Response After Repeated Ketamine Injections in a Rat Model of Neuropathic Pain

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Summary

Ketamine, an *N*-methyl-D-aspartate antagonist, reduces pain by decreasing central sensitization and pain windup. However, chronic ketamine use can cause tolerance, dependency, impaired consciousness, urinary symptoms, and abdominal pain. This study aimed to investigate the effects of repeated ketamine injections and ketamine readministration after discontinuation in a rat model of neuropathic pain. To induce neuropathic pain, partial sciatic nerve ligation (PSNL) was performed in 15 male Wistar rats, and these animals were divided into three groups: PSNL (control), PSNL + ketamine 5 mg/kg (K5), and PSNL + ketamine 10 mg/kg (K10; n=5 each). Ketamine was injected intraperitoneally daily for 4 weeks, discontinued for 2 weeks, and then readministered for 1 week. Following PSNL, the mechanical withdrawal threshold was determined weekly using the Von Frey. The K10 group showed a significant increase in the mechanical withdrawal threshold, presented here as the target force (in g), at 21 and 28 days compared to the time point before ketamine injection (mean±SE, 276.0±24.0 vs. 21.6±2.7 and 300.0±0.0 vs. 21.6±2.7, respectively; $P<0.01$) and at 14, 21, and 28 days compared to the control group (108.2±51.2 vs. 2.7±1.3, 276.0±24.0 vs. 2.5±1.5, and 300.0±0.0 vs. 4.0±0.0, respectively; $P<0.05$). However, in the K10 group, the ketamine effects decreased significantly at 7 days after readministration compared to those after 28 days of repeated injections ($P<0.05$). In the K10 group, repeated ketamine injections showed a significant increase in antinociceptive effect for >2 weeks, but this ketamine effect decreased after drug readministration.

Key words

Ketamine • Neuropathic pain • Rat • Partial sciatic ligation

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Introduction

Neuropathic pain, which is often severe and chronic, is a dysfunction of the central or peripheral somatosensory nervous system [1]. Chronic neuropathic pain activates *N*-methyl-D-aspartate (NMDA) receptors and increases the influx of calcium ions into cells, which in turn amplifies the pain signal, resulting in the neuronal plasticity and central sensitization [2]. Neuropathic pain may be a chronic, refractory disorder, and there are many patients with poor responses to various treatment approaches like nerve block and medication. Among suggested therapy options, ketamine has been used to treat neuropathic pain via intravenous injection or oral administration.

Ketamine, an NMDA antagonist binding to its phencyclidine site, reduces the channel opening frequency. This drug is also known to alleviate neuropathic pain by decreasing central sensitization and pain windup [3]. In addition, ketamine is a weak agonist of mu-opioid receptors by inducing dopamine release, which relieves pain; its opioid-sparing effect was also reported in a previous study [4]. In a rat model of neuropathic pain induced by partial sciatic nerve ligation (PSNL), thermal and mechanical allodynia was relieved

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after 2 weeks of ketamine injections [5]. Furthermore, pain relief can be achieved by injecting ketamine and magnesium for 2 weeks in patients with postherpetic neuralgia [2].

Ketamine has been used to treat neuropathic pain for many years. However, chronic neuropathic pain requires continuous treatment, and it is necessary to consider the unwanted effects that may occur with chronic ketamine use. Repeated administration of ketamine can affect dopaminergic, serotoninergic, and gamma-aminobutyric acid (GABA)ergic transmission in various ways [6], and according to the UK National Poisons Unit, the incidence of acute ketamine poisoning increased from 285 cases in 2000 to 1710 cases in 2009 [7]. Long-term ketamine use can cause impaired consciousness, ulcerative cystitis, kidney dysfunction, and intense abdominal pain called K-cramp. Since ketamine acts on the NMDA receptor which is associated with synaptic plasticity, its long-term use can affect working and episodic memory. In murine experiments, repeated ketamine injections of 5 mg/kg daily for 2 weeks damaged the fear memory and showed lasting effects on the encoding of sensory stimuli [8]. To date, guidelines specifying treatment duration and dose have not been established for the use of ketamine in patients with neuropathic pain.

Thus, the purpose of this study was to examine in a rat model of neuropathic pain the effects of repeated ketamine administration for 4 weeks with readministration after drug discontinuation and to evaluate the unwanted ketamine effects in this model.

