The Antiallodynic Effect of Nefopam on Vincristine-Induced Neuropathy in Mice

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Background: Chemotherapy-induced neuropathic pain is a disabling condition following cancer treatment. Vincristine has more neurotoxicity than other vinca alkaloid agents. This study evaluated the correlation of different doses of nefopam with antiallodynic effects in a mouse vincristine neuropathy model.

Methods: A peripheral neuropathic mouse model was made by intraperitoneal injection of vincristine (0.1 mg/kg/day; 5-day-on, 2-day-off schedule over 12 days). After the development of allodynia, mice were injected intraperitoneally with 0.9% normal saline (NS group) or various doses (10, 30, 60 mg/kg) of nefopam (Nefopam group). We examined allodynia using von Frey hairs pre-administration and at 30, 60, 90, 120, 180, 240 mins, and 24 hrs after drug administration. We also measured the neurokinin-1 receptor concentrations in the spinal cord to confirm the antiallodynic effect of nefopam after drug administration.

Results: The peripheral neuropathic mouse model showed prominent mechanical allodynia. Intraperitoneal nefopam produced a clear dose-dependent increase in paw withdrawal threshold compared with pre-administration values and versus the NS group. The concentration of neurokinin-1 receptor was significantly decreased in the Nefopam group (P<0.05).

Conclusion: Intraperitoneally administered nefopam yielded a dose-dependent attenuation of mechanical allodynia and decreased neurokinin-1 receptor concentration, suggesting that the neurokinin-1 receptor is involved in the antiallodynic effects of nefopam in vincristine neuropathy.

Keywords: allodynia, mice, nefopam, neurokinin-1, neuropathy, vincristine

Introduction
Chemotherapy-induced peripheral neuropathy (CIPN) is a disabling condition following cancer chemotherapy, characterised by sensory (hyperalgesia, paresthesia, dysesthesia, allodynia, spontaneous pain), motor (weakness, incoordination), and autonomic (altered thermoregulation, blood pressure, intestinal motility) symptoms. 1,2 Although the pathophysiology of CIPN is not clear, it probably results from loss of sensory terminals in the skin; alterations of membrane receptors; and changes in intracellular signaling, neurotransmission, excitability, and cellular metabolism. 3 A reduction in Meissner’s corpuscles and loss of epidermal nerve fibers are other possible mechanisms of CIPN. 4 The development of CIPN may lead to a reduction in dose or discontinuation of chemotherapy, which can result in increased cancer morbidity and/or mortality. 1,3 Because of these multiple mechanisms for the development of CIPN, so far, no agent has been reported to be successful in controlling CIPN optimally. Vincristine is one of the most commonly used chemotherapeutic agents for blood cancer and solid tumors. 5,6 Vincristine has
been known to induce microtubule destabilisation and deficits in axonal transport, and thus, has more neurotoxicity than other vinca alkaloid agents.\textsuperscript{7,8} Nefopam has drawn interest for its antinociceptive and antihyperalgesic effects; it acts by influencing monoamines, and the noradrenergic and/or serotonergic system, resulting in the prevention and reduction of central neural sensitisation.\textsuperscript{9–11} In a study with spinal nerve ligation models, intrathecal nefopam produced an antiallodynic effect by inhibition of microglial and astrocytic activation, which are reportedly involved in the release of nociceptive mediators and facilitation of the pain process pathway.\textsuperscript{12} However, the effect of nefopam on allodynia in vincristine neuropathy and the precise mechanism involved are not well understood. Gautam et al described that the neurokinin-1 (NK1) receptor is functionally associated with mechanosensitive nociceptors and neurotransmission at the spinal level.\textsuperscript{13} They reported that intrathecal administration of NK1 antagonist resulted in an antiallodynic effect in the post-incisional pain model.\textsuperscript{13} The NK1 receptor antagonist inhibits excitatory postsynaptic potential in the dorsal horn neurons, resulting in attenuation of spinothalamic neuron activation and prevention of nociceptive sensitisation.\textsuperscript{13,14} Therefore, we evaluated the correlation of different doses of nefopam with the antiallodynic effect in the mouse vincristine neuropathy model. In addition, the concentration of NK1 receptor in the spinal cord was assayed.

