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Original Article

The Synergistic Effect of Intrathecally Administered Dexmedetomidine and Ketorolac on Mechanical Allodynia in Rats with Spinal Nerve Ligation

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Objectives: This research was carried out to identify the synergistic effect of dexmedetomidine and ketorolac on neuropathic pain alleviation.
Methods: The anti-allodynic effect of intrathecal dexmedetomidine and ketorolac was investigated in rats after L5 spinal nerve ligation (SNL). Mechanical allodynia was assessed using Von Frey filaments. Every day for 3 consecutive days, beginning on the 10th day after SNL, behavioral tests were carried out at 1, 2, and 4 hr after drug injection.
Results: Significant increases in ipsilateral paw withdrawal thresholds (PWTs) were observed 1, 2, and 4 hr after drug injection in the groups of rats which received intrathecal injection of either dexmedetomidine (group D) or ketorolac (group K), compared to group S ($P < 0.05$). And group DK, which received simultaneous intrathecal injection of both dexmedetomidine and ketorolac, showed statistically significantly higher ipsilateral PWTs than groups D and K, which received only one of them ($P < 0.05$).
Conclusions: The results of this research demonstrated the anti-allodynic effect of dexmedetomidine and ketorolac on neuropathic pain induced by SNL in rats. They also suggest that synergistic analgesia can be induced by the simultaneous injection of dexmedetomidine and ketorolac, and that combination therapy is an effective approach to treating chronic neuropathic pain syndrome.

Key Words: Allodynia, Dexmedetomidine, Ketorolac, Neuropathic pain, Spinal nerve ligation

Neuropathic pain, a type of pathological pain that occurs in patients with a lesion or disease of the somatosensory nervous system, may be associated with dysesthesia (abnormal sensations), hyperalgesia (exaggerated pain sensation) and allodynia (pain produced by normally non-painful stimuli).[1] Little is known about the mechanism of neuropathic pain, but central or peripheral mechanism might possibly be involved. Normal drug therapy usually fails to alleviate neuropathic pain, and treatment methods for such patients are limited.[2]

Dexmedetomidine, a highly selective agonist of α2-adrenoceptor (α2AR), binds to the receptor with more than 8 times higher affinity compared to clonidine.[3] Dexmedetomidine induces sedation and enhanced analgesia in the spinal cord by stimulating α2ARs in the locus coeruleus.[4] Some

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preceding researches with neuropathic pain models demonstrated significant anti-nociceptive effects of systemic or intrathecal administration of dexmedetomidine.[4-6]

Ketorolac, a nonsteroidal anti-inflammatory drug (NSAID), has analgesic activity and its systemic administration is widely practiced for the clinical treatment of postoperative and cancer pain.[7] The induction of analgesia by intrathecal ketorolac has also been demonstrated in a variety of animal models of pain.[8,9]

The objective of this research is to identify the synergistic effect of intrathecal administration of dexmedetomidine and ketorolac on mechanical allodynia alleviation in SNL rats.

MATERIALS AND METHODS

The experimental protocol was approved by the Institutional Animal Care and Use Committee. A separate cage was used for each of them in a temperature controlled room (23 ± 0.5℃) with a 12/12-hr light/dark cycle. They were allowed free access to food and water. An established procedure was used for ligating the spinal nerve L5 of adult Sprague-Dawley rats (250 - 300 g).[10] The animals were anesthetized by delivering sevoflurane through a nose cone. Skin incision, at the midline over the lumbar spine, was carried out under aseptic condition. The left transverse process of the L6 vertebra was removed, followed by the dissection of the exposed left L5 spinal nerve from the underlying tissue. Then the left L5 spinal nerve, cut distally, was ligated using a 6-0 silk suture. After the surgery, the rats were again kept in the cages under the monitoring of their recovery.

7 days after the surgery, intrathecal catheterization was conducted by an established method so as to allow drug injection.[11] The catheter of a saline-filled polyethylene-10 tubing was implanted to thoracic vertebrae by being inserted (6-7 ㎝) into a small slit made on the atlanto-occipital membrane.

All the rats that had undergone SNL were divided into the following groups: Group S (n = 5), which received intrathecal injection of normal saline; group D (n = 5), intrathecal injection of dexmedetomidine; and group K (n = 5), intrathecal injection of ketorolac. Whereas group DK (n = 5) received simultaneous intrathecal injection of both dexmedetomidine and ketorolac.

