Korean J Anesthesiol 2009 Apr; 56(4): 425-32
DOI: 10.4097/kjae.2009.56.4.425

□ Experimental Research Article □

Comparison of antiallodynic effect of intrathecal morphine, brimonidine and rilmenidine between neuritis and ligation injury induced neuropathic pain

Department of Anesthesiology and Pain Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Young-Kug Kim, Jong-Yeon Park, and **Jai-Hyun Hwang**

Background: Mechanical allodynia is generally resulted from nerve damage by direct injury or inflammation. Thus, this study was designed to compare the antiallodynic effect of morphine, brimonidine and rilmenidine in two models of neuropathic pain, that is, induced by nerve ligation and neuritis.

Methods: Rats were prepared with tight ligation of the L_5/L_6 spinal nerves (SNL group) or with Freund's complete adjuvant (FCA) administration evoked sciatic inflammatory neuritis (SIN group). Antiallodynic effects by intrathecal morphine, brimonidine and rilmenidine were measured by applying von Frey filaments to the lesioned hind paw. Thresholds for withdrawal response were assessed and converted to % MPE to obtain an effective dose 50% (ED 50) and a dose response curve.

Results: Either SNL group or SIN group showed marked mechanical allodynia in the lesioned hind paw. Antiallodynic effects of morphine were different between two groups. That is ED 50 was 0.16 μg (SIN) and 8.12 μg (SNL), and dose response curve of the SIN group shifted left from that of the SNL group. The difference between SIN and SNL groups was statistically significant ($P < 0.05$). With the brimonidine or rilmenidine administration, ED 50 s were 0.12 μg (SNL) and 0.37 μg (SIN) and 2.16 μg (SIN) and 11.46 μg (SNL), respectively. And the shift to left of dose response curve from the SNL group is more prominent with rilmenidine administration.

Conclusions: These results suggest morphine and rilmenidine showed a better effect on reducing the mechanical allodynia induced by FCA administration. **(Korean J Anesthesiol 2009; 56: 425～32)**

Key Words: Allodynia, Brimonidine, Inflammation, Morphine, Rilmenidine, Spinal nerve ligation.

INTRODUCTION

Peripheral nerve injury and inflammation may result in a condition of extreme cutaneous sensitivity to normally innocuous mechanical stimulus, termed mechanical allodynia. Unilateral ligation of lumbar L_5/L_6 spinal nerves produces a profound and long-lasting allodynia for several weeks [1], which is abolished by surgical or chemical sympathectomy [2,3]. Signs of mechanical allodynia were most evident in the nerve ligation model among experimental animal models [4]. Recent studies reported that a focal inflammation of the sciatic nerve produces neuropathic pain sensations in a distant region [5,6].

Two pain models by direct nerve injury or nerve inflammation induces completely different responses: inflammation causes dramatic changes in dorsal horn neurons, whereas peripheral nerve injury induces changes mainly in the DRG neurons [7]. An important question is whether some of these changes may be involved either in inducing or in counteracting neuropathic pain. These changes may also explain why opiates are less efficient in treatment of neuropathic pain than in treatment of inflammatory pain. Although there is some controversy, morphine has an antiallodynic effect on neuropathic pain by inflammatory component [8]. The agents acting at α_2 adrenergic or imidazoline receptors, which are related with the sympathetic nervous system, have shown to effectively reduce the neuropathic pain [9-11]. Brimonidine is a relatively selective and potent α_2 adrenergic agonist and rilmenidine is a selective imidazoline receptor agonis [12,13].

Received: November 12, 2008.
Accepted: February 17, 2009.
Corresponding author: Jai-Hyun Hwang, M.D., Department of Anesthesiology and Pain Medicine, University of Ulsan College of Medicine, Asan Medical Center, 388-1 Pungnap-2dong, Songpa-gu, Seoul 138-736, Korea. Tel: 82-2-3010-3859, Fax: 82-2-470-1363, E-mail: jhhwang@amc.seoul.kr

Thus, we hypothesized that reduction of mechanical allodynia by intrathcal morphine, brimonidine and rilmenidine may be different in neuropathic pain state induced by either FCA administration or spinal nerve ligation. Therefore, this behavioral study was aimed to compare the antiallodynic effect of intrathecal morphine, brimonidine and rilmenidine in rats with neuropathic pain induced either by the administration of FCA around the sciatic nerve or by spinal nerve ligation.

MATERIALS AND METHODS

The following experiments were performed under a protocol approved by our Animal Care Committee. One hundred and ten male Sprague-Dawley rats weighing 160－200 g were used. They were housed 3 or 4 to a cage, given food and water ad libitum and kept in a temperature controlled vivarium (21 ± 1°C) and allowed to acclimate for three days in a 12/12-h light/dark cycle. Surgery was done on all rats under halothane anesthesia and a 1 : 1 flow ratio of N_2O and O_2. The rats recovered sufficiently from the surgical procedures to resume normal activity within 30 min after termination of the anesthesia.

