

좌골신경 만성협착손상 흰쥐에서 척수강 내로 투여된 Zaprinst의 항이질통 효과

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The Antiallodynic Effects of Intrathecal Zaprinst in Rats with Chronic Constriction Injury of the Sciatic Nerve

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Background: Zaprinst is an inhibitor of phosphodiesterase 5, 6 and 9. Phosphodiesterase inhibitors could produce anti-nociceptive effects by promoting the accumulation of cGMP. We hypothesized that intrathecal zaprinst could attenuate the allodynia induced by chronic constriction injury of the sciatic nerve in rat.

Methods: Sprague-Dawley rats were prepared with four loose ligations of the left sciatic nerve just proximal to the trifurcation into the sural, peroneal and tibial nerve branches. Tactile allodynia was measured by applying von Frey filaments to the lesioned hindpaw. The thresholds for the withdrawal responses were assessed. Zaprinst (3–100 μ g) was administered intrathecally by the direct lumbar puncture method to obtain the dose-response curve and the 50% effective dose (ED₅₀). Measurements were taken before and 15, 30, 45, 60, 90, 120, and 180 min after the intrathecal doses of zaprinst. The side effects were also observed.

Results: Intrathecal zaprinst resulted in a dose-dependent antiallodynic effect. The maximal effects occurred within 15–30 min and then they gradually decreased down to the baseline level over time in all the groups. There was a dose dependent increase in the magnitude and duration of the effect. The ED₅₀ value was 17.4 μ g (95% confidence intervals; 14.7–20.5 μ g). No severe motor weakness or sedation was observed in any of the rats.

Conclusions: Intrathecally administered zaprinst produced a dose-dependent antiallodynic effect in the chronic constriction injury neuropathic pain model. These findings suggest that spinal phosphodiesterase 5, 6 and 9 may play an important role in the modulation of neuropathic pain. (Korean J Pain 2009; 22: 16-20)

Key Words: allodynia, chronic constriction injury, intrathecal, neuropathic pain, zaprinst.

INTRODUCTION

Peripheral nerve injury induces neuropathic pain which

is associated with the development of a long-lasting hyperalgesia and allodynia.¹⁾ Bennett and Xie described a peripheral nerve ligation model in the rat which has many of the features of a neuropathic pain state similar to

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humans.²⁻⁴⁾ In this chronic constriction injury (CCI) model, the sciatic nerve on one side is loosely ligated with chromic cat gut. This damage is accompanied by hyperalgesia and allodynia which develop over the following 7–10 days.

Zaprinst is an inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase 5, 6 and 9. Several studies have proposed that phosphodiesterase inhibitors could produce anti-nociceptive effects by promoting accumulation of cGMP,⁵⁻⁷⁾ as this second messenger has several targets including cGMP dependent protein kinases (PKG), cGMP-regulated PDEs, and cyclic nucleotide-gated ion channels.⁸⁾ Elevated cGMP is especially interesting given the antinociceptive effect of zaprinst. It is already reported that intrathecally injected zaprinst alleviated the facilitated pain state as well as acute pain evoked by the formalin injection.⁹⁾ However, there is no study about the antiallodynic effect of intrathecal zaprinst especially in the neuropathic pain state. Thus, in this study, we investigated the hypothesis that intrathecal zaprinst also could attenuate the allodynia induced by chronic constriction injury of the sciatic nerve in the rat.

MATERIALS AND METHODS

This study was performed under a protocol approved by the Animal Use and Care Committee. The experiments were conducted in male Sprague-Dawley rats (weight 200–250 g), which were housed individually in a temperature controlled vivarium and allowed to acclimate for 3 days in a 12/12-hour light/dark cycle.