The amounts of intrathecally injected substances for individual groups are as follows: Group S received 10 ㎕ of normal saline; group D, the total volume of the solution made by mixing 1 ㎍ of dexmedetomidine with normal saline to a final volume of 10 ㎕; group K, the total volume of the solution made by mixing 50 ㎍ of ketorolac with normal saline to a final volume of 10 ㎕; and group DK, the total volume of the solution made by mixing 1 ㎍ of dexmedetomidine and 50 ㎍ of ketorolac with normal saline to a final volume of 10 ㎕. Every injection was followed by the injection of 10 ㎕ of normal saline to flush the catheter.

Behavioral tests were conducted for 3 consecutive days, beginning on the 10th day after SNL, each day at 1, 2, and 4 hr after drug injection.

For the tests, mechanical allodynia was induced on the plantar surface of the hindpaw between the foot pads. The animals were set on a mesh floor, with plastic domes, in a way that allowed full access to the test area. To induce mechanical allodynia, forces of approximately logarithmic increments were applied using a set of von Frey filaments(0.38, 0.57, 1.23, 1.83, 3.66, 5.93, 9.13, 13.1 g). Their responses to the mechanical stimuli were quantified by the up-down method, so as to determine mechanical PWTs.[12] The duration of the application of a von Frey filament to the test area was about 5 seconds. A 1.83 g stimulus was applied initially. Then if an animal had shown a positive response, the next smaller von Frey hair was used for the next application; whereas if an animal had shown a negative response, it was subjected to the application of the next higher force. Sudden paw withdrawal, shaking and licking were all considered as positive responses.

To minimize experimenter bias, the person who conducted the behavioral tests was blinded to individual animals' drug treatment conditions. The behavioral tests were conducted after the animals had undergone a 1-week procedure for acclimating them to the facilities. The animals were acclimated for 3 - 5 days before measurements were taken for baseline data, to minimize the variability of the behavioral outcome measures. In addition, on every test day, immediately prior to beginning a test, the animals underwent an about 30-minute procedure for habituating them to the test environment.

PWT was determined, using the formula of Dixon, by causing conversion between positive and negative responses to von Frey filament stimulation to a 50% threshold value.[13]

Data are presented as mean ± SEM. Statistical analysis was carried out using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Two-way ANOVA was used for data comparison between different groups, while the Tukey honestly significant difference test for post-hoc analysis was used for comparison between specific behavioral data points in ANOVA. Test data were considered to have statistical significance only when $P < 0.05$.

RESULTS

At 1, 2, and 4 hr after drug injection, on 3 consecutive days beginning on the 10th day after SNL, statistically significant increases in ipsilateral PWTs were shown by groups D and K compared to group S ($P < 0.05$), and by Group DK compared to groups D and K ($P < 0.05$) (Fig. 1). No significant change in contralateral PWT was observed after the treatments (Data not presented).

As for % pre-SNL in ipsilateral paw, significantly higher PWTs were shown by groups D and K compared to group S ($P < 0.05$), and by group DK compared to groups D and K SNL ($P < 0.05$) at 1, 2, and 4 hr after drug injection, on 3 consecutive