**Materials and Methods**

This study was reviewed and approved by the Animal Care and Use Committee of Seoul St. Mary’s hospital of the Catholic University of Korea. The animals were treated in accordance with the National Institute of Health and the International Association for the Study of Pain policies.

**Experimental Animals**

Adult male mice (age, 10 weeks old; weight, 25–30 g) were used in this study. All animal experiments were performed in a semi-pathogen-free barrier zone at the Catholic Laboratory Animal Research Center. The mice were housed 2 or 3 per cage for 7 days in humidity and temperature-controlled (21–23°C) environment, with a 12 hr light/dark cycle (7:00 am hour onset) and free access to food and water at all times. Behavioural testing and analgesiometry were performed according to the ethical guidelines, and the mice were euthanised after completion of the planned tests.

**Vincristine Injection**

The vincristine-induced peripheral neuropathy model was used in this experiment. Baseline response to mechanical stimulation of the hind paw was established on day 1. Either vincristine sulfate (Sigma Aldrich Co., St. Louis, MO, USA) or saline was administered by injection to create the neuropathy model, as described previously.\textsuperscript{15} In brief, 0.1 mg/kg/day vincristine was administered intraperitoneally for 5 days. Following cessation for 2 days, injection was continued for the next 5 days. On day 15, the paw withdrawal threshold (PWT) was measured with von Frey filaments (Semmes-Weinstein monofilaments, Stoelting Co., Wood Dale, IL, USA). If a foot withdrawal response occurred when a filament less than 0.6 g was applied to the hind paw, it was considered that mechanical allodynia had developed.

**Drug Administration**

Mice that showed mechanical allodynia were randomly divided into four groups before drug administration. The control group received distilled water 1 mL/kg (n=6). There were 3 experimental groups, namely, nefopam10, 30, and 60 (n=6, in each group), who were administered 10, 30, or 60 mg/kg nefopam (Acupan®, Pharmbio Korea, Seoul, Korea), respectively, intraperitoneally.

**Behavioural Tests**

All behavioural tests were conducted at fixed times (1:00–6:00 pm) to avoid circadian rhythm errors by the same examiner who was blinded to drug administration and dosing. After intraperitoneal drug administration, mice were placed on a metal mesh covered with a plastic dome (8 - 8 - 8 cm) for the assessment of mechanical allodynia. The PWT in response to mechanical stimulation was measured using the up-and-down method,\textsuperscript{16} applying a series of von Frey filaments ranging from 0.03 to 2.00 g. Brisk withdrawal and paw flinching were considered positive responses. If the mice showed a positive response, the next-less-stiff filament was used for the next trial, or the next stiffer one in cases where there was no withdrawal or licking. Mice that did not show allodynia (PWT less than 0.6 g) were excluded from the study. PWT was assessed before drug administration, and at 30, 60, 120, 180, 240 mins, and 24 hrs after drug administration.

**Immunohistochemistry**

We measured the NK1 receptor in the spinal cord to confirm the antiallodynic effect of nefopam. All the mice
in each of the groups were sacrificed, and the spinal cords (lumbar 4–6) were collected 30 min after nefopam and vehicle injection (n=6, in each group), according to the mechanical allodynia results. Mice injected with saline and mice treated with 60 mg/kg nefopam were anaesthetised and perfused transcardially with 50 mL of 4% paraformaldehyde dissolved in 0.01 M phosphate-buffered saline (PBS) with pH 7.2–7.4. The spinal cords of the mice were then dissected. All obtained tissues were postfixed, and immersed in a 30% sucrose solution overnight. Spinal cord segments were cut into 10 µm thick slices on a freezing microtome. The slices were incubated with a rabbit polyclonal antibody against the NK1 substance P (SP) receptor (1:1000; catalog no. AB5060; Chemicon, Temecula, CA, USA) overnight at 4°C. After the sections were washed with buffer, they were exposed to the secondary antibody, an anti-rabbit IgG antibody conjugated with Alexa-488 (1:500; Invitrogen, Carlsbad, CA, USA). The stained sections were cover-slipped and examined using a Zeiss LSM 510 Meta confocal microscope (Zeiss, Oberkochen, Germany), and the mean intensity was measured using Image-Pro Plus v.6.0 (Media Cybernetics, Inc., Rockville, MD, USA).

