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Anti-allodynic effect of bee venom on neuropathic pain in the rat

Bae Hwan Lee[1,†], Younbyoung Chae[2,†], Hye Jeong Hwang[2], Young-Kook Choi[2], Dae Hyun Hahm[2], Seung-Moo Han[3], Sung-Keel Kang[4], Hye-Jung Lee[2], Kwang-Ho Pyun[5] and Insop Shim[5,*]

[1]Department of Physiology, Brain Research Institute, Yonsei University College of Medicine; [2]Graduate School of East-West Medicine, Institute of Oriental Medicine, Kyung Hee University; [3]Department of Biomedical Engineering, Kyung Hee University; [4]Department of Acupuncture and Moxibustion, College of Oriental Medicine, Kyung Hee University; [5]Department of Integrative Medicine, College of Medicine, Catholic University of Korea, Seoul 137-701, Republic of Korea

SUMMARY

Neuropathic pain syndromes resulted from peripheral nerve injury appear to be resistant to conventional analgesics like opioids. However, it has been demonstrated that acupuncture including aqua-acupuncture may be effective in managing neuropathic pain. The present study was conducted to determine if bee venom injection into acupoint inhibits neuropathic pain, which is difficult to be treated by usual analgesics. Under pentobarbital anesthesia, male Sprague-Dawley rats were subjected to neuropathic surgery. Two weeks after nerve injury, mechanical and cold allodynia were tested in order to evaluate the antiallodynic effects of bee venom injection into an acupoint. Intraperitoneal injection of morphine inhibited mechanical allodynia dose-dependently. Bee venom injected into Zusanli acupoint significantly inhibited mechanical and cold allodynia. These results suggest that bee venom-acupuncture as well as morphine is very effective to inhibit mechanical allodynia.

Key words: Neuropathic pain; Bee venom; Zusanli, Acupuncture; Morphine

INTRODUCTION

It has been shown that peripheral nerve injury or soft-tissue injury can cause severe chronic pain in humans (Somerfield and Brandwein, 1988; Bonica, 1990). Abnormal pain states resulted from peripheral nerve injury are commonly referred to as neuropathic pain. These pain states are characterized by spontaneous pain, hyperalgesia (an increased sensitivity to painful stimuli), and allodynia (the perception of normally innocuous stimuli as painful).

Conventionally, opioids have been used to reduce acute or chronic pain. However, there is a controversy as to the effectiveness of opioids in treating neuropathic pain. In humans for example, intravenous administration of morphine results in a considerable relief of normal nociceptive input whereas chronic deafferentation pain is not affected (Arner and Meyerson, 1988). By contrast, intrathecal opioids relieve neuropathic pain in some patients (Portenoy *et al.*, 1990). Similarly in animal models of neuropathic pain, the effectiveness of opioids is quite different, ranging from a marked inhibition of neuropathic pain in some studies to little effects in others (Jazat and Guilbaud, 1991; Kupers and Gybel, 1995; Chung

*Correspondence: Insop Shim, Department of Integrative Medicine, College of Medicine, Catholic University of Korea, Seoul 137-701, Republic of Korea. Tel: +82-2-590-2971; Fax: +82-2-592-6359; E-mail: ishim@catholic. ac.kr
†These authors equally contributed to this work.

and Na, 1996). These observations lead to the other approaches to treat neuropathic pain.

According to our previous studies, electrical stimulation of the ventral periaqueductal gray (PAG) dramatically reduced mechanical allodynia and cold allodynia in a rat model of neuropathic pain employing injury to the sciatic nerve branches (Lee *et al*., 2000a). Antiallodynic effects of the PAG stimulation were reversed by pretreatment of naloxone, an opioid antagonist. This indicates that the endogenous descending opiate system may be involved in the modulation of neuropathic pain. Traditionally, it has been shown that acupuncture in meridian points inhibits pain by activating endogenous pain inhibition system. Thus, acupuncture may be effective in treating neuropathic pain. Treatment with bee venom (BV) has been utilized to reduce pain and arthritis (Billingham *et al*., 1973; Kwon *et al*., 2001b; Lee *et al*., 2001). Moreover, it has been demonstrated that injections of BV into a specific acupoint produced much more pronounced analgesic effects than those of acupuncture stimulation alone (Kwon *et al*., 2001b). However, the analgesic potential of BV has not been investigated in the neuropathic pain model by partial sciatic nerve injury in rats.

Therefore, the present study was conducted to determine if injections of BV into acupoint may be effective in alleviating neuropathic pain which is difficult to be treated by usual analgesics. In addition, the analgesic effect of BV therapy was compared that of morphine treatment.