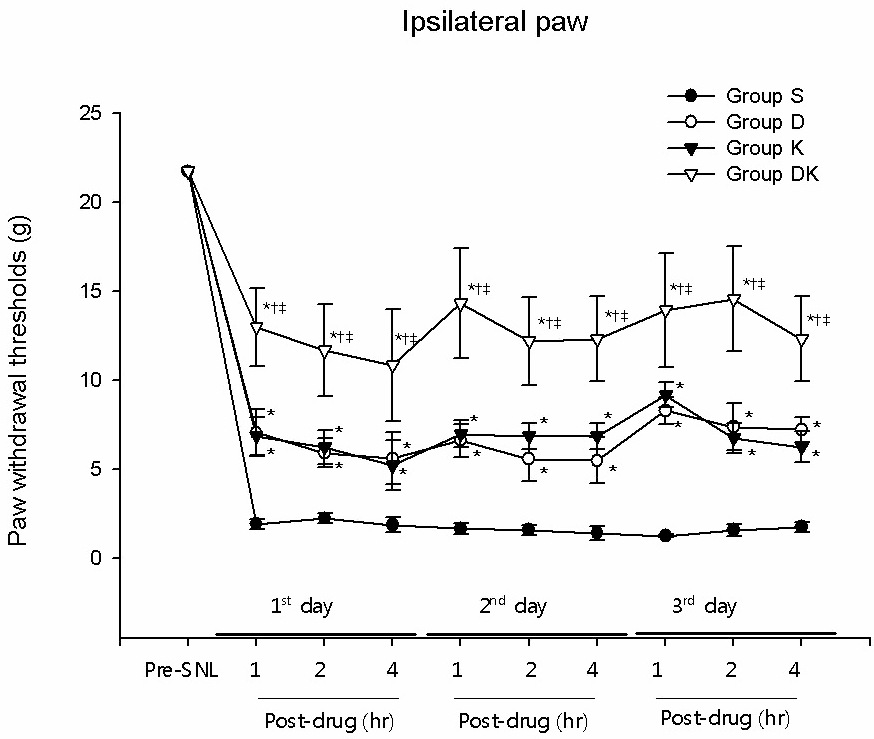

Fig. 1. The alleviating effect of intrathecal dexmedetomidine and ketorolac on mechanical allodynia in spinal nerve ligation rats. All the rats underwent SNL. Group S (n = 5) received intrathecal injection of normal saline, while groups D (n = 5) and K (n = 5) received intrathecal injection of dexmedetomidine and ketorolac, respectively. Group DK (n = 5) received simultaneous intrathecal injection of both dexmedetomidine and ketorolac. At 1, 2, and 4 hr after drug injection, on 3 consecutive days beginning on the 10th day after SNL, statistically significant increases in ipsilateral paw withdrawal thresholds were shown by groups D and K compared to group S (P < 0.05); and by Group DK compared to groups D and K (P < 0.05). *P < 0.05 compared to Group S, † P < 0.05 compared to Group D. ‡P < 0.05 compared to Group K.

days beginning on the 10th day after SNL (Fig. 2).

DISCUSSION

In this research involving rats, 3 consecutive days of measurements, beginning on the 10th day after SNL and taken at 1, 2, and 4 hr after intrathecal drug injection, all resulted in significant increase in PWT in the groups which received either dexmedetomidine or ketorolac compared to the group which received normal saline; while the group which received both dexmedetomidine and ketorolac simultaneously always showed significantly higher PWT than the groups which received only one of them. Thus it could be inferred that simultaneous administration of dexmedetomidine and ketorolac is more effective for neuropathic pain alleviation than the administration of only one of them. Although the mechanism of dexmedetomidine and ketorolac action is beyond the scope of the present research, some preceding researches on neuropathic pain concerned the mechanism of their action.

Neuropathic pain caused by peripheral nerve

injury is associated with a variety of changes in the sensory processing that involves primary afferent neurons, the spinal cord and supraspinal regions. There are only few drugs approved for neuropathic pain treatment, which include antidepressants and calcium channel $\alpha 2$-δ ligands.[14] They all act through a common pharmacological mechanism involving the activation of spinal α 2-adrenoceptors, which play a critical role in neuropathic pain suppression.[15,16]

Activated spinal $\alpha 2$-adrenoceptors directly inhibit nociceptive neurotransmission by suppressing the release of such neurotransmitters as substance P and glutamate from primary afferent terminals, and also by hyperpolarizing dorsal horn neurons via G-protein-mediated activation of potassium channels.[17,18]

Preceding researches suggest that intrathecal clonidine, an agonist of $\alpha 2$-adrenoceptor, enhances potency and efficacy against neuropathic pains in animals as well as in human.[19,20] It is considered that such an effect is mediated via downstream activation of inhibitory cholinergic interneurons. That is, an $\alpha 2$-adrenoceptor agonist, which inhibits spinal cholinergic interneurons in normal condition, rather excites them in response to nerve injury, and the consequent release of acetylcholine (ACh) is believed to be cru-

