200 μg/kg lost, on average, 12.5% of their body weight during the experiment. However, the weight was regained when the drug was stopped. In our study, VIN administered intravenously at 100 μg/kg dose caused cessation of physiological weight gain in rats. After vincristine withdrawal, the animals gradually regained weight.

Studies performed in recent years have emphasized the important contribution of inflammatory processes

in the development of neuropathic pain. Infiltration of inflammatory cells in response to nervous system damage leads to subsequent production and secretion of various cytokines, growth factors, or inflammatory mediators such as bradykinin, serotonin, prostaglandins (PGs), or nitric oxide (NO) [20]. Evidence of the involvement of PGs and NO in mechanisms underlying the development of neuropathic pain comes mainly from studies using animal models of peripheral nerve injury, such as chronic constrictive injury to the sciatic nerve [7, 18, 21], partial sciatic nerve ligation [18, 19, 27, 35], and L5/L6 spinal nerve ligation [10, 18, 23, 34, 35]. It was shown that, in neuropathic pain, PGs, synthesized by both COX-1 and COX-2, may play a role in the sensitization of nociceptors [18, 19, 26, 27] and in the sensitization of neurons in the central nervous system [10, 17, 28, 33]. Cata et al. [5] showed that ibuprofen (50.0 mg/kg *ip*) and rofecoxib (10.0 mg/kg *ip*) prevented mechanical hyperalgesia in a toxic neuropathy model. In the present study, both IND and CEX significantly reduced vincristine-induced hyperalgesia. After administration of lower doses of investigated drugs (0.1 mg/kg), antihyperalgesic action was weakly emphasized (data not shown). Moreover, the effect of CEX was somewhat more marked than the effect of IND.

It is commonly accepted that NO formed by nNOS, iNOS and eNOS may mediate some of the neuropathic pain syndromes following peripheral nerve injures [9, 15, 21, 34].

In the current study, a non-selective inhibitor of NOS, L-NOArg, and a selective inhibitor of iNOS, L-NIL, as well as the selective inhibitor of nNOS, 7-NI, completely prevented the VIN-hyperalgesia. Administration at a dose 10 times lower than the investigated drugs also ameliorated the development of VIN-induced hyperalgesia; however, the effects were less pronounced (data not shown). It was observed that, in neuropathic pain, NO was involved in the nociceptive processing both at the level of the peripheral [13–15] and at the central nervous system [8, 11, 16, 32].

There are not many studies linking NOS activity and VIN-induced hyperalgesia. The results of these studies are controversial. Aley and Levine [1] showed that a NOS inhibitor (L-NMA) administered intradermally into the dorsum of a rat hind paw significantly increased the paw withdrawal threshold in a VIN neuropathy model. The authors suggested that NO's peripheral action contributes markedly to VIN hyperalgesia. On the other hand, Kamei et al. [12] found that *sc* pretreatment with a substrate of NOS, L-arginine (30–300 mg/kg) or a cGMP analog (0.3– –3.0 nmol intrathecal) dose-dependently increased the tail-flick latencies in VIN-treated mice. This action was reversed by intrathecal (*it*) pretreatment with a nonselective inhibitor NO synthase (L-NAME 3–30 nmol). Furthermore, the protein level of nNOS, but not iNOS, in the spinal cord of VIN-treated mice decreased compared to that of naive mice. Authors suggested that dysfunction of the L-arginine/NO/cGMP cascade in the spinal cord may trigger VIN-induced thermal hyperalgesia.

A majority of studies suggest the involvement of NOS pathways in the transmission of nociceptive stimuli with a pronociceptive role of NO in neuropathy [22]. However, the results of Kamei et al. [12] are not surprising because a dual role of NO in nociception has been demonstrated [6, 25]. Niedbala et al. [22] showed that, in contrast to the results of Kamei et al. [12], L-arginine administered *ip* (5 μmol) along with a stable analog of cyclic guanosine monophosphate, 8-Br-cGMP, significantly increased nociceptive stimuli responses, but L-NAME *ip* (100–500 nmol) significantly decreased autonomous behavior in the sciatic and saphenous nerves transected rats. Sousa and Prado [25] showed that low doses of donor NO (SIN-1; 0.1–2.0 μg/10 μl), administered *it*, reduced responses, while higher doses (5 or 100 μg/10 μl) had no effect or increased (10 or 20 μg/10 μl) the mechanical allodynia induced by chronic ligature of the sciatic nerve in rats. Moreover, SIN-1 (0.1–100 μg/10 μl, *it*) produced only antinociceptive effects in the rat tail flick test. The NOS inhibitors (L-NOArg and L-NMMA) reduced the mechanical allodynia evoked by nerve injury and increased the tail flick latency. Choi et al. [6] observed an increase of NOS activity in the ipsilateral L5 and L6 dorsal root ganglions 2 weeks following ligation of the L5 and L6 nerve roots. On the other hand, NOS activity decreased bilaterally in the lumbar spinal cord. The same authors suggested that, a short time after nerve injury, NO production is increased and that increased NO level inhibits NOS *via* a negative feedback mechanism. The above-mentioned data indicate that the dual effect of NO in nociception may depend on the time and side of action, the amount of NO, or a kind of used nociceptive stimuli (i.e., mechanical or thermal).

