Steroid on Hyperalgesia in Nerve Ligation Induced Neuropathic Pain Rat Model

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**Background:** Neuropathic pain is resistant to conventional treatments, and may seriously affect the quality of life. Insufficient has been done on drug combination for the treatment of neuropathic pain. So we undertook to determine the effect of ketamine and steroid on mechanical hyperalgesia in rats with spinal nerve ligation.

**Methods:** Rats were administered L5 and L6 spinal nerve ligation to cause mechanical hyperalgesia. Control group (n = 6) were administered normal saline 5 ml intraperitoneally, the ketamine group (n = 6) ketamine 1mg/kg, and the steroid group (n = 6) ketamine 1 mg/kg and methylprednisolone 10 mg/kg. Mechanical hyperalgesia was assessed using a von Frey filament before injection, and then 15, 30, 60, 120, and 180 min after injection.

**Results:** Ketamine 1 mg/kg significantly attenuated mechanical hyperalgesia for 60 min. The combination of ketamine 1 mg/kg and methylprednisolone 10 mg/kg also significantly attenuated mechanical hyperalgesia. But the combination group did not exert a superior attenuating effect than ketamine alone.

**Conclusions:** The combination of ketamine and methylprednisolone did not exert a superior antinociceptive effect than ketamine alone in rats with spinal nerve ligation. (Korean J Anesthesiol 2004; 47: S 14 – S 17)

**Key Words:** combination, ketamine, methylprednisolone, neuropathic pain.

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**INTRODUCTION**

The main symptoms of neuropathic pain are spontaneous pain, hyperalgesia and allodynia. Intensive research has been focused on the mechanisms and treatment of neuropathic pain for the past decade. There has been trend that reduces side-effects of each drug and enhances analgesic effect by combination of various analgesics in the pain management.1

It has been proposed that N-methyl-D-aspartate (NMDA) receptor is involved in the development and maintenance of neuropathic pain.2-3 Ketamine, non-competitive NMDA receptor antagonist, 1.0 mg/kg attenuates mechanical hyperalgesia for 60 min after intraperitoneal injection in spinal nerve ligated rats. 25 mg/kg ketamine extends the degree and the period of anti-hyperalgesic activity compared to 1 mg/kg, but produces prolonged motor impairments.4

There is controversy about the efficacy of steroids in the treatment of neuropathic pain. While single intraperitoneal injection of methylprednisolone ranging from 0.01 to 12 mg/kg didn’t affect heat or mechanical withdrawal thresholds in normal rats,6 daily injection of dexamethasone reduced inflammatory reaction in rat models of neuropathic pain and blocked guarding behavior and thermal hyperalgesia.6 But glucocorticoid treatment is accompanied by serious side-effects, reflecting the symptoms of cortisol excess seen in Cushing’s syndrome patients.7

Interaction between steroids and NMDA receptors has been researched lately. While several pregn-5-ene steroids markedly potentiated NMDA-mediated [Ca²⁺] response, several steroids were identified that inhibited NMDA-induced elevations in [Ca²⁺].8-9

So the aim of this study is to investigate whether the addition of steroid to ketamine can reduce unwanted side-effects and enhance anti-hyperalgesic activity in neuropathic pain model.
MATERIALS AND METHODS

Animals

Sprague-Dawley male rats, weighing 130-160 g at time of operation, were used for the experiments. All animals were allowed to adapt to the environment 3-4 days before operation and free access to food and water until the time of the experiment. All of the testing was performed in accordance with the policies and recommendations of the International Association for the Standard of Pain (IASP) for the handling and use of laboratory animals, and approved by the Animal Care and Use Committee of the Catholic University.

Spinal nerve ligation

Nerve injury was induced using the procedure of Kim and Chung. Rats were first anesthetized with 1.5 % isoflurane in O₂ at a rate of 5 L/min. After surgical preparation, a partial excision of the left transverse process was made to expose dorsal vertebral column from L₄ to S₂. The left L₅ and L₆ spinal nerves were exposed and tightly ligated with 5-0 black silk distal to the dorsal root ganglion and proximal to the formation of the sciatic nerve. The incision was closed and the animals were allowed to recover for at least 5 days. Any rat that exhibited extensive motor deficiency (such as paw-dragging) after recovery from surgery or failed to exhibit subsequent tactile allodynia was excluded from further testing.

Behavioral tests and drug administration

At 7 days after surgery, all animals were behaviorally tested to determine the paw withdrawal threshold of injured hindpaws to mechanical stimuli. Animals were placed in a plastic cage with a wire mesh floor and allowed to explore and groom until they settled.