Statistical Analysis

All data were analysed using GraphPad Prism (version 5.0; GraphPad Software, Inc., San Diego, CA). Data are expressed as the mean ± standard error of mean (SEM). Time-response data are presented as PWT to mechanical stimulation. Variables measured at different time points were compared using the repeated-measures analysis of variance test, with the Bonferroni post hoc correction, performed when appropriate. A P-value of less than 0.05 was considered to indicate statistical significance.

Results

Effect of Vincristine Injection on the Nociceptive Threshold

None of the vincristine-injected mice showed any motor dysfunction or complications. The vincristine-injected mice did not gain weight during the injection schedule (data not shown). However, they looked healthy. Saline did not induce any hyperalgesia or allodynia. Vincristine injection produced significant mechanical alldyonia after the 12-day injection schedule (P<0.0001) (Figure 1). PWT was significantly reduced after vincristine treatment from day 5 to 15 compared with the baseline (Figure 1).

Mechanical Allodynia

The effects of nefopam on mechanical alldyonia are shown in Figure 2. Intraperitoneal nefopam produced a clear dose-dependent increase in the PWT compared with the NS group. There was no significant change in the PWT in the NS group at any time point after injection. The greater dose groups (nefopam30 and 60) showed a more sustained increase in PWT against mechanical stimuli compared with the nefopam10 group. The antialldyonic effect of nefopam was
maintained until 90 mins in nefopam10, 120 mins in nefopam30, and 180 mins in the nefopam60 group (\(P<0.0001\)).

**Neurokinin-1 Receptor**

Immunohistochemistry showed that the NK1 receptor components increased in vincristine-induced neuropathic mice (\(P=0.004\); Representative images in Figure 3B) than in a control mouse (Figure 3A). The increased immunoreactivity of the NK1 receptors was abolished after nefopam treatment in the vincristine-induced neuropathic spinal cord (\(P<0.01\)) (Figure 3C). The percentage of NK1 receptors was lower in mice that received nefopam treatment (\(P<0.05\)) (Figure 3D). The NK1 receptor concentration was 36.4±4.2 before vincristine injection, 80.6±7.9 after vincristine injection, and 45.6±4.7 after nefopam injection.

**Discussion**

In the present study, intraperitoneal nefopam produced a dose-dependent antiallodynic effect and attenuation of NK1 receptors in the dorsal root ganglia in vincristine-induced neuropathy mouse model. Our results suggest that nefopam promotes anti-nociception via the reduction of NK1 receptor, subsequently resulting in the inhibition of substance P (SP) and NK1 receptor signaling. However, we did not measure the circulating SP levels or NK1 receptor internalization; therefore, an additional study will be needed to investigate the change of NK1 receptor internalization and SP levels in mice with blunted NK1 receptor.

Cancer chemotherapeutic agents, such as vincristine, paclitaxel, and cisplatin are known to exert effects on the