MATERIALS AND METHODS

Subjects and surgery

Subjects were seventy Sprague-Dawley rats, weighing between 220 - 250 g at the time of the surgery. Under pentobarbital anesthesia (50 mg/kg, i.p.), a skin incision was made in the region of the thigh. The muscle was retracted and the tibial, sural, and common peroneal nerves were identified. In order to produce a neuropathic injury, the tibial and sural nerves were tightly ligated with a 6 - 0 silk thread and cut with fine scissors and the common peroneal nerve was left intact (Lee *et al*., 2000b). Complete hemostasis was confirmed and then the wound was sutured. Rats were allowed 24 h to recover from the surgery prior to behavioral testings. The experimental procedures were carried out according to the animal care guidelines of the NIH and the Kyung Hee University Institutional Animal Care and Use Committee.

Behavioral tests

Behavioral signs, representing different components of neuropathic pain (mechanical and cold allodynia) were postoperatively examined in all the rats for two weeks.

To measure mechanical allodynia, rats were placed on a metal mesh floor under a transparent plastic dome (8 × 8 × 18 cm), and innocuous mechanical stimulus was applied with a von Frey filament (8 mN of bending force) to the sensitive area of the hind paw. The most sensitive area was first determined by poking various areas of the paw with a von Frey hair. Then, the actual test was conducted by gently poking the spots with the filament. A von Frey filament was applied 10 times (once every 3 - 4 s) to each hind paw. The frequency of the foot withdrawal expressed in percentage was used as the index of mechanical allodynia.

To quantify cold allodynia, rats were placed on a metal mesh floor under a transparent plastic dome and the brisk paw withdrawal in response to acetone application was measured. Acetone was applied 5 times (once every 5 min) to each paw. The frequency of the paw withdrawal expressed in percentage was used as the cold allodynia index.

Experimental procedure

Two weeks after neuropathic surgery, mechanical and cold allodynia were tested in rats showing vivid neuropathic pain behaviors in order to

evaluate the antiallodynic effects of morphine or BV injection into an acupoint. Putative analgesics tested were as follows: morphine (2 and 10 mg/kg, i.p., Sigma, St. Louis, MO) and BV-acupuncture (33 and 66 μg/kg, s.c., Sigma, St. Louis, MO). The injected volume was 50 ml delivered over a period of 2 min. BV or saline were injected subcutaneously into injured side of Zusanli, which was located about 10 mm below the knee joint and about 5 mm lateral from the midline in the anterior surface of the hind leg. These putative analgesics were injected into each rat on different postoperatively days of 1, 7, 14, 17, 21, and 28. The sequence of injections was randomly determined. Behavioral test for mechanical or cold allodynia was conducted every 5 min for 2 h after drug injection.

Statistics

Data were expressed as mean ± standard error of the mean (S.E.M.). Differences in changes of neuropathic pain behaviors following nerve injury among the experimental groups were tested using one-way analysis of variance with repeated measures on the time factor, followed by post-hoc Tukey test. Statistical significance was inferred at the $P < 0.05$ level.

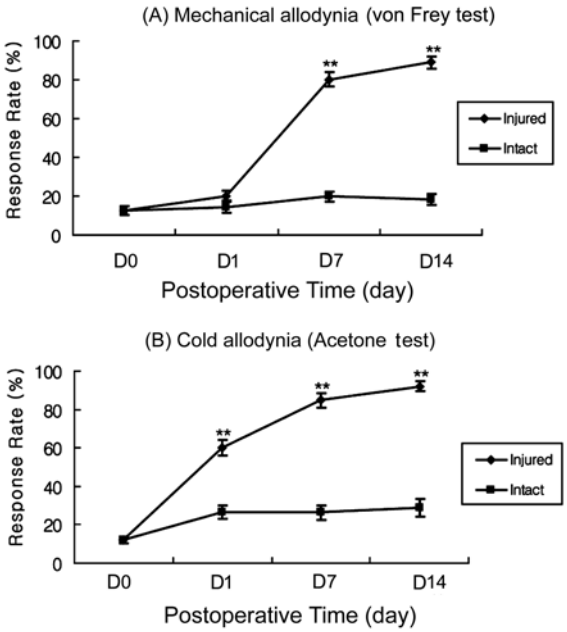

Fig. 1. Development of mechanical allodynia and cold allodynia in rats with injury to the tibial and sural nerves. Response rate to a von Frey filament or acetone was used as an index of mechanical allodynia (A) or cold allodynia (B), respectively. Data were expressed as mean ± S.E.M. (n = 38). Abscissa was marked as D_0 for preoperative control, and D_1 to D_{14} for postoperative days. Asterisks (*) indicate significant differences compared to preoperative control values ($^{**}P < 0.01$).

