Intrathecal Ketamine and Pregabalin at Sub-effective Doses Synergistically Reduces Neuropathic Pain without Motor Dysfunction in Mice

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Received August 28, 2012; accepted October 8, 2012

Peripheral or central nerve injury often leads to neuropathic pain. Although ketamine and pregabalin are first line options for the treatment of neuropathic pain, their clinical application is limited due to side effects such as sedation, dizziness and somnolence. We designed this model to determine whether the intrathecal (i.t.) co-treatment with ketamine and pregabalin at sub-effective low doses would elicit a sufficient pain relief without producing side effect in a neuropathic pain mouse model. At day 7 after chronic constriction injury (CCI) of sciatic nerve, dose dependent effects of i.t. ketamine (3, 10, 30, 100 µg) or i.t. pregabalin (10, 30, 100 µg) on mechanical allodynia and thermal hyperalgesia were measured. For combination treatment, 3 or 10 µg of ketamine and 30 µg of pregabalin were selected because these doses of drugs were not effective on neuropathic pain. Interestingly, combined i.t. treatment groups (ketamine 3 µg+pregabalin 30 µg and ketamine 10 µg+pregabalin 30 µg) produced strong analgesia on neuropathic pain although these doses of ketamine and pregabalin alone are not effective. Moreover, rota rod test revealed that normal motor function was not affected by combined treatment while i.t. ketamine at doses above 10 µg showed a significant motor dysfunction. Results of this study suggested that i.t. co-treatment with ketamine and pregabalin at sub-effect low doses may be a useful therapeutic method for the treatment of neuropathic pain patients.

Key words neuropathic pain; intrathecal; combination drug therapy; ketamine; pregabalin; mouse

Peripheral or central nerve injury by a cancer, trauma, and/or metabolic disease condition may evoke chronic neuropathic pain which is characterized by a presence of spontaneous pain (pain sensation without any stimulation), allodynia (pain sensation by a non-noxious stimulation), and hyperalgesia (enhanced pain by a noxious stimulation).1–4) The development and maintenance of neuropathic pain have been known to be closely associated with a variety of pathophysiologic changes, including peripheral and central sensitization.5–9) At the periphery, up-regulation of α2δ subunit of voltage-dependent calcium channel in the dorsal root ganglion correlates with the onset of tactile allodynia in spinal nerve-injured rats.10) Recently marketed drugs, gabapentin and pregabalin are used as a first line option for the treatment of neuropathic pain and its pain relief effect is induced by a blockade of Ca 2+ subunit Ca 2+ channel blockers has been expressed in the central nervous system and directly involved in the excitatory neurotransmission.10) NMDA receptor plays an important role in the development and maintenance of central sensitization, a state of long-lasting increase in synaptic transmission, and neuropathic pain.11) Spinally administered NMDA receptor antagonist including memantine and AP-5 remarkably attenuates spinal nerve ligation-induced allodynia. However, no antinociceptive effect is seen when NMDA antagonists are treated into the supra-spinal site (i.e. intracerebroventricular injection), suggesting that spinal NMDA receptor may be a main target to treat neuropathic pain.11) One of NMDA antagonists, ketamine has been used as an anesthetic or analgesic both in human and animal. However, serious side effects of NMDA antagonists have been reported including a motor impairment as well as sedation. In this regard, newer approach strategy using a NMDA blocker to treat neuropathic pain is required.

We hypothesized that the combining treatment with ketamine and pregabalin at sub-effective doses would suppress synergistically nerve injury-induced neuropathic pain without producing side effect. To examine this hypothesis, chronic constriction injury (CCI) neuropathic pain mouse model was employed. Ketamine or pregabalin was intrathecally injected to determine their individual dose-dependent analgesic effect. Based on the result of dose–response curve, these two agents were intrathecally co-administrated at sub-effective low doses. In addition, side effect such as motor dysfunction was examined by a rota-rod test.
MATERIALS AND METHODS

Animals Experiments were performed using male ICR mice (25–30 g), purchased from the Samtako Bio Korea (Kyoung-Ki, Korea). All animal experimentation adheres to the policy of the Chungnam National University regarding the use and care of animals. Animals were housed in a standard environment consisting of a 12-hour light/dark cycle, a constant room temperature (maintained between 20, 25°C), and 40–60% humidity. Food and water were given freely throughout the investigation.