![Figure 3](image_url) Effects of intraperitoneal nefopam on NK1 receptors after vincristine-induced neuropathy. Immunohistochemistry showed that NK1 receptor binding components were elevated in vincristine-induced neuropathic mice. (A) Representative spinal cord stained for NK1 receptor in a control mouse. (B) Representative spinal cord stained for NK1 receptor in a vincristine-induced neuropathic mouse. (C) Representative spinal cord stained for NK1 receptor in a vincristine-induced neuropathic mouse after 60 mg/kg nefopam treatment. (D) The percentage of NK1 receptors in the spinal cord was significantly lower in 60 mg/kg nefopam-injected mice (Nefopam) compared to saline-injected mice (Vincristine) in vincristine-induced neuropathic mouse models. *\(P<0.05\), **\(P<0.01\).
sensory nerve by altering the amplitude of the action potential, and conduction velocity, thereby, inducing neuropathic pain.\textsuperscript{4,19} In this pathophysiology, voltage gated-sodium, calcium, and potassium channels, glutamate activated N-methyl-D-aspartate (NMDA) receptors, which play a significant role in the nociceptive process,\textsuperscript{20} and increased SP release in the spinal cord are associated with cytotoxicity to the axons and neuronal cell bodies.\textsuperscript{19,21} The incidence of CIPN varies from 3\% to 38\% with combination protocols. It can occur as early as during the first treatment and may last indefinitely. If the painful neuropathy persists for more than 3 months after therapy has ended, it is regarded as refractory to improvement.\textsuperscript{4} Various drugs including anticonvulsants, tricyclic antidepressants, alpha-adrenergic agonists, ginkgo biloba extract, and opioids have been introduced to treat CIPN; however, the cause, persistence, optimal analgesics, and prevention regimes have not been fully documented.\textsuperscript{22–24} Long-term therapeutic effects have been reported with opioids,\textsuperscript{4} but the risks of tolerance, hyperalgesia, dependence, addiction, and immune suppression should be considered. Moreover, sensitivity to opioid analgesics may be reduced by the loss of opioid receptors on C-fibers, activation of NMDA receptors, or increased release of excitatory amino acids in some neuropathic pain states.\textsuperscript{22}

Nefopam is a non-opioid, non-steroidal benzoxazocine-derived analgesic that inhibits monoamine reuptake in the central nervous system.\textsuperscript{25,26} It indirectly modulates the NMDA receptor, inhibiting c-Fos expression in the dorsal horn of the spinal cord and relieving allodynia in rats.\textsuperscript{11} Nefopam was first introduced as an antidepressant and anticonvulsant and was then reported to be efficacious in postsurgical hyperalgesia.\textsuperscript{27} Several studies have demonstrated the anti-nociceptive effects of nefopam using a variety of routes of administration and for various indications.\textsuperscript{9–11,28–30} Nefopam reduced thermal hypersensitivity after acute and postoperative pain in rats.\textsuperscript{31} Intraperitoneal nefopam showed an antiallodynic effect mediated by adenosine triphosphate-sensitive potassium channel in rats with spinal nerve ligation-induced neuropathy.\textsuperscript{9} The study suggested that the prolonged preemptive effect of nefopam is a result of inhibition of central neuroplasticity mechanisms after nerve injury.\textsuperscript{9} Dam et al reported that intrathecal nefopam produced an antiallodynic effect by acting on the serotonin system (5-hydroxytryptamine) in neuropathic rats.\textsuperscript{29} However, the precise mechanism of action of nefopam in vincristine-induced neuropathy is not clear.

In our study, we used nefopam in doses starting from 10 mg/kg, and it showed an antiallodynic effect. Measuring the concentration of NK1 receptors in postsynaptic dorsal horn neurons has been used to indicate the release of SP into the spinal cord.\textsuperscript{32} SP is the main excitatory neurotransmitter of afferent C-fibers and contributes to the development and maintenance of neuropathic pain.\textsuperscript{33,34} SP is an important molecule in the neurogenic inflammation pathway and plays a key role in eliciting pain sensation in the central and peripheral nervous system.\textsuperscript{35} SP release increases in the spinal dorsal horn following the stimulation of peripheral nociception.\textsuperscript{36} Elevated SP levels have been reported in the cerebrospinal fluid of patients with fibromyalgia and a chronic pain disorder.\textsuperscript{37,38} The biologic actions of SP can be mediated through activation of the NK1 receptor, which is known to have a higher affinity for SP than the NK2 and NK3 receptors.\textsuperscript{39} The released SP can mediate its effect on the NK1 receptor via two different effector systems, and promote neuronal hyperpolarization, which is associated with inflammatory allodynia and hyperalgesia.\textsuperscript{35,39} Several studies have attempted to clarify the mechanism underlying the SP mediated responses and NK1 receptor internalization to investigate the feasibility of using NK1 receptor antagonist as a potential analgesics.\textsuperscript{35,40,41} Our study demonstrated the analgesic effect of nefopam by validating NK1 receptor attenuation, which may reduce SP and NK1 signaling and lead to decreased nociceptive response.