RESULTS

Development of neuropathic pain behaviors

The results of the behavioral tests for mechanical and cold allodynia are shown in Fig. 1. Prior to the surgery, the rats were rarely responsive to a von Frey filament or acetone. After the injury of the tibial and sural nerves, responsiveness was gradually increased in injured paw from the first day postoperatively and reached at higher level of response rate on the 7^{th} day postoperatively (mechanical allodynia, $P < 0.01$ either on 7 or 14^{th} day; cold allodynia, $P < 0.01$ either on 1, 7 or 14^{th} day). In contrast, contralateral intact paw was remained unresponsive.

Effects of morphine injection

To determine whether morphine can inhibit neuropathic pain, morphine was injected intraperitoneally. Fig. 2 shows the effect of intraperitoneally injected morphine on mechanical allodynia. Injections of morphine inhibited mechanical allodynia dose-dependently. Pain responses were significantly reduced in low ($P < 0.05$ at 60 and 120 min) or high morphine-treated groups ($P < 0.01$ at 10, 30, 60, 120 min, respectively). Physiological saline did not change mechanical allodynia.

Effects of BV aqua-acupuncture

Figs. 3 and 4 show the effects of BV aqua-acupuncture on mechanical (Fig. 3) and cold allodynia (Fig. 4).

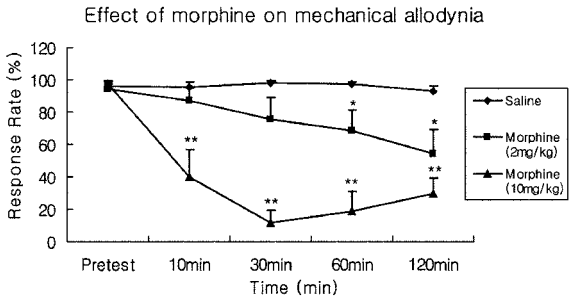

Fig. 2. Effects of intraperitoneally injected morphine on mechanical allodynia. Morphine reduced mechanical allodynia dose-dependently (n = 11). Asterisks (*) indicate significant differences compared to saline injected control group ($^{*}P < 0.05$, $^{**}P < 0.01$).

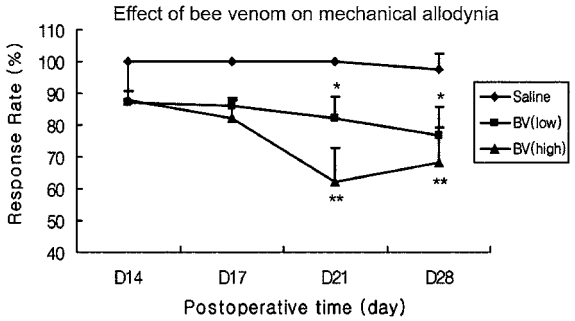

Fig. 3. Effects of bee venom aqua-acupuncture on mechanical allodynia on 14, 17, 21, 28 postoperative days. BV aqua-acupuncture was effective in reducing mechanical allodynia (n = 21). Asterisks (*) indicate significant differences compared to saline injected control group ($^{*}P < 0.05$, $^{**}P < 0.01$).

BV injected repeatedly on 21, 28 days into Zusanli acupoint significantly inhibited mechanical allodynia ($P < 0.01$ at 21 or 28 days). Low dose of BV also inhibited mechanical allodynia ($P < 0.05$ at 21th and 28th day). High dose of BV was more effective in reducing mechanical allodynia than low dose. Physiological saline did not change mechanical allodynia.

In order to determine whether BV is effective in reducing cold allodynia, BV was injected into Zusanli and cold allodynia was tested. Compared to saline, BV aqua-acupuncture significantly reduced cold allodynia ($P < 0.05$, Fig. 4) but the pain-reducing effects were not prominent than on mechanical allodynia (Fig. 4).