For creating two neuropathic pain rat models, each surgical procedure was performed according to the method devised by either Kim and Chung [1] or Eliav et al [5]. Under anesthesia, a dorsal midline incision was made from L_3 to S_2 vertebral level. The left L_6/S_1 posterior interarticular process was exposed and resected. A partial excision of the L_6 transverse process was made and the left L_5 and L_6 spinal nerves were gently isolated and ligated tightly with 6－0 black silk just distal to the dorsal root ganglion and proximal to the formation of the sciatic nerve (SNL group, n = 60). In the SIN group (n = 50), rats were anesthetized and the sciatic nerve was isolated. Freund's complete adjuvant (FCA; Sigma, St. Louis, MO, USA), killed *Mycobacterium butyricum* suspended in mineral oil, was applied around the sciatic nerve by wrapping with a band of FCA-soaked absorbable gelatin sponge gelfoam (2 × 15 mm; Spongostan®, Johnson & Johnson, UK). The gelfoam, saturated with 50 μl of FCA, is loosely around the nerve. After each surgical procedure, complete hemostasis was confirmed and the wound was sutured closely.

For spinal drug administration, implantation of the intrathecal catheter was performed if the rat showed a withdrawal threshold of 4.0 g or less postoperatively. As devised by Yaksh and Rudy [14], intrathecal PE-10 tubing was passed caudally from the cistern magna to the spinal cord level of lumbar enlargement. The catheter was externalized through the skin. Proper location was confirmed by a temporary motor block of both hind limbs after injection of 2% lidocaine 7 μl, followed by 10 μl saline. Only rats with no evidence of neurologic deficit after the operation were examined. At least a five-day recovery period was allowed before the rats were used in experiments.

The drugs were given by using a microinjection syringe over a 60-s interval in a volume of 10 μL, followed by a 10 μL flush. For the determination of % maximal possible effect (%MPE) and the 50% effective dose (ED50) for each drug, morphine sulfate (Sigma, USA), brimonidine (Sigma, USA) and rilmenidine (Sigma, USA) were administered intrathecally. The doses of morphine were 0.1, 0.3, 1, 3, 10 and 30 μg (5 to 10 rats per subgroup) in both groups. The doses of brimonidine were 0.03, 0.1, 0.3, 1, and 3 μg (9 to 12 rats per subgroup) in the SNL group and 0.3, 1, 3, 10 and 30 μg (4 to 12 rats per subgroup) in the SIN group and those of rilmenidine were 1, 3, 10, 30 and 100 μg (6 to 10 rats per subgroup) in the SNL group and 3, 10, 30 and 100 μg (7 to 12 rats per subgroup) in the SIN group, respectively. Although some of rats received two or three injections, there was at least a five-day interval between drug injections of successive experiment to minimize any possibility of tolerance development and to eliminate the residual effects of a drug.

Behavioral testing was performed during the day portion of the circardian rhythm (9:00 AM to 3:00 PM). To undertake the measurements of withdrawal response to tactile stimuli, each rat was placed under a transparent plastic box on a metal mesh floor. Ten minutes were allotted for behavioral accommodation before starting the testing. Four to six animals were tested simultaneously. Measurements were taken before and 15, 30, 60, 120, and 180 min after an intrathecal dose of the drug (s). Baseline threshold value for each rat at each drug trial was determined by checking responses to von Frey filaments on the same day just before drug injection.

Tactile threshold was measured by applying a series of eight calibrated von Frey filaments (0.40, 0.70, 1.20, 2.00, 3.63, 5.50, 8.50, and 15.1 g; Stoelting Co., Wood Dale, IL, USA) to the midplantar surface of the hind paw until a positive sign for pain behavior was elicited. Each filament was applied to ipsilateral hind paw with sufficient force to cause slight bending against the paw and it was held for six seconds. A brisk withdrawal or paw flinching and/or licking of the paw was considered as a positive response, in which case the next fila-

ment tested was the next lower force. In the absence of such responses, the next filament tested was the next greater force. In the absence of a response at 15 g pressure, the animals were assigned to this cutoff value. The tactile stimulus producing a 50% likelihood of withdrawal was determined by using the up-down method [15].

Withdrawal threshold data from von Frey hair testing were obtained as the actual threshold in grams and were converted to %MPE using the formula: %MPE for antiallodynia = ([postdrug threshold - baseline threshold] / [15 g - baseline threshold]) × 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 these data were used to plot a %MPE versus log dose curve. The dose response curve obtained from the mean %MPE of each drug. The ED50 values, slopes, and 95% confidence intervals are calculated from the %MPE at each concentration of each drug by using dose response data [16].

Side effects were simply assessed by observing 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 abnormal ambulation. Although the drugs given were blinded to the experimenter, data collection could not be done in a blind fashion because animals in the SNL group showed a characteristic mild foot deformity of the lesioned hind paw.

Data in dose response curve were expressed as the mean ± standard error (SE) in each group. The differences between two chronic pain models were compared using an unpaired *t*-test or a Mann-Whitney Rank Sum test if equal variance test or normality test is failed (SigmaStat 3.1). P value less than 0.05 was considered to be statistically significant.