For creating the CCI neuropathic rat model, a surgical procedure was performed.³⁾ Under enflurane anesthesia, the left sciatic nerve was exposed at the middle level of rat thigh just proximal to the trifurcation into the sural, peroneal and tibial nerve branches, thereafter four ligatures (4–0 chromic gut) were performed loosely with microsurgical techniques. Intervals among ligatures were about 1 mm. The same investigator created CCI animals to avoid variation. After 15 days of postoperative period, if the rats showed a withdrawal threshold of less than 4.0 g, these rats were defined as demonstrating mechanical allodynia, and used in behavioral testing.

For spinal drug administration, to avoid the potential effects of inflammation or surgically implanting a foreign body in the spinal cord on antiallodynic effect of zaprinst, we used the direct lumbar puncture method.¹⁰⁾ Rats were anesthetized with enflurane in oxygen via nose cone. The lumbar region was shaved, prepared with Betadine solution, and the intervertebral spaces widened by placing the animal on a plexiglas tube. Animals were then injected at the L5-6 interspace using a 0.5-inch 30-gauge needle (Becton-Dickinson, USA) connected to a Hamilton syringe. The Hamilton syringe was preloaded with 5 μ l air and then filled with test drug 10 μ l. The needle plunger was then slowly lowered just 10 μ l and the needle was immediately pulled out to prevent injection of air. Correct subarachnoid positioning of the tip of the needle was verified by a tail-or paw-flick test.¹⁰⁾ Animals then recovered in their home cage before analgesic testing.

For the determination of the time to peak effect and the ED₅₀ which is estimated to produce 50% maximal possible effect (%MPE), zaprinst (molecular weight 271.27, Sigma, USA) was administered intrathecally. The doses of 3, 10, 30 and 100 μ g (n = 8 per subgroup) were injected for zaprinst, and the doses given were blind to the experimenter. Zaprinst was dissolved in dimethyl sulfoxide (DMSO, minimum 99.5%; Sigma, USA) and diluted with 0.9% sodium chloride solution.

Behavioral testing was carried out in a quiet room between 9:00 and 1:00 p.m. The rats were placed in an individual plastic cage with a wire mesh bottom. After 20 min of stabilizing the rats, mechanical threshold was measured by applying a series of 8 calibrated von Frey filaments (0.4, 0.6, 1.0, 2.0, 4.0, 6.0, 8.0, and 15.0 g; Stoelting Co., USA) to the midplantar surface of the hindpaw ipsilateral to the nerve injury. Filament was held for 6 seconds until a positive sign for pain behavior was elicited. A brisk withdrawal or paw flinching was considered as positive responses, in which case the adjacent filament with lower force was tested. In the absence of such response, the adjacent filament with higher force was tested. In case of the absence of a response at 15 g of pressure, the animals were assigned to this cutoff value. The mechanical stimulus producing a 50% likelihood of withdrawal was determined by using the up-down

method.¹¹⁾ Measurements were taken before and 15, 30, 45, 60, 90, 120, and 180 min after an intrathecal doses of zaprinast. Baseline responses to von Frey filaments for each animal was determined on the same day just before drug injection.

Withdrawal threshold data from von Frey filament testing were obtained as the actual threshold in grams and were converted to %MPE using the formula: %MPE for antiallodynia = $(\text{postdrug threshold} - \text{baseline threshold}) / [15 \text{ g} - \text{baseline threshold}] \times 100$, where postdrug threshold = the largest threshold observed after intrathecal injection. The cutoff value was defined as a stimulus intensity of 15 g for the tactile threshold (i.e., %MPE = 100). The peak drug effect was used to calculate a %MPE, and %MPE were plotted in curve versus log dose.

Side effects were simply assessed by observing the presence of sedation and motor weakness. Severe sedation was defined as a significant decrease in spontaneous activity and a loss of the orienting response to the light touch stimulation. Motor weakness was evaluated by observing the righting and placing/stepping reflexes, abnormal weight bearing, and ambulation.

Data were expressed as mean \pm SEM because of the small number of rats in each group. The ED₅₀ values, slopes, and 95% confidence intervals were calculated using dose-response data. Variances and its 95% confidence intervals for the theoretical ED₅₀ may also be calculated from the variances of each component administered alone.¹²⁾ Statistical significance ($P < 0.05$) was determined using one-way repeated-measures ANOVA followed by a Tukey's test for multiple comparisons between groups.