**Limitations**

This study had some limitations. We did not examine cold allodynia. Also, we did not evaluate other inflammatory chemical mediators including pro-inflammatory cytokines (tumor necrosis factor α, interleukin (IL)-1, IL-6, and interferon α) and chemokines related with CIPN.\textsuperscript{42} Further studies are needed to investigate the effectiveness of vincristine-induced neuropathy in cancer patients and related chemical mediators.

**Conclusion**

This study is the first to investigate the effects of intraperitoneal nefopam on allodynia in vincristine-induced neuropathy. The increased concentration of NK1 receptors as a result of vincristine administration coincided with the development of mechanical allodynia, and treatment with nefopam effectively decreased the NK1 receptor concentration and increased the PWT. These results have interesting applications for better understanding of the mechanism of
vincristine-related peripheral neuropathy and the relationship between nefopam and the NK1 receptor.

Data Sharing
The authors are willing to share the data in this article. The data can be accessible by contacting the corresponding author. These will be sent by means of the attached files.

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Author Contributions
JYL and HJP designed the study, analyzed data, and wrote the manuscript. NRC and BWK collected data. WSS and JYM contributed to data analysis and revision of the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure
The authors declare no competing interests.

References
1. Seretny M, Currie GL, Sena ES, et al. Incidence, prevalence, and predictors of chemotherapy-induced peripheral neuropathy: a systematic review and meta-analysis. Pain. 2014;155(12):2461–2470. doi:10.1016/j.pain.2014.09.020
2. Hou S, Huh B, Kim HK, Kim KH, Abdi S. Treatment of chemotherapy-induced peripheral neuropathy: systematic review and recommendations. Pain Physician. 2018;21(6):571–592.
3. Schloss J, Colosimo M, Vitetta L. New insights into potential prevention and management options for chemotherapy-induced peripheral neuropathy. Asia Pac J Oncol Nurs. 2016;3(1):73–85. doi:10.4103/2347-5625.170977
4. Boyette-Davis JA, Cata JP, Driver LC, et al. Persistent chemoneuropathy in patients receiving the plant alkaloids paclitaxel and vincristine. Cancer Chemother Pharmacol. 2013;71(3):619–626. doi:10.1007/s00280-012-2047-z
5. Zhu B, Yu L, Yue Q. Co-delivery of vincristine and quercetin by nanocarriers for lymphoma combination chemotherapy. Biomed Pharmacother. 2017;91:287–294. doi:10.1016/j.biopha.2017.02.112
6. Moore RJ, Groninger H. Chemotherapy-induced peripheral neuropathy in pediatric cancer patients. Curr Neurol Neurosci Rep. 2013;13(5):66. doi:10.1007/s11910-012-0124-4
7. Nama N, Barker MK, Kwan C, et al. Vincristine-induced peripheral neurotoxicity: a prospective cohort. Pediatr Hematol Oncol. 2019;1–14. doi:10.1080/08880018.2019.1677832.
8. Park BY, Park SH, Kim WM, Yoon MH, Lee HG. Antinociceptive effect of memantine and morphine on vincristine-induced peripheral neuropathy in rats. Korean J Pain. 2010;23(3):179–185. doi:10.3344/kjp.2010.23.3.179
9. Koh WU, Shin JW, Bang JY, Kim SG, Song JG. The antiallodynic effects of nefopam are mediated by the adenosine triphosphate-sensitive potassium channel in a neuropathic pain model. Anesth Analg. 2016;123(3):762–770. doi:10.1213/ane.0000000000001411