RESULTS

After spinal nerve ligation or FCA administration, all rats displayed a normal general behavior and resulted in an allodynic state with the mean threshold of 2.42 g (± 0.35). The means (± SE) of baseline values of morphine, brimonidine and rilmenidine were 2.623 g (± 0.146), 2.715 g (± 0.121), 2.48 g (± 0.138) in the SNL group and 2.172 g (± 0.146), 2.145 g (± 0.169), 2.13 g (± 0.17) in the SIN group, respectively. The level of general activity was indistinguishable from that of a normal rat. The allodynic states in both SNL and SIN groups occurred within 1－5 days, maintained 1－2 weeks and then gradually decreased over time. The time-effect courses of three drugs at each concentration are shown in both neuropathic pain

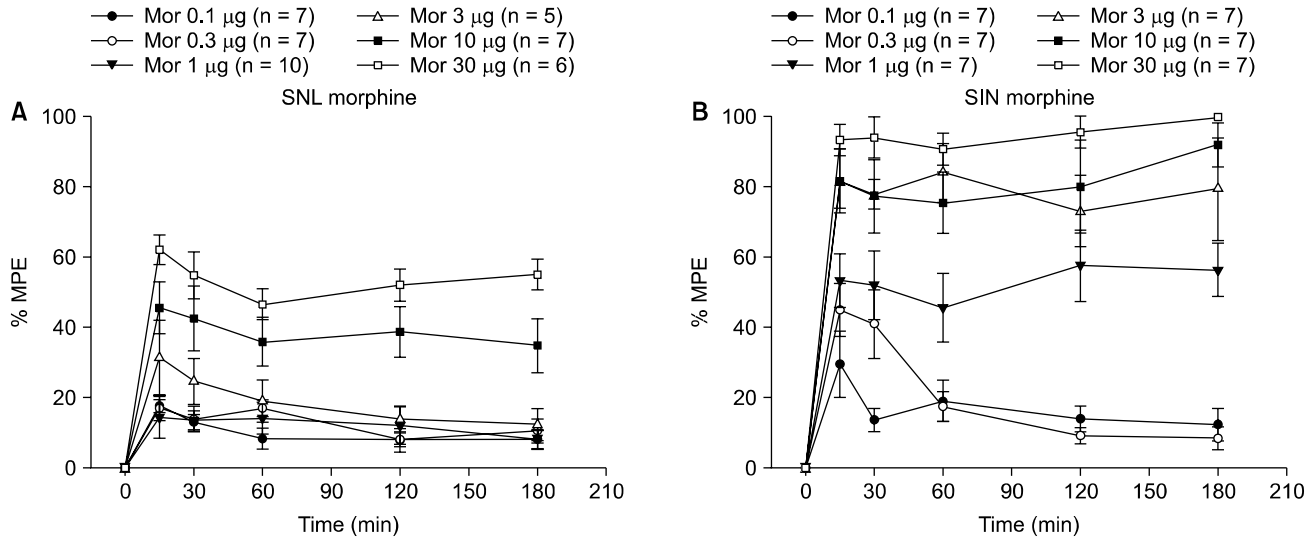

Fig. 1. Time course of the antiallodynic effects of morphine administered intrathecally in rats made allodynic by L_5/L_6 spinal nerve ligation in the SNL group (A) and FCA induced inflammation in the SIN group (B). Data are expressed as the mean ± SEM in each dose group. These curves show a dose dependent antiallodynic effect. Time (min) is represented on the x-axis and peak %MPE is represented on the y-axis. SNL: spinal nerve ligation, SIN: sciatic nerve inflammation neuritis.