RESULTS

There were no significant differences in rat weights in any of the animals used in this study. Most rats displayed normal weight gain and general behavior except for the pain behavior. The thresholds for evoking hindpaw withdrawal were in the range of 1–4 g for all rats. The unilateral mechanical allodynia was maintained for up to 60 days.

Intrathecal zaprinast resulted in a dose-dependent anti-

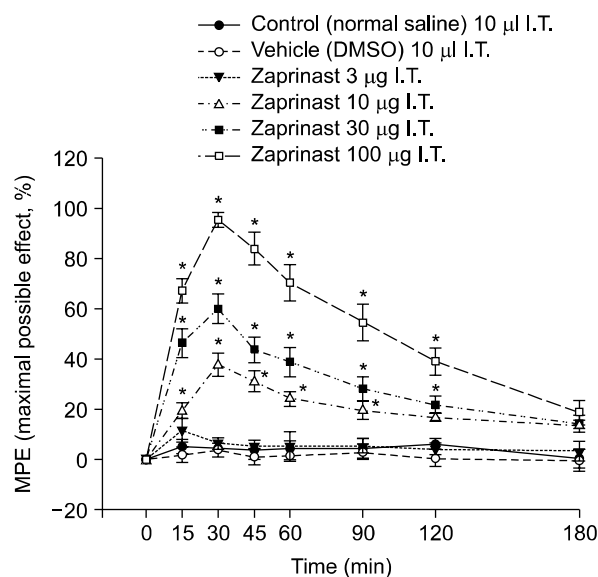


Fig. 1. Time course of antiallodynic effects by intrathecal injection of zaprinast (8 rats per each dose). These curves show a dose-dependent antiallodynic effect in each dose. Data are expressed as mean \pm SEM. Asterisks indicate that mean percent of maximal possible effect (%MPE) at that time point is significant compared with baseline value of its own group. * $P < 0.05$; one-way repeated-measures analysis of variance followed by multiple comparisons (Tukey's method). I.T.: intrathecal, DMSO: dimethyl sulfoxide. %MPE for antiallodynia = $(\text{postdrug threshold} - \text{baseline threshold}) / [15 \text{ g} - \text{baseline threshold}] \times 100$, where postdrug threshold = the largest threshold observed after intrathecal injection.

allodynic effect (Fig. 1). For all groups, the maximal effects occurred within 15–30 min and then gradually decreased down to the previous baseline level over whole observation periods for all doses of each group. There was a dose dependent increase in magnitude and duration of the effect. Intrathecal normal saline and DMSO (vehicle groups) produced only a slight increase in withdrawal threshold, which means that vehicles do not have an effect on the antiallodynic action (Fig. 1). The ED₅₀ value (95% confidence intervals) was 17.4 (14.7–20.5) μg and slope (95% confidence intervals) was 57.4 (50.2–64.6) (Fig. 2). No severe motor weakness or sedation was observed in any rats.

DISCUSSION

We found that intrathecal zaprinast produced a dose-dependent increase of withdrawal threshold for a spinally