10. Nam JS, Cheong YS, Karm MH, et al. Effects of nefopam on streptozotocin-induced diabetic neuropathic pain in rats. Korean J Pain. 2014;27(4):326–333. doi:10.3344/kjp.2014.27.4.326
11. Verleye M, Andre N, Heulard I, Gillardin JM. Nefopam blocks voltage-sensitive sodium channels and modulates glutamatergic transmission in rodents. Brain Res. 2004;1013(2):249–255. doi:10.1016/j.brainres.2004.04.035
12. Kim KH, Byeon GJ, Kim HY, Baek SH, Shin SW, Koo ST. Mechanical antiallodynic effect of intrathecal nefopam in a rat neuropathic pain model. J Korean Med Sci. 2015;30(8):1189–1196. doi:10.3346/jkms.2015.30.8.1189
13. Gautam M, Prasoon P, Kumar R, Reeta KH, Kaler S, Ray SB. Role of neurokinin type 1 receptor in nociception at the periphery and the spinal level in the rat. Spinal Cord. 2016;54(3):172–182. doi:10.1038/sc.2015.206
14. Jensen DD, Lieu T, Halil ML, et al. Neurokinin 1 receptor signaling in endosomes mediates sustained nociception and is a viable therapeutic target for prolonged pain relief. Sci Transl Med. 2017;9:392. doi:10.1126/scitranslmed.aal3447
15. Weng HR, Cordella JV, Dougherty PM. Changes in sensory processing in the spinal dorsal horn accompany vincristine-induced hyperalgesia and allodynia. Pain. 2003;103(1–2):131–138. doi:10.1016/S0304-3959(02)00445-1
16. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods. 1994;53(1):55–63. doi:10.1016/0165-2470(94)90144-9
17. Clark CG, Sun Z, Meininger GA, Potts JT. Atomic force microscopy to characterize binding properties of alpha7-containing nicotinic acetylcholine receptors on neurokinin-1 receptor-expressing medullary respiratory neurons. Exp Physiol. 2013;98(2):415–424. doi:10.1113/expphysiol.2012.067660
18. Bennett VJ, Simmons MA. Analysis of fluorescently labeled substance P analogs: binding, imaging and receptor activation. BMC Chem Biol. 2001;1(1):1. doi:10.1186/1472-6769-1-1
19. Jaggi AS, Singh N. Mechanisms in cancer-chemotherapeutic drugs-induced peripheral neuropathy. Toxicol. 2012;291(1–3):1–9. doi:10.1016/j.tox.2011.10.019
20. Fundytus ME. Glutamate receptors and nociception: implications for the drug treatment of pain. CNS Drugs. 2001;15(1):29–58. doi:10.2165/00002321-200115010-00004
21. Peters CM, Jimenez-Andrade JM, Kusowski MA, Ghilardi JR, Mantyh PW. An evolving cellular pathology occurs in dorsal root ganglia, peripheral nerve and spinal cord following intravenous administration of paclitaxel in the rat. Brain Res. 2007;1168:46–59. doi:10.1016/j.brainres.2007.06.066
22. Bujalska M, Makulaska-Nowak H, Gumulka SW. Magnesium ions and opioid agonists in vincristine-induced neuropathy. Pharmacoephol Rep. 2009;61(6):1096–1104. doi:10.1016/S1734-1140(09)70172-0
23. Park HJ, Lee HG, Kim YS, et al. Gingko biloba extract attenuates hyperalgesia in a rat model of vincristine-induced peripheral neuropathy. Anesth Analg. 2012;115(5):1228–1233. doi:10.1213/ANE.0b013e318262ce170
24. Lynch JJ 3rd, Wade CL, Zhong CM, Mikusa JP, Honore P. Attenuation of mechanical allodynia and hyperalgesia in a rat model of vincristine-induced peripheral neuropathic pain. J Pain. 2004;110 (1–2):56–63. doi:10.1016/j.pain.2004.03.010
25. Kim EM, Jeon JH, Chung MH, et al. The effect of nefopam infusion during laparoscopic cholecystectomy on postoperative pain. Int J Med Sci. 2017;14(6):570–577. doi:10.7150/ijms.19021
26. Alfonsi P, Adam F, Passard A, Guignard B, Sessler DJ, Chauvin M. Nefopam, a non sedative benzoxazocine analgesic, selectively reduces the shivering threshold in unanesthetized subjects. Anesthesiology. 2004;100(1):37–43. doi:10.1097/00000542-200401000-00010