Methods

Animals and animal care

Male Wistar rats (aged 6–8 weeks, weighing 150 g) used in the neuropathic pain model were provided by the Inha University Experimental Animal Center (Oriental Bio, Korea). Prior to experimental procedures, the rats were individually housed in ventilated cages for 7 days at a room temperature of 22±0.5 °C and humidity of 60 % for environmental adaptation. The study was approved by the Institutional Animal Care and Use Committee of Inha University, and all experimental procedures and breeding involving animals were approved by the Committee of Research Facilities at Inha University. The rats were treated in accordance with the guidelines of the Ethics Committee for Animal Research and the Inha University Experimental Animal Center Guidelines.

Partial sciatic nerve ligation, a rat model of neuropathic pain

PSNL was performed as follows: All rats were anesthetized with a mixture of isoflurane and oxygen. For surgery, the right hind paw of the rat was positioned on a sterile operating table. An incision was made 1 cm distal to the trochanter of the right femur, and the muscle was bluntly dissected to expose the sciatic nerve near the trochanter just distal to the point at which the posterior biceps semitendinosus nerve branches off the common sciatic nerve. The surrounding tissue was carefully removed to expose the sciatic nerve, and half of the right sciatic nerve was ligated with an 8-0 silk suture. The wound was then closed with two muscle and four to five skin sutures [9].

Hind paw sensitivity to tactile stimulation determined with the von Frey test

To quantify mechanical nociception in the PSNL model, Von Frey filaments (Stoelting, Wood Dale, IL, USA) with incremental stiffness (1.65, 2.46, 2.44, 2.83, 3.22, 3.61, 3.84, 4.08, 4.17, 4.31, 4.56, 4.74, 4.93, 5.07, 5.18, 5.46, 5.88, 6.10, 6.45, and 6.65) were applied to the lateral surface of the right hind paw on a wire mesh experimental table with an eye size of 2×2 mm. The Dixon up-and-down method was used to assess mechanical allodynia [10]. If rats responded to a filament of a certain force(g) by lifting the injured paw to avoid the unpleasant stimulus, a von Frey filament with a lower force was used next. If the rats did not respond, a von Frey filament with a higher force was applied.

A cutoff pressure of 300 g was used to avoid tissue damage. The force (g) at which the paw was moved was recorded as the tactile threshold, and the percentage of maximal possible effect (%MPE) was calculated as follows: *% MPE = [{post-drug threshold (g) − post-injury baseline threshold (g)} / {cutoff threshold (300 g) − post-injury baseline threshold (g)}] × 100* [7].

Experimental protocols

Pretreatment

After adapting to the environment for 7 days, a 6.65 von-filament test was performed on both paws, and as a result, it was confirmed that all rat avoided or did not guard when the filament touched them.

The rats were divided into three groups: PSNL (control) (n=5), PSNL + ketamine 5 mg/kg (K5) (n=5),

and PSNL + ketamine 10 mg/kg (K10) (n=5).

After inducing neuropathic pain in model rats, the presence of neuropathic pain was confirmed by the detection of pain signs such as guarding and avoidance behavior of the injured paw or an avoidance reaction for stimuli of <26 g at 7 days after PSNL surgery.

Posttreatment

K5 and K10 were injected intraperitoneally daily for 28 days after PSNL induction, discontinued for 14 days, and then injected again for 7 days. A chart of the study design is shown in Fig. 1. The von Frey test was performed on days 7, 14, 21, 28, 35, 42, 49, and 56 after PSNL surgery.

Statistical analysis

All variables are expressed as means and standard errors. The Friedman rank-sum test and the Nemenyi post-hoc test were used to compare the effects of ketamine in rats before and after drug injection. The effects of ketamine over time in each group were compared using repeated-measures analysis of variance (P<0.05). For three-group comparisons at all time points, the Kruskal-Wallis test was used. The post-hoc Nemenyi multiple comparison test was used to compare differences at all time points between groups with significant differences in the Kruskal-Wallis test. All statistical analyses were performed using R software version 4.0.1. Statistical significance was set at P<0.05.