Mechanical stimulation is applied with von Frey filaments (bending force 47 mN) onto the ventral surface of the hindpaws. The 47-mN filament can activate nociceptors. A withdraw of the hindpaw immediately after the von Frey stimulation is recorded as a response. A single trial consists of 10 applications within approximately 30 s. A % response frequency is calculated for each trial using the following formula: (no. of foot withdraws/10) × 100 = % response frequency. Three trials are carried on per time point and a mean % response frequency is calculated.

After baseline measurement, rats were divided into three groups, first group administered intraperitoneally with ketamine 1 mg/kg, second group with ketamine 1 mg/kg and methylprednisolone 10 mg/kg, and control group with normal saline 5 ml. Behavioral tests were conducted before injection, 15 min, 30 min, 60 min, 120 min and 180 min after injection.

All behavioral data are expressed as mean ± SEM. The statistical analyses were carried out by repeated measures analysis of variance (ANOVA), with Bonferroni adjustment using LS means for multiple comparisons. Significance is indicated when P value < 0.05.

RESULTS

Following surgery, stimulation with the 47 mN von Frey filament elicited robust responses when applied to the experimental hindpaw. By the end of the first week post-surgery, the mean % response frequency following stimulation with this filament had increased to 64 ± 30% for saline group, 72 ± 25% for ketamine group, and 68 ± 29% for ketamine and methylprednisolone group.

Intraperitoneal injection of ketamine 1 mg/kg significantly attenuated the mechanical hyperalgesia; however, saline had no effect on behavioral responses (Fig. 1). When compared to pre-injection levels, ketamine significantly reduced the withdrawal response for at least 60 min, after which the withdrawal response returned to pre-injection levels. Intraperitoneal injection of ketamine 1 mg/kg and methylprednisolone 10 mg/kg.

Fig. 1. Histograms showing the effect of normal saline, ketamine, ketamine and steroid on mechanical hyperalgesia of neuropathic rats. Responses are expressed as mean ± SEM. *: P < 0.05 compared with control group. also significantly attenuated the mechanical hyperalgesia, but
did not have superior effect than ketamine alone.  
All rats administered with ketamine recovered within 5 minute and showed normal behavioral activity.

DISCUSSION

In the present study, intraperitoneal ketamine 1 mg/kg reduced mechanical hyperalgesia for 60 min without significant motor impairment, consistent with the experiment of Qian et al. Ketamine has been used as an anesthetic-anaesthetic agent for more than 30 years.

Its mechanism of action has been disputed but it is now generally agreed that one pharmacological mechanism of the antinociceptive action is specific binding to the phencyclidine (PCP) site of the NMDA receptor-gated ion channel.10

Because administration of glucocorticoid alone was not tried in the present experiment, it is not clear whether single intraperitoneal injection of methylprednisolone is effective in neuropathic pain model. Traditionally, glucocorticoids have been thought to exhibit their actions by binding to intracellular receptors and stimulating or inhibiting the transcription of the gene and hence the expression of proteins and transcription factors.11 But lately, there has been reported that glucocorticoids have a rapid and transient effect on NMDA receptor mediated Ca$^{2+}$ signalling12 and increase the level of extracellular aspartate and glutamate levels13 in rat hippocampus.

The goal of this study was to investigate whether additive effect occurs in spinal nerve ligated models by co-administration of ketamine 1 mg/kg and methylprednisolone 10 mg/kg, reducing side effects.

The rationale underlying the practice of combining drugs in pain management is based mainly on two considerations. First, combining drugs that act at different receptors and on different pain mechanisms may enhance pain relief. Second, drug combinations may allow reduction in the amount of the single components to achieve the same analgesic effect with a lower incidence of side-effects.11

No superior effect of ketamine and methylprednisolone on ketamine can be explained in several ways. First, it might be possible that the duration of methylprednisolone administration was too short that it couldn’t produce anti-hyperalgesic activity. Kingery et al.14 suggested that glucocorticoid serum concentrations must be continuously maintained at an effective level for an extended period of time to achieve anti-hyperalgesia in the complex regional pain syndrome (CRPS) model. Second, the explanation is also possible that present animal models have little inflammatory component at the time of drug administration and behavioral testing. The level of tumor necrosis factor receptor (TNFR)1 and TNFR2, products increased during inflammatory reaction, showed biphasic increase in L5 dorsal root ganglion at 6 h and 12 h after spinal nerve ligation.15

In conclusion, we showed that systemic ketamine reduced mechanical hyperalgesia in rats with spinal nerve ligation. But the combination of ketamine and methylprednisolone didn’t exhibit superior effect than ketamine alone. So we hypothesize that different doses, durations and routes of drug administration must be used to produce better results.

REFERENCES

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