Induction of Neuropathic Pain: Chronic Constriction Injury (CCI) Model CCI neuropathic pain model was originally introduced by Bennett and Xie in rats19,20 and recently modified for mice.21,22 Briefly, mice were anesthetized with Zoletil® (5 mg/kg, tiletamine HCl+zolazepam HCl, Virbac Laboratories, Carros, France) and Rompun® (0.75 mg/kg, Xylazine HCl, Bayer Korea, Ansan, Korea). The right sciatic nerve was exposed at the mid-thigh level, and 3 loose ligatures of 4-0 chromic gut were placed with a 0.5-mm interval until the brief twitch was elicited in the right hind paw. During recovery, mice were housed in a temperature-controlled plastic cage with a thick layer of sawdust bedding.

Intrathecal Treatment with Ketamine and Pregabalin All behavioral experiment was performed on a day 7 after CCI induction. Ketamine (3, 10, 30, 100 µg) and pregabalin (10, 30, 100 µg) were prepared by diluting with artificial cerebrospinal fluid (aCSF, in mM: NaCl 126; NaHCO3 26; KCl 5; NaH2PO4 2.4; CaCl2 2.4; MgCl2 1.2; glucose 10; pH was 7.3–7.4). For combination treatment tests, ketamine (3 µg)+pregabalin (30 µg) or ketamine (10 µg)+pregabalin (30 µg) was prepared.

The intrathecal (i.t.) injection was performed according to the previously described method.23 Briefly, a volume of 10 µL solution was intrathecally injected into lumbar spinal area using a 30-G Hamilton syringe attached with a 30-gauge needle. The gentle flinching behavior of tail was considered indicative of a successful i.t. administration. The control group mice received an i.t. injection of vehicle (aCSF).

Behavior Assessments Prior to the main experiment, pain behavior such as mechanical allodynia and thermal hyperalgesia was examined to confirm the development of neuropathic pain in CCI model. The number of paw withdrawal responses to normally innocuous mechanical stimuli was measured by using a von Frey filament of 2.0 g (North Coast Medical, Morgan Hill, CA, U.S.A.). In a preliminary experiment, von Frey hairs producing forces of 0.07, 0.16, 0.6, 2.0, 6.0, and 10.0 g were examined to determine a sub-threshold mechanical force. The 2.0-g hair was selected for further testing because it did not induce a paw withdrawal response, whereas both the 6.0-g and 10.0-g filaments produced withdrawals. Briefly, mice were placed on a metal mesh grid under a plastic chamber, and von Frey filaments were applied from underneath the metal mesh floor to each hind paw for 10 trials at approximately 10-s intervals. The number of paw withdrawal responses after each stimulus was then counted. The results of mechanical alldyinic behavior in each experimental animal were presented as a percentage of withdrawal response frequency (WRF, %).

To assess nociceptive responses to heat stimuli, we measured paw withdrawal response latency (WRL) by using the procedure previously described.24 Briefly, mice were placed in a plastic chamber (20 cm in diameter and length) with a glass floor and allowed to acclimate for 10 min before testing. A radiant heat source was positioned under the glass floor beneath the hind paw to be tested, and withdrawal latency was measured to the nearest 0.1 s by using a photoelectric cell connected to a digital clock. The intensity of the light source was calibrated to produce withdrawal response within 12 to 15 s in normal animals. The test was duplicated in each hind paw at each time point, and mean withdrawal latency was calculated. Cut-off time in the absence of a response was 20 s to prevent the possible tissue damage.