27. Zhao T, Shen Z, Sheng S. The efficacy and safety of nefopam for pain relief during laparoscopic cholecystectomy: a meta-analysis. *Medicine (Baltimore)*. 2018;97(10):e0089. doi:10.1097/md.0000000000010089
28. Moon JY, Choi SS, Lee SY, et al. The effect of nefopam on postoperative fentanyl consumption: a randomized, double-blind study. *Korean J Pain*. 2016;29(2):110–118. doi:10.3344/kjp.2016.29.2.110
29. Dam LJ, Hai L, Ha YM. Role of the 5-HT(7) receptor in the effects of intrathecal nefopam in neuropathic pain in rats. *Neurosci Lett*. 2014;566:50–54. doi:10.1016/j.neulet.2014.02.021
30. Kim Do Y, Chae JW, Lim CH, et al. The role of spinal dopaminergic transmission in the analgesic effect of nefopam on rat inflammatory pain. *Korean J Pain*. 2016;29(3):164–171. doi:10.3344/kjp.2016.29.3.164
31. Girard P, Pansart Y, Coppe MC, Gillardin JM. Nefopam reduces thermal hypersensitivity in acute and postoperative pain models in the rat. *Pharmacol Res*. 2001;44(6):541–545. doi:10.1006/phrs.2001.0886
32. Li X, Guo R, Sun Y, et al. Botulinum toxin type A and gabapentin attenuate postoperative pain and NK1 receptor internalization in rats. *Neurochem Int*. 2018;116:52–62. doi:10.1016/j.neuint.2018.03.010
33. Cahill CM, Coderre TJ. Attenuation of hyperalgesia in a rat model of neuropathic pain after intrathecal pre- or post-treatment with a neurokinin-1 antagonist. *Pain*. 2002;95(3):277–285. doi:10.1016/S0304-3959(01)00410-9
34. Takemura M, Sugiyo S, Moritani M, Kobayashi M, Yonehara N. Mechanisms of orofacial pain control in the central nervous system. *Arch Histol Cytol*. 2006;69(2):79–100. doi:10.1679/ahc.69.79
35. Chang CT, Jiang BY, Chen CC. Ion channels involved in substance P-Mediated nociception and antinociception. *Int J Mol Sci*. 2019;20(7):1596. doi:10.3390/ijms20071596
36. Duggan AW, Hendry IA, Morton CR, Hutchison WD, Zhao ZQ. Cutaneous stimuli releasing immunoreactive substance P in the dorsal horn of the cat. *Brain Res*. 1988;451(1–2):261–273. doi:10.1016/0006-8993(88)90771-8
37. Vaeroy H, Helle R, Forre O, Kass E, Terenius L. Elevated CSF levels of substance P and high incidence of Raynaud phenomenon in patients with fibromyalgia: new features for diagnosis. *Pain*. 1998;32(1):21–26. doi:10.1016/0304-3959(88)90019-x
38. Russell JJ, Orr MD, Littman B, et al. Elevated cerebrospinal fluid levels of substance P in patients with the fibromyalgia syndrome. *Arthritis Rheum*. 1994;37(11):1593–1601. doi:10.1002/art.1780371106
39. Satake H, Aoyama M, Sekiguchi T, Kawada T. Insight into molecular and functional diversity of tachykinins and their receptors. *Protein Pept Lett*. 2013;20(6):615–627. doi:10.2174/0929866511320060002
40. Wang Y, Lei J, Jha RK, Kiven S, Gupta K. Substance P modulates electroacupuncture analgesia in humanized mice with sickle cell disease. *J Pain Res*. 2019;12:2419–2426. doi:10.2147/jpr.S210196
41. Fujita M, Fukuda T, Sato Y, Takasusuki T, Tanaka M. Allopregnanolone suppresses mechanical allodynia and internalization of neurokinin-1 receptors at the spinal dorsal horn in a rat postoperative pain model. *Korean J Pain*. 2018;31(1):10–15. doi:10.3344/kjp.2018.31.1.10
42. Boyette-Davis J, Xin W, Zhang H, Dougherty PM. Intradermal nerve fiber loss corresponds to the development of taxol-induced hyperalgesia and can be prevented by treatment with minocycline. *Pain*. 2011;152(2):308–313. doi:10.1016/j.pain.2010.10.030