It is concluded that both COX-1 and COX-2, as well as iNOS and nNOS, are involved in the develop-

ment of VIN neuropathy. These findings allow for differentiation between VIN-produced hyperalgesia and diabetic hyperalgesia due to streptozotocin administration since, in the latter, nNOS is not involved.

References:

1. Aley KO, Levine JD: Different peripheral mechanisms mediate enhanced nociception in metabolic/toxic and traumatic painful peripheral neuropathies in the rat. Neuroscience, 2002, 111, 389–397.
2. Aley KO, Reichling DB, Levine JD: Vincristine hyperalgesia in the rat: a model of painful vincristine neuropathy in humans. Neuroscience, 1996, 73, 259–265.
3. Bujalska M, Tatarkiewicz J, deCordé A, Gumułka SW: Effect of cyclooxygenase and nitric oxide synthase inhibitors on streptozotocin-induced hyperalgesia in rats. Pharmacology, 2008, 81, 151–157.
4. Bujalska M, Tatarkiewicz J, Gumułka SW: Effect of bradykinin receptors antagonists on vincristine and streptozotocin induced hyperalgesia in rat model of chemotherapy-induced and diabetic neuropathy. Pharmacology, 2008, 81, 158–163.
5. Cata JP, Weng HR, Dougherty PM: Cyclooxygenase inhibitors and thalidomide ameliorate vincristine-induced hyperalgesia in rats. Cancer Chemother Pharmacol, 2004, 54, 391–397.
6. Choi Y, Raja SN, Moore LC, Tobin JR: Neuropathic pain in rats is associated with altered nitric oxide synthase activity in neural tissue. J Neurol Sci, 1996, 138, 14–20.
7. Durrenberger PF, Facer P, Casula MA, Yiangou Y, Gray RA, Chessell IP, Day NC et al.: Prostanoid receptor EP1 and Cox-2 in injured human nerves and a rat model of nerve injury: a time-course study. BMC Neurol, 2006, 6, 1–11.
8. Guan Y, Yaster M, Raja SN, Tao YX: Genetic knockout and pharmacologic inhibition of neuronal nitric oxide synthase attenuate nerve injury-induced mechanical hypersensitivity in mice. Mol Pain, 2007, 3, 1–10.
9. Hao JX, Xu XJ: Treatment of a chronic allodynia-like response in spinally injured rats: effects of systemically administered nitric oxide synthase inhibitors. Pain, 1996, 66, 313–319.
10. Hefferan MP, O'Rielly DD, Loomis CW: Inhibition of spinal prostaglandin synthesis early after L5/L6 nerve ligation prevents the development of prostaglandin-dependent and prostaglandin-independent allodynia in the rat. Anesthesiology, 2003, 99, 1180–1188.
11. Inoue T, Mashimo T, Shibata M, Shibuta S, Yoshiya I: Rapid development of nitric oxide-induced hyperalgesia depends on an alternate to the cGMP-mediated pathway in the rat neuropathic pain model. Brain Res, 1998, 792, 263–270.
12. Kamei J, Tamura N, Saitoh A: Possible involvement of the spinal nitric oxide/cGMP pathway in vincristine-induced painful neuropathy in mice. Pain, 2005, 117, 112–120.
13. Levy D, Höke A, Zochodne DW: Local expression of inducible nitric oxide synthase in an animal model of neuropathic pain. Neurosci Lett, 1999, 260, 207–209.
14. Levy D, Kubes P, Zochodne DW: Delayed peripheral nerve degeneration, regeneration, and pain in mice lacking inducible nitric oxide synthase. Neuropathol Exp Neurol, 2001, 60, 411–421.
15. Levy D, Zochodne DW: Local nitric oxide synthase activity in a model of neuropathic pain. Eur J Neurosci, 1998, 10, 1846–1855.
16. Lui PW, Lee CH: Preemptive effects of intrathecal cyclooxygenase inhibitor or nitric oxide synthase inhibitor on thermal hypersensitivity following peripheral nerve injury. Life Sci, 2004, 75, 2527–2538.
17. Ma W, Du W, Eisenach JC: Role for both spinal cord COX-1 and COX-2 in maintenance of mechanical hypersensitivity following peripheral nerve injury. Brain Res, 2002, 937, 94–99.
18. Ma W, Eisenach JC: Cyclooxygenase 2 in infiltrating inflammatory cells in injured nerve is universally upregulated following various types of peripheral nerve injury. Neuroscience, 2003, 121, 691–704.
19. Ma W, Eisenach JC: Morphological and pharmacological evidence for the role of peripheral prostaglandins in the pathogenesis of neuropathic pain. Eur J Neurosci, 2002, 15, 1037–1047.
20. Moalem G, Tracey DJ: Immune and inflammatory mechanisms in neuropathic pain. Brain Res Rev, 2006, 51, 240–264.
21. Naik AK, Tandan SK, Kumar D, Dudhgaonkar SP: Nitric oxide and its modulators in chronic constriction injury-induced neuropathic pain in rats. Eur J Pharmacol, 2006, 530, 59–69.
22. Niedbala B, Sánchez A, Feria M: Nitric oxide mediates neuropathic pain behavior in peripherally denervated rats. Neurosci Lett, 1995, 188, 57–60.
23. O'Rielly DD, Loomis W: Increased expression of cyclooxygenase and nitric oxide isoforms, and exaggerated sensitivity to prostaglandin E_2 in the rat lumbar spinal cord 3 days after L5-L6 spinal nerve ligation. Anesthesiology, 2006, 104, 328–337.
24. Randall LO, Selitto JJ: A method for measurement of analgesic activity on inflamed tissue. Arch Int Pharmacodyn Ther, 1957, 111, 409–419.
25. Sousa AM, Prado WA: The dual effect of a nitric oxide donor in nociception. Brain Res, 2001, 897, 9–19.
26. Sűleyman H, Dermican B, Karagöz Y: Anti-inflammatory and side effects of cyclooxygenase inhibitors. Pharmacol Rep, 2007, 59, 247–258.
27. Syriatowicz JP, Hu D, Walker JS, Tracey DJ: Hyperalgesia due to nerve injury: role of prostaglandins. Neuroscience, 1999, 94, 587–594.
28. Takahashi M, Kawaguchi M, Shimada K, Konishi N, Furuya H, Nakashima T: Cyclooxygenase-2 expression in Schwann cells and macrophages in the sciatic nerve after single spinal nerve injury in rats. Neurosci Lett, 2004, 363, 203–206.
29. Tallarida RJ, Murray RB: Manual of Pharmacologic Calculation with Computer Programs, 2nd edn., New York, Springer, 1986, 113–117.