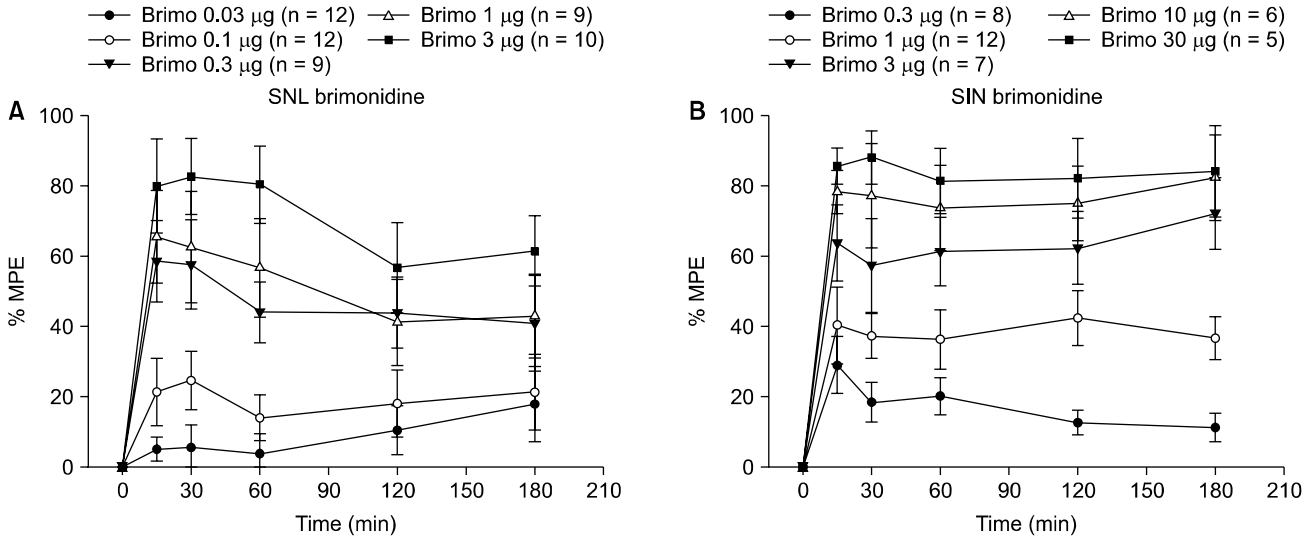

Fig. 2. Time course of the antiallodynic effects of brimonidine administered intrathecally in rats made allodynic by L_5/L_6 spinal nerve ligation in the SNL group (A) and FCA induced inflammation in the SIN group (B). Data are expressed as the mean ± SEM in each dose group. These curves show a dose dependent antiallodynic effect. Time (min) is represented on the x-axis and peak %MPE is represented on the y-axis. SNL: spinal nerve ligation, SIN: sciatic nerve inflammation neuritis.

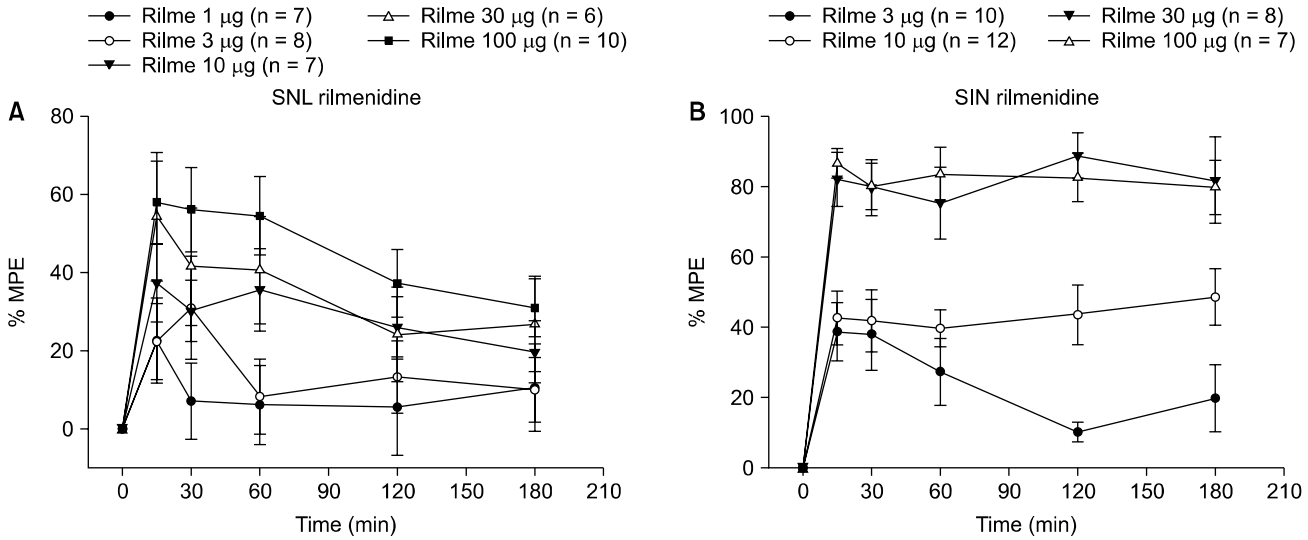

Fig. 3. Time course of the antiallodynic effects of rilmenidine administered intrathecally in rats made allodynic by L_5/L_6 spinal nerve ligation in the SNL group (A) and FCA induced inflammation in the SIN group (B). Data are expressed as the mean ± SEM in each dose group. These curves show a dose dependent antiallodynic effect. Time (min) is represented on the x-axis and peak %MPE is represented on the y-axis. SNL: spinal nerve ligation, SIN: sciatic nerve inflammation neuritis.

models (Fig. 1－3). FCA administration as well as nerve ligation injury produced a marked mechanical allodynia in the lesioned hind paw.

As shown in Fig. 1, intrathecal morphine, brimonidine and rilmenidine in both groups showed the antiallodynic effects in a dose dependent manner (Fig. 4). ED50 values and slopes are shown in the Table 1.

Antiallodynic effect of each drug was different between the SIN and the SNL groups. In the morphine subgroups, ED 50 values were 0.18 μg (SIN) and 8.12 μg (SNL). The dose response curve of the SIN group was more left located than that of the SNL group. The difference between SIN and SNL

Table 1. ED50s and Slopes (95% Confidence Interval) of Intrathecally Administered Drugs

Drug	SNL model (n = 45)			SIN model (n = 45)		
	N	ED50 (μg)	Slope	N	ED50 (μg)	Slope
Morphine	42	8.12 (3.93－16.76)	19.4 (13.9－24.8)	42	0.18 (0.09－0.32)	27.2 (20.9－33.5)
Brimonidine	52	0.12 (0.05－0.26)	29.6 (17.3－41.9)	38	0.37 (0.17－0.78)	32.2 (22.8－41.6)
Rilmenidine	38	11.46 (3.55－36.97)	22.4 (6.9－37.9)	37	2.56 (0.73－8.86)	32.1 (16.5－47.7)

SNL: spinal nerve ligation, SIN: sciatic inflammatory neuritis, N: number of rats tested; some rats received two or three injections with at least a five-day interval between drug injections.