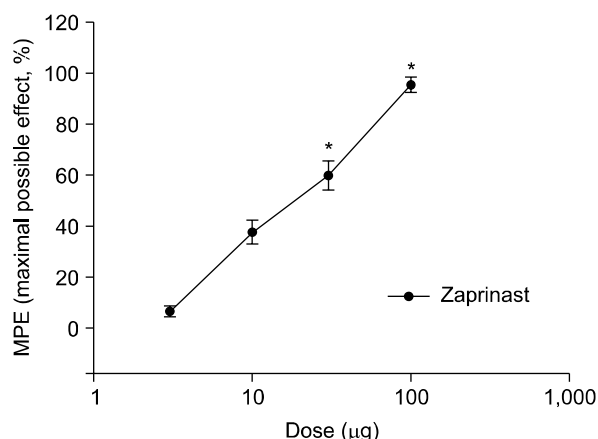


Fig. 2. Dose-response curves from the peak effects of percent maximal possible effect (%MPE) of the zaprinast (8 rats per each dose). This curve shows a dose-dependent antiallodynic effect. Data are expressed as mean \pm SEM. Doses (μ g) are represented logarithmically on the x axis and peak %MPE is represented on the y axis. Asterisks indicate that the mean %MPE of each group is significant compared with that of 3 μ g dosing group. * $P < 0.05$.

mediated mechanical allodynia in the CCI neuropathic pain model. These findings suggest that spinal phosphodiesterase 5, 6 and 9 may play an important role in the modulation of neuropathic pain state.

Phosphodiesterase enzymes are present widely in many biological systems and are found in mammalian tissues.¹³⁾ At least eleven distinct nucleotide phosphodiesterase isoenzymes^{14,15)} have been identified on the basis of their functional characteristics, such as their substrate specificity, cellular distribution and susceptibility to selective inhibitors.¹⁶⁾ It has been reported that phosphodiesterases 5, 6 and 9 have been to be specific for cGMP.¹⁷⁾ cGMP-specific phosphodiesterase catalyzes the hydrolysis of cGMP to GMP. In particular, cGMP may play an important role in the modulation of nociception. This proposal was based on the observation that a local injection of dibutyryl-cGMP produced antinociception in a modification of the Randall-Selitto hyperalgesia.¹⁷⁾ In addition, intrathecal 8-bromo-cGMP reduced the mechanical allodynia in neuropathic rats of CCI model.¹⁸⁾ This indicates that cGMP level could be increased by inhibiting this enzyme, thereby producing antiallodynic effect.

At the spinal level, cGMP dose-dependently either inhibits or facilitates nociceptive transmission. It is reported

that spinally delivered 8-Br-cGMP showed a dual effect in the rat formalin test; low doses reduced nociceptive behavior while high doses caused hyperalgesia.¹⁹⁾ Antinociception appeared to be the primary effect, since the antinociceptive effects were produced in the low cGMP concentration. Intrathecal application of nitric oxide (NO) donors also showed this dual cGMP effect;¹⁸⁾ low doses of NO donors induced antinociception while high doses increased the nociceptive response. It is also reported that spinally administered sildenafil, another phosphodiesterase inhibitor, activated the NO-cGMP pathway in the rat CCI model, and high doses (100–200 μ g) caused hyperalgesia.⁶⁾ There are some reports about the injection doses or injection routes of zaprinast. In the rat formalin test, the ED₅₀ values (95% confidence intervals) of intrathecal zaprinast was 44 (24–81) μ g in phase 1, and 62 (39–100) μ g in phase 2.⁹⁾ In the rat paw model of carrageenan-induced (250 μ g) hyperalgesia, 50 μ g/10 μ l of zaprinast was injected per paw and which had no effect when administered alone.¹⁴⁾ On the other hand, in this study, the ED₅₀ values (95% confidence intervals) of intrathecal zaprinast was 17.4 (14.7–20.5) μ g and it was relatively small dose compared to other studies. Although intrathecal zaprinast (100 μ g) showed antiallodynic effect in the present study, intrathecal sildenafil (100–200 μ g) produced mechanical hyperalgesia.⁶⁾ Such a discrepancy between this study and those reported in the literature may be caused by the route of the drugs given, the kinds of animal, and the different pain models used.

In conclusion, intrathecally administered zaprinast produced a dose-dependent antiallodynic effect in the CCI neuropathic pain model. These findings suggest that spinal phosphodiesterase 5, 6 and 9 may play an important role in the modulation of neuropathic pain state. Future studies are needed to further explain the sites and mechanisms of these actions. Also, clinical investigations are needed to identify specific settings and patient populations in which intrathecal zaprinast may be useful.

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