Rota-Rod Test The rota-rod test is a commonly used screening procedure to detect motor incoordination and/or ataxia in rodents.25 Using a rota-rod tester (SciTech Korea Inc., Seoul, Korea), mice were placed on a cylindrical platform (12-cm wide; 6-cm diameter) suspended 33 cm above the bottom of the apparatus. Escape to either side was prevented by Plexiglas walls, and falls were cushioned by wood shaving bedding. Rota-rod test has done before and at every 0, 30, 60 and 90 min after i.t. injection of test drugs. Spent time on a rotating rod (constant speed of 3 rpm) was measured at each time point and cut-off time for this test was 2 min.

Statistics Data are expressed as the mean±standard error of mean. The statistical significance of differences between control and treated-groups was determined by two-tailed Dunnnett’s test following one-way analysis of variance (ANOVA). A p value less than 0.05 was considered to be statistically significant.

RESULTS

CCI-Induced Neuropathic Pain and Effects of Intrathecal Ketamine and Pregabalin From 1 d after CCI induction, prominent neuropathic pain responses such as mechanical allodynia in CCI-induced neuropathic pain at day 7 after CCI induction in further experiments.

At day 7 after CCI induction, i.t. ketamine at a dose of 100 µg significantly reduced both of mechanical allodynia (Fig. 1C, ***p<0.001 vs. control) and thermal hyperalgesia (Fig. 1D, *p<0.05 vs. control). However, lower doses (3, 10 µg) of ketamine did not produce any noticeable analgesic effect.

In a separate experiment, i.t. pregabalin significantly reduced mechanical allodynia (Fig. 1E, *p<0.05 and **p<0.01 vs. control), however analgesic effect of pregabalin on thermal hyperalgesia was less than the effect of mechanical allodynia (Fig. 1F, **p<0.01 vs. control). Previous report indicates that the analgesic effects of pregabalin is not particularly dose-dependent, with the middle dose often producing greater effects than the highest dose, and occasionally the lowest dose producing the greatest effects.10 This pattern suggests that pregabalin may be producing an inverted-U or bell shaped dose response curve, as has been noted for other analgesics.26,27

Effect of Co-administrated Ketamine and Pregabalin on Neuropathic Pain and Motor Function Interestingly, combination of i.t. ketamine with pregabalin (3 µg ketamine+30 µg pregabalin or 10 µg ketamine+30 µg pregabalin) remarkably suppressed CCI-induced mechanical allodynia (Fig. 2A,
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**p < 0.05, **p < 0.01 and ***p < 0.001 vs. control) and thermal hyperalgesia (Fig. 2B, *p < 0.05, **p < 0.01 and ***p < 0.001 vs. control). These results strongly suggested that combination treatment by using i.t. ketamine and pregabalin at sub-effectice doses may be a useful method to treat neuropathic pain.

Spent time on a rotating rod was significantly decreased by i.t. ketamine at doses above 3 µg (Fig. 2C, *p < 0.05 and ***p < 0.001 vs. control), however combination treatment of ketamine with pregabalin (3 µg ketamine + 30 µg pregabalin and 10 µg ketamine + 30 µg pregabalin) did not alter the normal motor function (Fig. 2D).

**DISCUSSION**

Neuropathic pain patients commonly suffer from severe and intractable pain such as spontaneous pain and abnormal tactile and thermal responses to stimuli. Since the efficacy of conventional analgesics for the treatment of neuropathic pain is not sufficient and unwanted side effects limit their clinical use, development of newer analgesic or treatment method is required.128 In this regard, combination drug therapy may have greater advantages to treat neuropathic pain patient because multiple mechanism of action enhances therapeutic...
efficacy and reduced treatment dosage decreases the incidence of side effects. Results of this study revealed that combining treatment with i.t. ketamine (3 or 10 \( \mu \)g) and i.t. pregabalin (30 \( \mu \)g) significantly reduced CCI-induced neuropathic pain although these doses of ketamine and pregabalin alone were ineffective. In addition, these combined treatments did not alter the normal motor function as compared with higher doses of ketamine (10, 30, 100 \( \mu \)g). Taken together, i.t. co-treatment of ketamine and pregabalin at sub-effective lower doses exhibited a synergic antinociceptive effect on CCI mice model without producing unwanted side effect such as a motor dysfunction, suggesting this combination drug therapy may be useful to cure neuropathic pain patient.