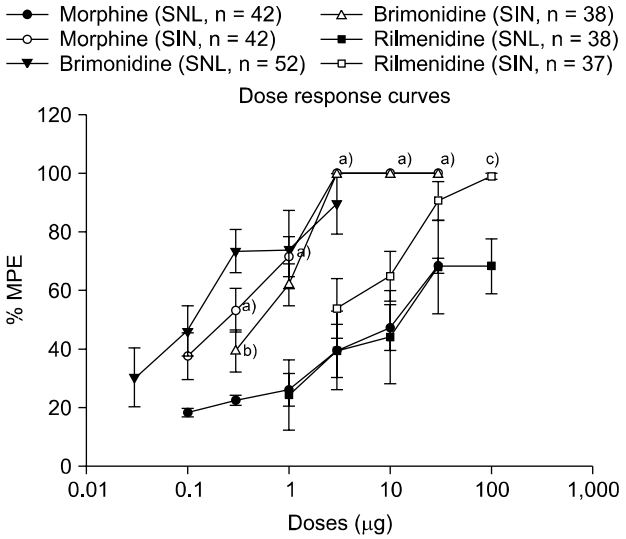

Fig. 4. Dose response curves from the peak effects of percent maximal effect (%MPE) for the antiallodynia by intrathecal morphine, brimonidine and rilmenidine in the spinal nerve ligation (SNL) group and the FCA induced sciatic nerve inflammation neuritis (SIN) group. These curves show a dose dependent antiallodynic effect. Data are expressed as the mean ± SEM. Doses (μg) are represented logarithmically on the x-axis and peak %MPEs are represented on the y-axis. [a)]$P < 0.005$ compared with morphine of the SNL group. [b)]$P = 0.005$ compared with brimonidine of the SNL group. [c)]$P < 0.05$ compared with rilmenidine of the SNL group.

groups was statistically significant at the dose range of 0.3－30 μg ($P < 0.005$). In the brimonidine subgroups, ED 50 values were 0.12 μg (SNL) and 0.37 μg (SIN). Dose response curve was more left shifted in the SNL group. The difference between SIN and SNL groups was statistically significant only at the dose of 0.3 μg ($P = 0.005$). In the rilmenidine subgroups, ED 50 values were 2.56 μg (SIN) and 11.46 μg (SNL). Dose response curve of the SIN group was existed in the left side. The difference between SIN and SNL groups was statistically significant only at the high dose of 100 μg ($P < 0.05$).

No abnormal behavior or foot deformity was seen in the SIN group but four rats in the SNL group were excluded due to flaccidity and severe deformity from the spinal nerve damage. In the SNL group, most rats showed a slight foot deformity of the lesioned hind paw after surgery. The foot of the lesioned side was moderately everted on the injured side and the toes were held together. None of the rats was showed a severe sedation to affect the experiment after drug administration. All the included animals in both SNL and SIN groups produced allodynic response without a lack of general activity and any signs of motor weakness.

DISCUSSION

There are two important observations in this study. First, either the nerve inflammation induced by administration of FCA around the sciatic nerve or nerve injury induced by spinal nerve ligation can produce a mechanical allodynia.

Second, as shown a statistically significant difference between two neuropathic pain models, the efficacy of intrathecal morphine and rilmenidine was better than that of brimonidine.

Neuropathic pain including mechanical allodynia is generally resulted from nerve damage by direct injury or inflammation. Signs of mechanical allodynia were most evident in the nerve ligation model among experimental animal models [4]. Freund's complete adjuvant (FCA), an inflammatory agent, is widely used in an animal model of chronic pain [17]. Many investigators used to administer FCA into the foot or ankle to induce a peripheral inflammatory condition [17,18]. Although exact mechanism is not known, activation of sympathetic nervous system is at least partly involved in induction and maintenance of neuropathic pain induced by inflammation [19]. However, recent studies reported that the sciatic inflammatory neuritis (SIN) produces neuropathic pain sensations in a distant region and this is not due to axonal damage [5,6]. Thus, we performed

this behavioral study in the spinal nerve ligation model and the sciatic inflammatory neuritis model.

Intrathecal clonidine has an antiallodynic effect in rats with spinal nerve ligation injury [20]. Clonidine is not pure α_2 adrenergic receptor agonist but is also able to combine with nonadrenergic imidazoline recepto [21]. The agents acting at α_2 adrenergic or imidazoline receptors have shown to effectively reduce the neuropathic pain [9-11]. Spinal α_2 adrenergic receptor agonists inhibit preganglionic neurons and diminish sympathetic outflow, resulting in their antiallodynic action [22]. Brimonidine is a relatively selective and potent α_2 adrenergic agonist and rilmenidine is a selective imidazoline receptor agonist [12,13]. Imidazoline receptor is more related in sympathetic activity than α_2 adrenergic receptor [13]. Although there is some controversy, morphine has an antiallodynic effect on neuropathic pain by inflammatory cause [8]. Therefore, we chose morphine, brimonidine and rilmenidine in two different neuropathic pain rat models.