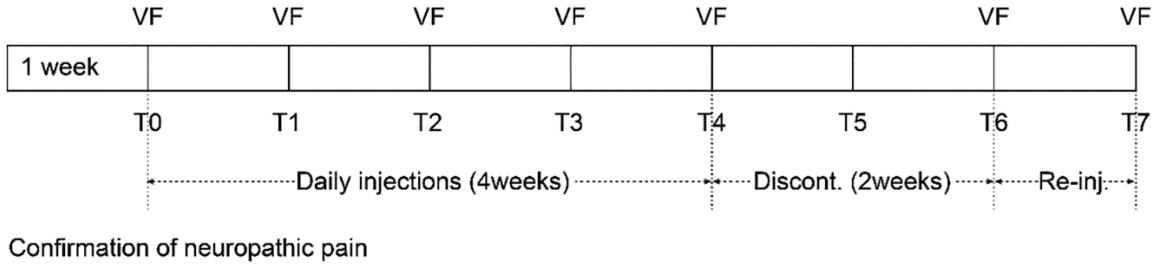

Fig. 1. Schematic timeline of the experimental design. VF: von Frey test.

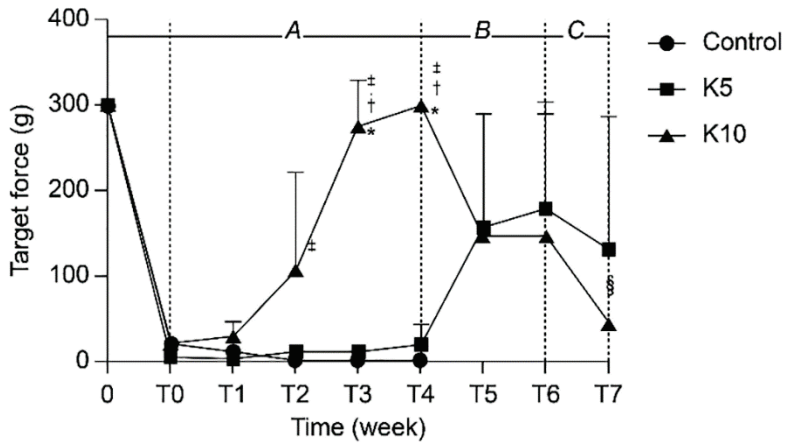

Fig. 2. Time course of paw withdrawal responses to von Frey filaments as the target force after partial sciatic nerve ligation (PSNL). Data are presented as the withdrawal threshold (g). The mean ± SEM of all values is shown for each time point. Statistical significance is determined by comparing data with those at T0 in each group (*P<0.01) and with those at T1 in the K10 group (†P<0.01). Significant differences between K10 and control groups are indicated for each time point (‡P<0.05). In the K10 group, the target force is significantly decreased at T7 compared to T4 (§P<0.05). T0: neuropathic rat model confirmed, T1: 1 week after ketamine injection, T2: 2 weeks after ketamine injection, T3: 3 weeks after ketamine injection, T4: 4 weeks after ketamine injection, T5: 1 week after ketamine discontinuation, T6: 1 week after ketamine discontinuation, T7: 1 week after ketamine readministration, **A**: repeated ketamine injections, **B**: ketamine discontinuation, **C**: ketamine readministration.

Results

Comparison of ketamine effects by time point in each group

The control group showed no tendency indicating that the pain perception deteriorated over time. Similarly, no statistically significant ketamine effects over time were observed in the K5 group. However, the K10 group showed a statistically significant increase in the mechanical withdrawal threshold, presented as the target force, at T2 the target force showed a tendency to increase compared K5 group and control group and at time points T3 and T4 compared to T0 (276.0 g vs. 21.6 g and 300.0 g vs. 21.6 g, respectively; *P<0.05) and T1

(276.0 vs. 32.6 and 300.0 vs. 32.6, respectively; †P<0.01; Fig. 2). The percentage of the maximal possible effect, %MPE, also showed significant differences at time points T3 and T4 compared to T1 in the K10 group (91.24 % vs. 3.13 % and 100 % vs. 3.13 %, respectively; †P<0.05). In the K10 group, the target force and %MPE were significantly decreased at time point T7 compared to the corresponding values at time point T4 (45.2 vs. 300.0 [§P<0.05] and 8.63 % vs. 100 % [‡P<0.05], respectively).

Comparison of ketamine effects between groups at each time point

The target force was significantly different in the K10 group compared to the control group at the time points T2 (108.0 vs. 2.7, ‡P<0.05), T3 (276.0 vs. 2.5, ‡P<0.05), and T4 (300.0 vs. 4.0, ‡P<0.05). The %MPE values in the K10 group also were significantly different compared to those in the control group at time points T3 and T4 (-4.91 % vs. 91.24 % and -3.86 % vs. 100 %, respectively; †P<0.05; Fig. 3). By contrast, there was no statistically significant ketamine effect in the K5 group in comparison to the control group at any time point.