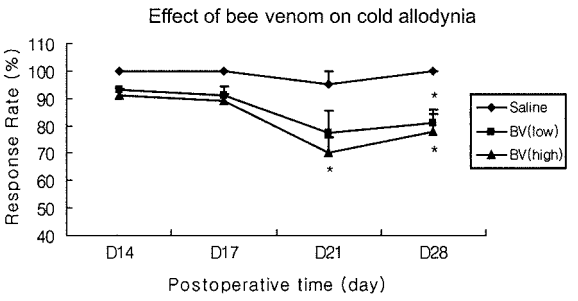

Fig. 4. Effects of bee venom aqua-acupuncture on cold allodynia on 14, 17, 21, 28 postoperative days. BV aqua-acupuncture was effective in reducing cold allodynia (n = 21). Asterisks (*) indicates significant differences compared to saline injected control group ($^{*}P < 0.05$).

DISCUSSION

In this study, we have found that intraperitoneally injected morphine inhibited mechanical allodynia. Similarly, Chung and Na (Chung and Na, 1996) showed that intraperitoneal injection of a high dose of morphine was effective in reducing neuropathic pain behaviors reflecting evoked but not ongoing pain and the responses to innocuous stimuli applied to the uninjured side remained unchanged. In the present study, we aimed to produce reliable and profound neuropathic pain behaviors and to determine whether this animal model is dependant on opioid system. We also tried to compare antiallodynic effects between bee venom and morphine. As far as we have known, this is first report to demonstrate that morphine has an antiallodynic effect in this neuropathic pain model. Administration of morphine significantly reduced behavioral response of neuropathic pain. These results suggest that opioid system is involved in modulating neuropathic pain with injury to the tibial and sural nerves while leaving the common peroneal nerve intact.

Several clinical studies demonstrated the poor efficacy of opioids in neuropathic pain that was sometimes evoked by the nerve injury (Arner and Meyerson, 1988; Iadarola and Caudle, 1997). Even

though morphine was reported to have relatively little effect on neuropathic pain, there were instances where this substance was effective in treating such conditions, both in humans (Portenoy *et al*., 1990) and in animal models (Kayser *et al*., 1990; Chung and Na, 1996). Similarly, the present study has clearly demonstrated the beneficial effects of morphine on mechanical allodynia. Morphine also significantly inhibited cold allodynia (data not shown). The reasons for these discrepancies from studies to studies as to the effects of opioids on neuropathic pain are not known. In the present animal model of neuropathic pain, therefore, i.p. morphine may exert analgesic action through the descending pain inhibition system. Consequently, the effectiveness of opiates may depend on multiple factors such as types of injury, different levels of the dosage of opiates, properties of neuropathic pain behaviors, and the timing of administration after injury.

Acupuncture has been used as an important tool to cure chronic pain syndromes. Clinically, the alternative forms of acupoint stimulation including electroacupuncture and acupressure appear to have more potent analgesic effects than manual needle acupuncture (Tsui and Leung, 2002).

BV can produce immediate single phase of persistent spontaneous nociceptive responses accompanied by primary heat and mechanical hyperalgesia in the injection site and a spread of hyperalgesia in the non-injected site when administered subcutaneously into the plantar surface of one hind paw in rats (Chen *et al.*, 1999). Thus BV may produce pain. Alternatively, however, BV can inhibit pain. Traditionally in the Orient, BV has been used to relieve pain and to treat inflammatory diseases such as rheumatoid arthritis in humans (Billingham *et al*., 1973). For example, pretreatment of BV suppressed both the paw edema and thermal hyperalgesia evoked by carrageen (Lee *et al*., 2001). Furthermore, it was reported that BV administered into the specific acupoint can produce more potent antinociception than non-acupoint injection in the formalin test (Kwon *et al*., 2001a) and Freund's adjuvant-induced arthritis model (Kwon *et al*., 2001b). Long-term BV treatment inhibited the induction of experimental arthritis in animals (Hadjipetrou-Kourounakis and Yiangou, 1984; Somerfield and Brandwein, 1988). Consistent with these observations, the present results strongly demonstrated that BV injected into Zusanli acupoint significantly inhibited mechanical and cold allodynia. Therefore, BV therapy may be an alterative treatment for the chronic pain symptoms. However, the mechanism underlying the beneficial effects of BV on pain response should be investigated further. In addition, it is known that BV contains a variety of different peptides including melittin, apamin, adolapin and mast cell degranulating peptide (Billingham *et al*., 1973). Future studies are also needed to examine the antiallodynic effects of these components in the neuropathic pain model.

In summary, the present study demonstrated that morphine inhibited mechanical allodynia dose-dependently. It was also shown that BV injection into an acupoint, ST36 significantly reduced cold allodynia but the pain-reducing effects were not prominent than on mechanical allodynia. These results suggest that BV aqua-acupuncture as well as morphine is very effective to inhibit mechanical allodynia.

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