The efficacy of morphine in neuropathic pain states is somewhat controversial. Some investigators have suggested that morphine is ineffective against neuropathic pain in animal studies [23,24], whereas others have found that opioids may alleviate neuropathic pain [25]. Unlike our finding of intrathecal morphine in the SNL group, previous studies reported that intrathecal morphine was not efficacious against mechanical allodynia in rats with ligation of L5/6 nerve roots [23,24] and this was due to the lack of a generalized loss of μ opioid receptors [26]. However, intrathecal morphine is effective in reducing neuropathic pain induced by inflammatory cause [8] and our results in the SIN group are consistent with this description. Inflammation of the nerve by FCA administration created a similar profile of pain facilitation as did nerve ligation. In brief, both manipulations increased the frequency of withdrawal response by tactile stimulus, suggesting that FCA administration on the sciatic nerve causes an allodynia. The study by Kim and Chung [1] reported that mechanical allodynia by spinal nerve ligation injury exist for several weeks in the affected foot. In the present study, an allodynic response to tactile stimulus was well maintained during the experiment of 2－3 weeks in the unilateral lesioned side.

Chronic pain, that is associated with prolonged tissue damage or injuries to the peripheral and central nervous system, results from a number of complex changes in nociceptive pathways. The resultant increase in neural excitability can be reduced with receptor selective drugs that block peripheral and central chemical mediators or that control ectopic activity or cellular phenotype changes. Direct interactions of sympathetic nerves or sympathetic transmitters with afferent fibers have not been easy to demonstrate [27]. During inflammation, afferent fibers may be sensitized by prostanoids released from sympathetic fibers, and following peripheral nerve injury, sympathetic nervous stimulation or the administration of noradrenaline can excite some Aβ- and C- fiber afferent via α adrenoceptor [28]. These findings may partially explain causalgia and sympathetically maintained pain, since these conditions may be ameliorated by sympathectomy or α_2 adrenoceptor agonist, clonidine. Previous study reported that spinal nerve ligation triggers sprouting of myelinated sympathetic fibers in the dorsal root ganglion and result in a functional coupling between sprouted sympathetic fibers and sensory neurons [29,30]. Goff et al [31] demonstrated the reorganization of the spinal dorsal horn in three models of chronic pain. Considering together, our results from three drugs in both groups can be explained. Spinal nerve ligation model generally reflects the sympathetic component [2,3], whereas chronic constriction injury and partial nerve injury models relatively do the inflammatory component [32]. Our results from intrathecal brimonidine and rilmenidine in the SNL group are consistent with the former.

In the case of inflammatory pain animal models, FCA is widely used in a model of chronic pain [17,18,33]. The exact mechanism of neuropathic pain by FCA administration is not known in the this study, but the inflammatory mediators may sensitize acutely inflamed nerve fibers to mechanical and thermal stimuli [34,35]. Another probable mechanism is that an inflammation-induced neuropathy (neuritis) by FCA administration initiates an immune mechanism and also produces a destructive caseous local inflammation. Therefore, unspecific mechanisms such as nerve compression by the surrounding granuloma or direct destruction of the nerve by the inflamed caseous tissue might be involved the development of the allodynia. Several studies reported that a neuroimmune interaction contributes to the genesis of painful peripheral neuropathies [5,6,36]. An inflammation reaction, an immune cell infiltration and increased endoneurial levels of pro-inflammatory cytokines have been detected at the site of nerve injury in animal models of painful peripheral neuropathy [37,38]. Watkins et al [39] demonstrated that the neuropathic pain produced by the neuritis was accompanied by minor structural damage to axons or glia and application of FCA to the surface of the nerve evoked an endoneu-

rial inflammation characterized by evidence for plasma extravasation and the infiltration of immune cells. Also, Maves et al [40] suggested that a neuroimmune interaction in the endoneurial compartment plays a key role in producing the neuritis-evoked neuropathic pain. A new model of sciatic inflammatory neuritis induced unilateral and bilateral mechanical allodynia following acute unilateral peri-sciatic immune activation in rats [6], whereas a focal inflammation of the sciatic nerve produced neuropathic pain on the ipsilateral side [5].

Morphine and rilmenidine but not brimonidine showed a significantly different 50% effective dose (ED50) in these two neuropathic pain models. The dose response curve of the SIN group was more left located than that of the SNL group. This means that morphine and rilmenidine is more effective in the reduction of allodynic state caused by inflammation than by nerve injury.