Unwanted effects

One rat in the K5 group showed hematuria symptoms after 25 days of ketamine injections. No other ketamine-related symptoms were observed during the study period.

Behavior was observed during ketamine treatment in neuropathic pain rat, but no special test was conducted.

Neuropathic pain rats showed spontaneous pain to reluctance to foot weight in the injured paw after the procedure. In the all ketamine-injected group, they supported their body with both paws after 2 weeks and showed a comfortable appearance during the experiment compared to the control group.

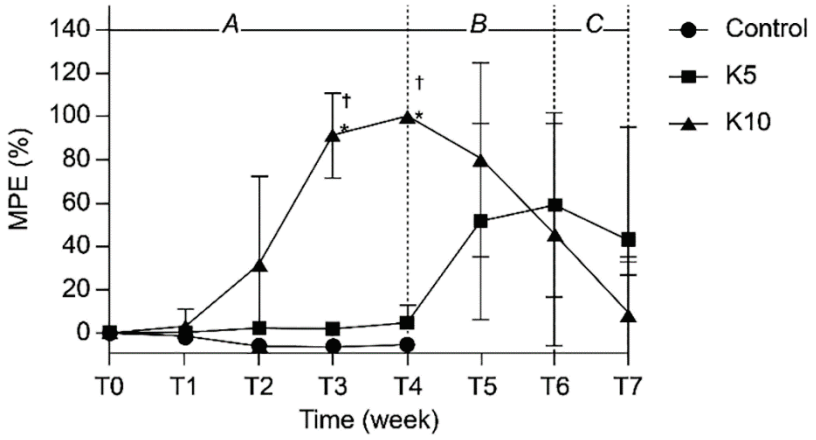

Fig. 3. Data are presented as the percent of maximal possible effect (%MPE). Each point on the graph represents the mean ± SEM of 5 rats. Statistical significance is determined by comparison with T1 in each group (*P<0.01). Significant differences between K10 and sham-ligated groups are indicated for each time point (†P<0.05). In the K10 group, the %MPE value is statistically significantly decreased at T7 compared to T4 (‡P<0.05). T0: neuropathic rat model confirmed, T1: 1 week after ketamine injection, T2: 2 weeks after ketamine injection, T3: 3 weeks after ketamine injection, T4: 4 weeks after ketamine injection, T5: 1 week after ketamine discontinuation, T6: 1 week after ketamine discontinuation, T7: 1 week after ketamine readministration, **A**: repeated ketamine injections, **B**: ketamine discontinuation, **C**: ketamine readministration.

Discussion

This study investigated the antinociceptive effects of repeated ketamine injections, followed by discontinuation for 2 weeks and readministration for 1 week, in a rat model of neuropathic pain. The main findings were that repeated ketamine injections in the K10 group showed a significant increase in antinociceptive effect for >2 weeks, but this effect was decreased after ketamine had been administered for another 1 week following 2-week treatment discontinuation.

In this study, the antinociceptive effect in the K10 group increased for nearly 3 weeks to its maximum compared to the control group and to the conditions before ketamine injection in the K10 group. From the time T2, the target force showed a tendency to increase, and the avoidance behavior also decreased significantly in the K10 group**.** These findings are not consistent with those of a previous study that in the formalin test, acute pain in rats is relieved immediately by ketamine injection and the total time spent on pain-related behavior decreases significantly even in the group injected with only 5 mg/kg ketamine [3]. These differences may be attributed to the fact that ketamine might have different mechanisms of action depending on the type of pain or that the appropriate pain control dose has not been reached. In future studies, different therapeutic doses of

ketamine should be applied depending on the type of pain, and an appropriate dosage standard must be established.

In the K5 group, the antinociceptive effect was not significantly altered according to the duration of ketamine treatment; however, the pain intensity decreased after ketamine had been discontinued. In the K10 group, after discontinuation of ketamine, the antinocicetpive effect gradually decreased, even after re-injection of ketamine for 1 week. These results suggest the development of ketamine tolerance.

However, the time may have been insufficient for the ketamine effects to fully develop, and tolerance to ketamine may be influenced by various mechanisms. It was reported that when ketamine was pretreated for 14 days in rats, the anesthetic dose of ketamine was increased, but it was the case of high concentration of 100 mg/kg injected, and there was no significant difference between the control group in the case of the 32 mg/kg group [11]. This indicates that ketamine tolerance may vary depending on ketamine dose or experimental conditions.