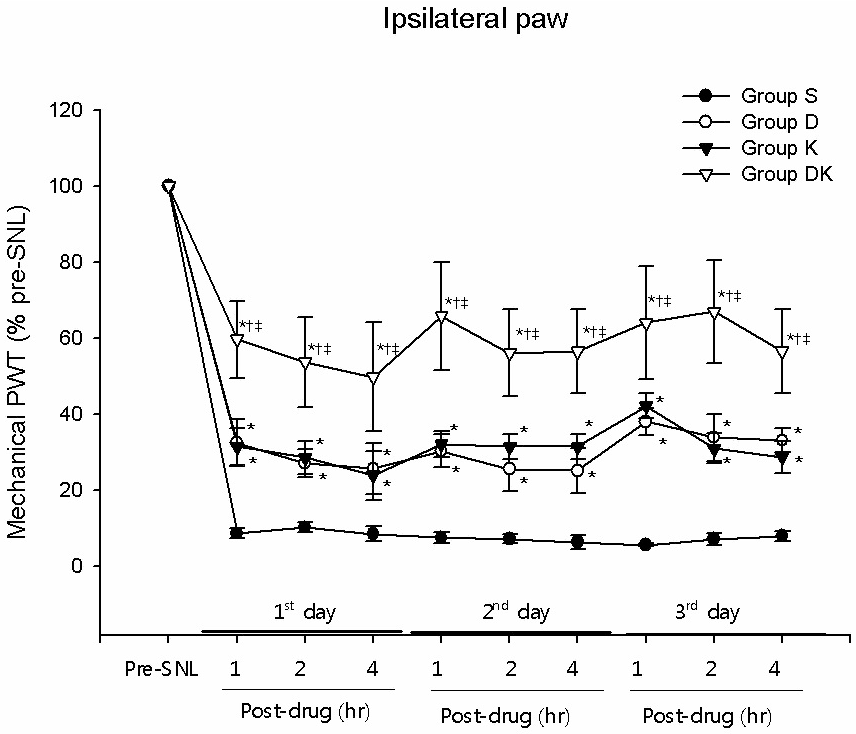

Fig. 2. Increases in ipsilateral paw withdrawal thresholds in spinal nerve ligation rats caused by intrathecal dexmedetomidine and ketorola. Regarding % pre-SNL in ipsilateral paw, significantly higher PWTs were shown by groups D and K compared to group S at 1, 2, and 4hr after drug injection, on 3 consecutive days beginning on the 10th day after SNL ($P < 0.05$); and by group DK compared to groups D and K ($P < 0.05$). *$P < 0.05$ compared to Group S, †$P < 0.05$ compared to Group D. ‡$P < 0.05$ compared to Group K.

cial in the analgesic mechanism involving the activation of spinal α2-adrenoceptors.[21]

Using in vivo microdialysis, Kimura et al.[5] showed that intrathecally injected dexmedetomidine, a selective agonist of α2-adrenoceptor, caused an increase of ACh level in the lumbar spinal cord of SNL rats, but not in that of normal rats. This suggests that, in SNL rats, increase of ACh level in the spinal cord is critical for the anti-hyperalgesic mechanism involving the activation of spinal α2-adrenoceptor.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are most commonly used for the pharmacologic treatment of pain. Their actions are considered to primarily involve the inhibition of cyclooxygenase and the consequent reduction of prostaglandin production at peripheral sites of inflammation. However, prostaglandin synthesis has been shown to occur in the spinal cord, increasing in response to high threshold afferent input.[22] Many researches with animals showed that the expression and activity of spinal cord cyclooxygenase are highly responsible for a pain state, and that spinally injected NSAIDs inhibited pain behaviors.[23,24]

Prostaglandins play an important role in the spinal level of augmented processing of pain information.[23] Intrathecal injection of prostaglandins E2 (PGE2) and D2 (PGD2) was shown to induce hyperalgesia and allodynia in mice.[25] Whereas NSAIDs was also shown to alleviate the behavioral hyperalgesia caused by the spinal action of N-methyl-D-aspartate (NMDA) and substance P.[23]

There have been researches on the analgesic activity of intrathecal ketorolac in various pain models, which include formalin test in rats as well as acetic acid writhing test or p-phenylquinone test in mice.[8,9,26,27] According to the result of the formalin test, intrathecal ketorolac inhibited phase 2 pain response, but with only a limited effect on phase I pain response.[9] Morphine and alpha2 agonist (ST-91) showed different effects, resulting in dose-dependent suppression of phase 1 and phase 2 pain responses.[9] Importantly, the result of the above formalin test demonstrated a significant synergistic effect of ketorolac, morphine and ST-91 on phase 1 and 2 pain responses.[9]

Miranda et al.[26] demonstrated that pretreatment with neither naloxone nor indomethacin could antagonize the antinociceptive activity of intrathecal ketorolac. Their data suggest that the antinociceptive mechanism of ketorolac may not involve opioid receptors or prostaglandin biosynthesis. Accordingly, it would be reasonable to conclude that the analgesic mechanism of ketorolac in the rat model of spinal nerve ligation is rather complex. The elucidation of the central action mechanism of ketorolac requires further investigation.

On the other hand, the combined use of several drugs with similar therapeutic effects or synergistic interactions may also enable the reduction of side effects by decreasing the dose of individual drug. This study was conducted only on a single

volume of dexmedetomidine and ketorolac. Thus, those in other volumes should be performed in further studies, and it is considered necessary to study adverse effects or toxicity caused by the volume of drug in the future. In order to develop therapeutic agent for patients with neuropathic pain, clinical research should be conducted. To this end, various studies are needed in the future.

In conclusion, this research demonstrated that simultaneous intrathecal administration of ketorolac and dexmedetomidine could induce effective synergistic analgesia in the rat model of neuropathic pain. Synergistic interaction between these drugs is expected to have a great therapeutic significance in the future as a means of reducing the dose of individual drug required for achieving a desired level of analgesia. Accordingly, follow-up researches would have to concern the complex administration of various other drugs for the treatment of neuropathic pain, as well as the mechanisms of their interactions.

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