30. Tanner KD, Levine JD, Topp KS: Microtubule disorientation and axonal swelling in unmyelinated sensory axons during vincristine-induced painful neuropathy in rat. J Comp Neurol, 1998, 395, 481–492.
31. Topp KS, Tanner KD, Levine JD: Damage to the cytoskeleton of large diameter sensory neurons and myelinated axons in vincristine-induced painful peripheral neuropathy in the rat. J Comp Neurol, 2000, 424, 563–576.
32. Verge VM, Xu Z, Xu XJ, Wiesenfeld-Hallin Z, Hökfelt T: Marked increase in nitric oxide synthase mRNA in rat dorsal root ganglia after peripheral axotomy: in situ hybridization and functional studies. Proc Natl Acad Sci U S A, 1992, 89, 11617–11621.
33. Willingale HL, Gardiner NJ, McLymont N, Giblett S, Grubb BD: Prostanoids synthesized by cyclo-oxygenase isoforms in rat spinal cord and their contribution to the development of neuronal hyperexcitability. Br J Pharmacol, 1997, 122, 1593–1604.
34. Yoon YW, Sung B, Chung JM: Nitric oxide mediates behavioral signs of neuropathic pain in an experimental rat model. Neuroreport, 1998, 9, 367–372.
35. Zhu X, Eisenach JC: Cyclooxygenase-1 in the spinal cord is altered after peripheral nerve injury. Anesthesiology, 2003, 99, 1175–1179.

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