Thus, further studies are required considering various conditions. On the other hand, in the K5 group, antinociceptive effect appeared after discontinuation. It is possible that the effect was delayed because a lower dose was administered compared to K10, and further studies on the appropriate dose and administration period of ketamine are needed.

The pathophysiology of neuropathic pain involves ectopic nerve injury and central sensitization. Ectopic stimuli continually occur in nociceptive pathways, leading to changes in nociceptive thresholds. In addition, secondary allodynia and hyperalgesia may occur in adjacent areas, causing structural damage to the central nervous system and the development of central sensitization.

The release of excitatory neuropeptides from peripheral afferent fibers causes changes in postsynaptic NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors. The inhibition of GABAergic interneurons in the spinal horn is decreased, resulting in pain exacerbation [12]. Because of the various mechanisms involved, sufficient pain relief for neuropathic pain can hardly be expected, and treatment options are limited.

Ketamine can act as an antagonist of GABA receptors, prevent pain windup, relieve pain by strengthening inhibitory pathways, and have opioid-sparing effects [8]. Low-dose ketamine has also been used for treating complex regional pain syndromes, and its combination with benzodiazepines has been used for acute pain management and treatment of psychosis-like effects [13]. In one study, ketamine was continuously administered for 3 weeks in patients with phantom limb pain, and pain control was reported for up to 5 months [14]. In another study, refractory pain subsided with ketamine 5 mg administered via an epidural catheter in patients with postherpetic neuralgia [15]. Future studies are necessary to investigate the different mechanisms of ketamine and whether these mechanisms act differently depending on the type of pain or the ketamine dose.

A meta-analysis published in 2018 showed high heterogeneity in both duration and dosage of ketamine treatment; ketamine treatments in humans varied from 1 to 90 days [16]. In a study examining the ketamine effects in rats with neuropathic pain, intraperitoneal injection of ketamine (5 mg/kg) followed by subcutaneous injection of magnesium (5 mg/kg) significantly reduced the antinociceptive response [3]. In another study investigating the antinociceptive effects of ketamine in a model of chronic constriction injury, the latency of the pressure reaction was increased only in the group treated with 50 mg/kg ketamine [6]. Various studies suggest different ketamine doses for the treatment of pain with different underlying mechanisms. Thus, a standardized therapeutic dose according to the pain mechanism should be established and verified in large-scale randomized controlled trials.

Neuropathic pain that progresses to chronic pain requires continuous treatment. However, long-term ketamine treatment can cause various adverse effects, such as cognitive impairment and urinary dysfunction, as well as secondary side effects when used with benzodiazepines. Although many studies investigated the antinociceptive effects of ketamine, very few studies have examined unwanted effects associated with ketamine long-term treatment. In one study on side effects, impairments in fear memory were reported when 5 mg/kg ketamine was injected daily for 4 weeks [8]. In the study by Ng et al., ketamine users who visited the emergency department reported cognitive dysfunction, lower urinary tract symptoms, abdominal pain, and dizziness [17].

Recently, studies have investigated the synergistic effects of ketamine and magnesium, which both act as NMDA receptor antagonists [3]. Reducing the ketamine dose through combined administration with magnesium has the advantage of minimizing unwanted

ketamine effects. This drug combination is associated with decreased pain-related behavior [1]. In patients with postherpetic neuralgia, reduced pain was reported after two weeks of treatment with ketamine (1 mg/kg) and magnesium sulfate (5 mg/kg) [2]. Additionally, it is necessary to study the effects of ketamine administered via various routes, such as injection into the epidural space, which can reduce systemic absorption, or oral ketamine delivery.

This study has some limitations. The results might have been affected by the study procedure because the rats were subjected to the stress of repeated injections. It was also difficult to confirm the occurrence of unwanted effects such as tolerance or physical dependence because the period of ketamine readministration was short, so a longer research period will be needed in future studies.

Conclusions

This study investigated the effects of continuous ketamine injection in rats with neuropathic pain. Repeated injections of 10 mg/kg ketamine resulted in a significant pain reduction for >2 weeks; however, when ketamine was readministered for 1 week after 2 weeks of discontinued treatment, the antinociceptive effect decreased.

For ketamine to be applied clinically in the future for neuropathic pain control, its unwanted and adverse effects during long-term treatment must be identified, appropriate therapeutic doses must be established, and different treatment guidelines according to the pain mechanism must be implemented. The rat model of neuropathic pain was used for the guidelines for ketamine dose and duration of treatment, and it can be a basis to develop guidelines for the treatment of chronic pain patients in the future.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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