Diluted Bee Venom Injection Reduces Ipsilateral Mechanical Allodynia in Oxaliplatin-Induced Neuropathic Mice

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Oxaliplatin, which is used as one of anti-cancer drugs, commonly induces peripheral neuropathic pain. We have previously reported that an injection of diluted bee venom (DBV) produced a significant anti-nociceptive effect in several pain models of mice or rats. In this study, we evaluated time- and dose-dependent development of oxaliplatin-induced mechanical allodynia in bilateral hind paws of mice, and investigated the effect of DBV injection on this mechanical allodynia. DBV (0.1 mg/kg) was subcutaneously injected into the Zusanli acupoint 2 weeks after oxaliplatin (10 mg/kg) injection. One hour after DBV injection, we observed a significant reduction of mechanical allodynia in the ipsilateral hind paw, but not in the contralateral hind paw to DBV injection site. We subsequently examined whether this effect of DBV was related to the activation of peripheral nerves in DBV injected site, and then whether it was mediated by the activation of spinal cord alpha-2 adrenoceptors or opioid receptors. Subcutaneous pre-injection of 2% lidocaine (40 mg/kg) into the Zusanli acupoint completely blocked the anti-allodynic effect of DBV. Intrathecal pretreatment with yohimbine (25 µg/mouse), an alpha-2 adrenoceptor antagonist, also prevented the anti-allodynic effect of DBV, whereas pretreatment with naloxone (20 µg/mouse), an opioid receptor antagonist, did not block the effect of DBV. Taken together, these findings demonstrate that DBV injection into the Zusanli acupoint significantly reduces ipsilateral mechanical allodynia generated by oxaliplatin in mice, and also suggest that this anti-allodynic effect is dependent on the peripheral nerve activation in injected site and spinal cord alpha-2 adrenoceptors.

Key words bee venom; oxaliplatin; mechanical allodynia; alpha-2 adrenoceptor

Peripheral neuropathy is one of the most common side effects following chemotherapy and results in prominent sensory disability and persistent pain such as allodynia (pain as a result of a stimulus that does not normally provoke pain).1–4 Oxaliplatin is the third-generation platinum-based compound used as the primary therapy for metastatic colorectal cancer and other malignancies such as lung, breast, and ovarian cancers.5–8 Oxaliplatin also induces prominent neuropathic pain that is characterized by pronounced cold and mechanical hypersensitivity and spontaneous pain.9–11 Several types of analgesics, anticonvulsants, and antioxidants have been tested in preventing oxaliplatin-induced neuropathic pain, but no definite effects have been found in clinical studies.12 Thus, it still remains a high priority to identify safe and effective approaches to prevent or ameliorate oxaliplatin-induced neuropathic pain.

Chemical acupuncture point (acupoint) stimulation with diluted bee venom (DBV), termed apipuncture, has been used clinically in traditional oriental medicine to produce a potent anti-inflammatory and anti-nociceptive effect in human patients.13 Previous experimental studies have shown that DBV treatment into acupoint produced a prominent anti-nociceptive effect in several animal models of pain including the formalin test, the writhing test and the arthritis model.14–16 Recently we has also demonstrated that the injection of DBV into Zusanli acupoint alleviated thermal hyperalgesia and/or mechanical allodynia in the rat sciatic nerve chronic constricive injury (CCI) model of neuropathic pain.17–18 We have further shown that this DBV-induced anti-nociceptive effect is mediated by the activation of descending coeruleospinal noradenergeic pathways, which in turn activate spinal alpha-2 adrenoceptors.17–19 Despite this anti-nociceptive effectiveness of DBV injection, no data are available on the potential effect of DBV treatment in oxaliplatin-induced mechanical allodynia, and the underlying mechanism is also unclear.

Thus, we evaluated time- and dose-dependent development of oxaliplatin-induced neuropathic pain, i.e., mechanical allodynia in bilateral hind paws of mice, and then investigated the relieving effect of DBV acupoint injection on this mechanical allodynia. In addition, we determined whether the effect of DBV was associated with the activation of peripheral nerves in DBV injected site, and whether it was also mediated by the activation of opioid receptors and/or alpha-2 adrenoceptors in the spinal cord.