Marked deformity of the lesioned hind paw causes an important problem due to abnormal weight bearing balance and bias of a blinded experimenter. The spinal L_5 and L_6 nerve ligation model mostly reflected a mechanical allodynia; nevertheless, this model has a problem in behavioral testing because a blinded experimenter knows which is the lesioned hind paw and may have a bias when testing responses to stimuli. Although a minor foot deformity of the lesioned hind paw is still remained, segmental spinal nerve ligation model could solve a problem of abnormal weight bearing. Furthermore, all rats in the SIN group showed no foot deformity in the lesioned side. Thus, the absence of foot deformity is an advantage compared to the SNL model because a blind experimenter has no bias.

There are two considerations on the selection of each pain model and dosages of each drug. First, among many neuropathic pain models producing allodynia, we just chose the spinal nerve ligation model as a direct injury model and FCA induced neuritis evoked model as an inflammatory model although any of them could not reflect a complete clinical neuropathic pain model in humans. But one must not extrapolate the results of an animal model. Second, the rationale of administration of high dosages, especially morphine and rilmenidine, in SIN group was to compare the efficacy of each drug between two groups on a dose basis. However, such experiment should be done under consideration of ethical issues of animal experiment.

In the present study, there are several limitations to define the difference between two models. First, we did not examine the ongoing spontaneous pain that is one of the typical signs of neuropathic pain and only checked static component of mechanical allodynia. Second, we administered only one of many similar drugs acting on each receptor. Third, we did not use a purely selective agents acting on each receptor. Thus, it cannot be sure that such difference is definitive until it is not further validated pharmacologically. Both positive and negative reference compounds should be tested with various methods to confirm the difference.

In conclusion, the present study demonstrates that local administration of FCA on the sciatic nerve as well as spinal nerve ligation injury induces mechanical allodynia and that intrathecal morphine, brimonidine and rilmenidine are effective in reducing mechanical allodynia in rats. At the spinal level, rilmenidine and especially morphine have a better effect on reducing the mechanical allodynia in the FCA induced neuropathic pain than in the spinal nerve injury induced neuropathic pain.

ACKNOWLEDGEMENT

This work was supported by Korea Research Foundation Grant (KRF-2004-041-E00245).

REFERENCES

1. Kim SH, Chung JM. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 1992; 50: 355-63.
2. Kim SH, Na HS, Sheen K, Chung JM. Effects of sympathectomy on a rat model of peripheral neuropathy. Pain 1993; 55: 85-92.
3. LoPachin RM, Rudy TA. The effects of intrathecal sympathomimetic agents on neural activity in the lumbar sympathetic chain of rats. Brain Res 1981; 224: 195-8.
4. Kim KJ, Yoon YW, Chung JM. Comparison of three rodent neuropathic pain models. Exp Brain Res 1997; 113: 200-6.
5. Eliav E, Herzberg U, Ruda MA, Bennett GJ. Neuropathic pain from an experimental neuritis of the rat sciatic nerve. Pain 1999; 83: 169-82.
6. Chacur M, Milligan ED, Gazda LS, Armstrong C, Wang H, Tracey KJ, et al. A new model of sciatic inflammatory neuritis (SIN): induction of unilateral and bilateral mechanical allodynia following acute unilateral peri-sciatic immune activation in rats. Pain 2001; 94: 231-44.
7. Hökfelt T, Zhang X, Xu Z, Ru-Rong J, Shi T, Corness J, et al. Cellular and synaptic mechanisms in transition of pain from acute to chronic. In: Proceedings of the 8th wolrd congress on pain. Edited by Jensen TS, Turner JA, Wiesenfelder-Hallin Z: Seattle,

