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

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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 L₅/L₆ 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 L₅/L₆ 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 α₂ 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 α₂ adrenergic agonist and rilmenidine is a selective imidazoline receptor agonist [12,13].
Thus, we hypothesized that reduction of mechanical allodynia by intrathecal 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 N₂O and O₂. 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 Kim and Chung [1] or Eliav et al [5]. Under anesthesia, a dorsal midline incision was made from L₃ to S₂ vertebral level. The left L₄/S₁ posterior interarticular process was exposed and resected. A partial excision of the L₅ transverse process was made and the left L₅ and L₆ spinal nerves were gently isolated and ligated tightly with 6−0 black silk just distal to the dorsal root ganglion and proximal to the foramen 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 circadian 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-
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Fig. 1. Time course of the antiallodynic effects of morphine administered intrathecally in rats made allodynic by L5/L6 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.

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...
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

<table>
<thead>
<tr>
<th>Drug</th>
<th>SNL model (n = 45)</th>
<th>SIN model (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>ED50 (μg)</td>
</tr>
<tr>
<td>Morphine</td>
<td>42</td>
<td>8.12 (3.93 – 16.76)</td>
</tr>
<tr>
<td>Brimonidine</td>
<td>52</td>
<td>0.12 (0.05 – 0.26)</td>
</tr>
<tr>
<td>Rilmenidine</td>
<td>38</td>
<td>11.46 (3.55 – 36.97)</td>
</tr>
</tbody>
</table>

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.

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**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 α2 adrenergic receptor agonist but is also able to combine with nonadrenergic imidazoline receptor [21]. The agents acting at α2 adrenergic or imidazoline receptors have shown to effectively reduce the neuropathic pain [9-11]. Spinal α2 adrenergic receptor agonists inhibit preganglionic neurons and diminish sympathetic outflow, resulting in their antiallodynic action [22]. Brimonidine is a relatively selective and potent α2 adrenergic agonist and rilmenidine is a selective imidazoline receptor agonist [12,13]. Imidazoline receptor is more related in sympathetic activity than α2 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 α2 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-
The difference between two models. First, we did not examine

In the present study, there are several limitations to define

experiment. Done under consideration of ethical issues of animal
groups on a dose basis. However, such experiment should be

high dosages, especially morphine and rilmenidine, in SIN
model in humans. But one must not extrapolate the results of

of them could not reflect a complete clinical neuropathic pain
models producing allodynia, we just chose the spinal nerve
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 sig-
nificantly 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 impor-
tant problem due to abnormal weight bearing balance and
bias of a blinded experimenter. The spinal L5 and L6 nerve
ligation model mostly reflected a mechanical allodynia; never-
thless, 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 le-
sioned side. Thus, the absence of foot deformity is an advan-
tage 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 neuro-
pathic 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

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 me-
cechanical 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 refer-
ence compounds should be tested with various methods to con-
firm the difference.

In conclusion, the present study demonstrates that local ad-
ministration of FCA on the sciatic nerve as well as spinal
erve ligation injury induces mechanical allodynia and that in-
trathecal morphine, brimonidine and rilmenidine are effective in
reducing mechanical allodynia in rats. At the spinal level, ril-
menidine and especially morphine have a better effect on re-
ducing the mechanical allodynia in the FCA induced neuro-
pathic pain than in the spinal nerve injury induced neuropathic
pain.

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