MATERIALS AND METHODS

Animals The experiments were performed using male C57BL/6 mice (25–30 g; Central Lab. Animal Inc., Seoul, Korea) housed in colony cages with free access to food and water, and maintained in temperature- and light-controlled rooms (23±2°C, 12/12-hour light/dark cycle with lights on at 07:00) for at least 1 week prior to the experiment. The experimental protocols for animal usage were reviewed and approved by the Kyung Hee University Institutional Animal Care and Use Committee and conformed to National Institutes
Oxaliplatin Administration Oxaliplatin (Tocris Biosciences, Bristol, U.K.) was prepared by diluting to 1 mg/mL in saline (0.9%) from a stock solution (5 mg/mL in 5% dextrose) and injected intraperitoneally at each dosage of 3, 5 or 10 mg/kg. Control animals received an equivalent volume of vehicle, which consisted of 5% dextrose and saline in the same final concentration as the oxaliplatin solution.

DBV and Drugs Treatment Bee venom from Apis mellifera (Sigma, St. Louis, MO, U.S.A.) was dissolved in physiologic saline (20 µL) at a dose of 0.1 mg/kg (DBV). DBV was subcutaneously administered into the Zusanli acupoint (ST36) of the right hind limb (DBV-Zu) or non-acupoint (an arbitrary position on the back, DBV-Back). The Zusanli point is one of the most frequently used of all acupuncture points, and is certainly the most intensively studied in pain medicine in association with the complementary and alternative medicine. A lot of literatures have reported that acupuncture therapy into Zusanli point using manual and electrical stimulation as well as chemical stimulation could produce significant antinociceptive effects in several pain animal models and human patients. The Zusanli point was located on the lateral side of the stifle joint adjacent to the anterior tubercle of the tibia as previously described. Animals in the control group received an injection of saline into the Zusanli acupoint (saline).

To verify the effect of DBV was mediated by peripheral neuronal activation, lidocaine (Sigma, St. Louis, MO, U.S.A.), a common local anesthetic, was dissolved in 20% ethanol solution (2% lidocaine in 50 µL volume, 40 mg/kg) and was subcutaneously pre-injected into the same Zusanli point 10 min before DBV (Lido-DBV) or saline injection (Lido-saline).

The involvement of spinal alpha-2 adrenoceptors or opioid receptors in effect of DBV was examined by intrathecal (i.t.) pretreatment with an alpha-2 adrenergic or an opioid receptor antagonist. Thus, yohimbine (YOH, Sigma; 25 µg/mouse diluted in D.W.); an alpha-2 adrenoceptor antagonist, or naloxone (NAL, Tocris; 20 µg/mouse diluted in saline), a non-selective opioid receptor antagonist, were i.t. injected 10 min before DBV (YOH-DBV and NAL-DBV) or saline treatment (YOH-saline and NAL-saline), respectively. Moreover, it was assessed whether i.t. pretreatment with vehicle (D.W. or saline) affected DBV-induced effect (vehicle-saline and vehicle-DBV groups).

Mechanical Allodynia Test Mechanical withdrawal threshold was determined for all mice 0 d prior oxaliplatin injection to obtain normal baseline values of withdrawal threshold to mechanical stimuli (Day 0 in Fig. 1). For 14 d post-oxaliplatin injection, all experimental animals were behaviorally tested to confirm the development of mechanical allodynia by high dose (10 mg/kg) of oxaliplatin. **p<0.01 and ***p<0.001 vs. vehicle.
allodynia (Day 1, 3, 7, 10 and 14 in Fig. 1). At 15 or 16 d after oxaliplatin injection, mice were randomly assigned and the effect of single DBV injection was subsequently examined. Mice were placed in an acrylic cylinder (6.5 cm in diameter, 17 cm height) on an elevated metal mesh grid and allowed to acclimate for 30 min before testing. We used an ascending series of von Frey filaments (North Coast Medical, Morgan Hill, CA, U.S.A.) that delivered approximately logarithmic incremental forces (range of bending force: 0.008, 0.02, 0.04, 0.07, 0.16, 0.4 and 0.6 g). Each monofilament, starting with the lowest force (0.008 g), was applied six times to the mid-plantar region of hind paw before the next higher force monofilament. The monofilament that produced a response of paw withdrawal, flinching, or licking in 3 out of the 6 applications was defined as the mechanical withdrawal threshold as previously described.27,28) The behavioral investigator was blinded to the treatment of animals during the experiments.