At the spinal dorsal horn, metabotropic and ionotropic glutamate receptors are closely associated with the development and maintenance of neuropathic pain. Among these various glutamate receptors, the actions of excitatory amino acids such as glutamate on the NMDA receptor of second order neuron is known to be critical to produce the phenomenon of ‘wind up’ and neuronal hyperexcitability which can lead to neuropathic pain. This suggests that drugs, capable of inhibiting NMDA receptor activity, may have potential therapeutic effect on neuropathic pain. Several uncompetitive NMDA receptor antagonists including dextromethorphan, amantadine, memantine, and ketamine have been reported to relieve pain in various neuropathic pain states including phantom limb pain, central neuropathic pain, postherpetic neuralgia, and peripheral neuropathic pain. Despite NMDA receptor plays a critical role in the development and maintenance of neuropathic pain, clinical application of NMDA antagonists has been limited. Clinical study shows that epidural ketamine can be used to alleviate neuropathic pain at a sub-anesthetic dosage due to the sedation and other side effects when it is applied at higher dose. These previous reports suggested that the clinical utility of NMDA antagonists for the treatment of neuropathic pain is compromised by their small therapeutic window and a newer treatment strategy is needed for the safe use of NMDA antagonist such as ketamine to cure the neuropathic pain patient. In the present study, i.t. ketamine attenuates nerve injury-induced mechanical allodynia and thermal hyperalgesia on a day 7 after CCI. However, rota-rod test revealed that the higher doses of ketamine (10, 30, 100 \( \mu \)g) significantly produced motor dysfunction.

Pregabalin was originally marketed as an anticonvulsant and recently emerging as first-line option to treat neuropathic pain. Although pregabalin was designed to act as an agonist for gamma aminobutyric acid (GABA) receptor, its major effect on neuropathic pain is likely to be mediated by binding to the \( \alpha2\delta \) subunit of voltage-gated Ca\(^{2+}\) channels and subsequent inhibition of glutamate release in the spinal dorsal horn.

Fig. 2. Effect of Intrathecally Co-administered Ketamine (Ket) and Pregabalin (Pre) on Nerve Injury-Induced Mechanical Allodynia (A) and Thermal Hyperalgesia (B)

Effect of ketamine only (C) and ketamine+pregabalin co-treatment (D) on motor function. *\( p<0.05 \), **\( p<0.01 \) and ***\( p<0.001 \) vs. control.
Similar results revealed that i.t. pregabalin produces greater efficacy than systemic treatment, suggesting that the spinal cord may be a main target area of pregabalin. However, its full mechanism of pain relieving effect on neuropathic pain is still not clear. Results of this study showed that analgesic effect of i.t. pregabalin (100 µg) on CCI-induced mechanical allodynia was greater than thermal hyperalgesia, consistently with a previous report. This indicates that additional drug treatment with pregabalin may be needed to suppress various nerve injury-related pain symptoms. Although pregabalin shows improved efficacy than gabapentin, relatively high dose of pregabalin is still needed to successfully relieve neuropathic pain. This indicates that incidence of side effects by pregabalin such as dizziness and somnolence would be also increased due to high dose of drug for sufficient pain relief.

In conclusion, results of this study revealed that combination drug therapy by using i.t. ketamine and pregabalin at sub-effective lower doses (3 µg ketamine+30 µg pregabalin and 10 µg ketamine+30 µg pregabalin) sufficiently decreased CCI-induced neuropathic pain in mice and this combined treatment may be useful for the treatment of neuropathic pain patient since dual action mechanism of ketamine and pregabalin can synergistically produce antinociception. Moreover these combined treatments did not alter the normal motor function as compared with higher dose of ketamine (10, 30, 100 µg), suggesting that the i.t. co-treatment with ketamine and pregabalin at sub-effective doses may be useful method to cure neuropathic pain patients.

Acknowledgements This study was supported by research fund of Chungnam National University in 2009 (2009-0971).

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