IASP Press. 1997, pp 133-53.
8. Dellemijn P. Are opioids effective in relieving neuropathic pain? Pain 1999; 80: 453-62.
9. Ali Z, Ringkamp M, Hartke TV, Chien HF, Flavahan NA, Campbell JN, et al. Uninjured C-fiber nociceptors develop spontaneous activity and alpha-adrenergic sensitivity following L6 spinal nerve ligation in monkey. J Neurophysiol 1999; 81: 455-66.
10. Fairbanks CA, Nguyen HO, Grocholski BM, Wilcox GL. Moxonidine, a selective imidazoline-alpha2 -adrenergic receptor agonist, produces spinal synergistic antihyperalgesia with morphine in nerve-injured mice. Anesthesiology 2000; 93: 765-73.
11. Stone LS, Fairbanks CA, Wilcox GL. Moxonidine, a mixed alpha(2)-adrenergic and imidazoline receptor agonist, identifies a novel adrenergic target for spinal analgesia. Ann N Y Acad Sci 2003; 1009: 378-85.
12. Cambridge D. UK-14,304, a potent and selective alpha2-agonist for the characterisation of alpha-adrenoceptor subtypes. Eur J Pharmacol 1981; 72: 413-5.
13. Khan ZP, Ferguson CN, Jones RM. alpha-2 and imidazoline receptor agonists. Their pharmacology and therapeutic role. Anaesthesia 1999; 54: 146-65.
14. Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976; 17: 1031-6.
15. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994; 53: 55-63.
16. Tallarida RJ, Murray RB. Manual of pharmacologic calculations with computer programs. 2nd ed. New York, Springer-Verlag 1987, pp 153-9.
17. Leem JW, Hwang JH, Hwang SJ, Park H, Kim MK, Choi Y. The role of peripheral N-methyl-D-aspartate receptors in Freund's complete adjuvant induced mechanical hyperalgesia in rats. Neurosci Lett 2001; 297: 155-8.
18. Millan MJ, Czlonkowski A, Morris B, Stein C, Arendt R, Huber A, et al. Inflammation of the hind limb as a model of unilateral, localized pain: influence on multiple opioid systems in the spinal cord of the rat. Pain 1988; 35: 299-312.
19. Dray A, Urban L, Dickenson A. Pharmacology of chronic pain. Trends Pharmacol Sci 1994; 15: 190-7.
20. Pan HL, Chen SR, Eisenach JC. Intrathecal clonidine alleviates allodynia in neuropathic rats: interaction with spinal muscarinic and nicotinic receptors. Anesthesiology 1999; 90: 509-14.
21. Hieble JP, Ruffolo RR Jr. Possible structural and functional relationships between imidazoline receptors and alpha 2-adrenoceptors. Ann N Y Acad Sci 1995; 763: 8-21.
22. Yaksh TL, Pogrel JW, Lee YW, Chaplan SR. Reversal of nerve ligation-induced allodynia by spinal alpha-2 adrenoceptor agonists. J Pharmacol Exp Ther 1995; 272: 207-14.
23. Bian D, Nichols ML, Ossipov MH, Lai J, Porreca F. Characterization of the antiallodynic efficacy of morphine in a model of neuropathic pain in rats. Neuroreport 1995; 6: 1981-4.
24. Nichols ML, Bian D, Ossipov MH, Lai J, Porreca F. Regulation of morphine antiallodynic efficacy by cholecystokinin in a model of neuropathic pain in rats. J Pharmacol Exp Ther 1995; 275: 1339-45.
25. Yu W, Hao JX, Xu XJ, Wiesenfeld-Hallin Z. Comparison of the anti-allodynic and antinociceptive effects of systemic; intrathecal and intracerebroventricular morphine in a rat model of central neuropathic pain. Eur J Pain 1997; 1: 17-29.
26. Porreca F, Tang QB, Bian D, Riedl M, Elde R, Lai J. Spinal opioid mu receptor expression in lumbar spinal cord of rats following nerve injury. Brain Res 1998; 795: 197-203.
27. McMahon SB. Mechanisms of sympathetic pain. Br Med Bull 1991; 47: 584-600.
28. McLachlan EM, Janig W, Devor M, Michaelis M. Peripheral nerve injury triggers noradrenergic sprouting within dorsal root ganglia. Nature 1993; 363: 543-6.
29. Chung K, Yoon YW, Chung JM. Sprouting sympathetic fibers form synaptic varicosities in the dorsal root ganglion of the rat with neuropathic injury. Brain Res 1997; 751: 275-80.
30. Lekan HA, Chung K, Yoon YW, Chung JM, Coggeshall RE. Loss of dorsal root ganglion cells concomitant with dorsal root axon sprouting following segmental nerve lesions. Neuroscience 1997; 81: 527-34.
31. Goff JR, Burkey AR, Goff DJ, Jasmin L. Reorganization of the spinal dorsal horn in models of chronic pain: correlation with behaviour. Neuroscience 1998; 82: 559-74.
32. Bridges D, Thompson SW, Rice AS. Mechanisms of neuropathic pain. Br J Anaesth 2001; 87: 12-26.
33. LaBuda CJ, Fuchs PN. A behavioral test paradigm to measure the aversive quality of inflammatory and neuropathic pain in rats. Exp Neurol 2000; 163: 490-4.
34. Michaelis M, Vogel C, Blenk KH, Arnarson A, Janig W. Inflammatory mediators sensitize acutely axotomized nerve fibers to mechanical stimulation in the rat. J Neurosci 1998; 18: 7581-7.
35. Sweitzer SM, Colburn RW, Rutkowski M, DeLeo JA. Acute peripheral inflammation induces moderate glial activation and spinal IL-1beta expression that correlates with pain behavior in the rat. Brain Res 1999; 829: 209-21.
36. Bennett GJ. Does a neuroimmune interaction contribute to the genesis of painful peripheral neuropathies? Proc Natl Acad Sci USA 1999; 96: 7737-8.
37. DeLeo JA, Colburn RW, Rickman AJ. Cytokine and growth factor immunohistochemical spinal profiles in two animal models of mononeuropathy. Brain Res 1997; 759: 50-7.
38. Clatworthy AL, Illich PA, Castro GA, Walters ET. Role of peri-axonal inflammation in the development of thermal hyperalgesia and guarding behavior in a rat model of neuropathic pain. Neurosci Lett 1995; 184: 5-8.
39. Watkins LR, Maier SF, Goehler LE. Immune activation: the role of pro-inflammatory cytokines in inflammation, illness responses and pathological pain states. Pain 1995; 63: 289-302.
40. Maves TJ, Pechman PS, Gebhart GF, Meller ST. Possible chemical contribution from chromic gut sutures produces disorders of pain sensation like those seen in man. Pain 1993; 54: 57-69.