Statistical Analysis All values are expressed as the mean±S.E.M. Statistical analysis was performed using Prism 5.0 (Graph Pad Software, San Diego, CA, U.S.A.). Repeated measured two-way ANOVA was performed to determine overall differences at each time point in 50% paw withdrawal threshold. Post-hoc analysis was performed using the Bonferroni’s multiple comparison test in order to determine the p value among experiment groups. A p<0.05 was considered statistically significant.

RESULTS

Dose-Dependent Induction of Mechanical Allodynia by Oxaliplatin No significant change in the paw withdrawal threshold was observed in vehicle-treated animals (n=5) for 14 d as compared to pre-treated value (Figs. 1A, B). In contrast, oxaliplatin injection dose-dependently decreased the 50% paw withdrawal threshold in both hind limbs from 7 or 10 d to 14 d after injection, indicating the remarkable induction of mechanical allodynia by high dose (10 mg/kg) of oxaliplatin (Figs. 1A, B, ** p<0.01 and *** p<0.001 as compared to those in vehicle-treated mice).

Region-Dependent Effect of DBV Injection The results of DBV injection are summarized in Figs. 2A and B. These figures illustrate the effect of saline (n=6) versus DBV (DBV-Zu, n=6) injection into the Zusanli acupoint and the effect of DBV injection into a non-acupoint (DBV-Back, n=5) on mechanical allodynia in right hind paw (Fig. 2A) and left hind paw (Fig. 2B). No statistically significant differences were detected between the values for the paw withdrawal threshold before and after treatment in the saline and the DBV-Back
Interestingly, 1h after DBV injection, significant increases of paw withdrawal threshold were detected in the right hind paw of the DBV-Zu group (the ipsilateral side to DBV-Zu injection) as compared to those in saline-treated group (Fig. 2A, ***p < 0.001). In contrast, there is no effect of DBV treatment in the left hind paw (the contralateral side of DBV-Zu injection, Fig. 2B).

**Involvement of Peripheral Nerve Activation**

Peripheral vehicle pretreatment into acupoint did not modify DBV-induced increase of paw withdrawal threshold (vehicle-DBV, n=6), which was similar to the result illustrated in Fig. 2A.
Chemotherapy treatment with oxaliplatin produces painful neuropathy characterized by reduced thresholds to mechanical stimuli. In the first set of experiments, we examined that an injection of oxaliplatin dose-dependently induced significant decreases of paw withdrawal threshold, i.e., mechanical allodynia in both hind limbs of mice. High dose of oxaliplatin (10 mg/kg) was shown to produce more consistent mechanical allodynia in both hind paws 14 d after oxaliplatin injection as compared to those in lower doses of oxaliplatin (3 or 5 mg/kg). Based on these data, subsequent experiments were performed using neuropathic mice generated by 10 mg/kg dose of oxaliplatin.

The present study demonstrated that DBV injection into the Zusanli acupoint reduced significantly oxaliplatin-induced mechanical allodynia in the ipsilateral hind paw to DBV injected site. In contrast, there was no effect in DBV into non-acupoint treated animals. Until now we have demonstrated that DBV injection into Zusanli acupoint, but not non-acupoint, could evoke an anti-nociceptive effect in several pain animal models including peripheral neuropathic pain.\(^{14,15,17}\) In addition, DBV stimulation into Zusanli acupoint activates capsaicin-insensitive afferents, maybe it is considered as an A\(\delta\) and C fiber.\(^{30}\) These results demonstrated that DBV injection into acupoint could reduce the nociceptive behaviors in acute pain sensation and mechanical allodynia in chronic pain condition, indicating the injection site-specific analgesic effect of DBV in experimental pain animal models.

In order to verify the anti-nociceptive effect of DBV and its underlying mechanism, we have used the formalin-induced pain model, the carrageenan-induced inflammatory pain model and the peripheral nerve injury-induced neuropathic pain model.\(^{14,15,17}\) These animal models represent the unilateral pain behavior in injured or stimulated hind limb, while the oxaliplatin-induced neuropathic pain model used in the present study is characterized by a robust mechanical allodynia in both hind limbs. In this regard, the present study could explain whether DBV injection into a single side of hind limb reduces the pain response in only treated side (ipsilateral) hind paw or in the bilateral hind paws. Interestingly, the present study showed that an injection of DBV into acupoint could modulate the mechanical allodynia in ipsilateral hind paw to DBV-injected side, whereas the mechanical allodynia in contralateral hind paw to injection side was not reduced by DBV injection. These results demonstrated that DBV-induced anti-allodynic effect might be dependent on the treated side of DBV injection in oxaliplatin-induced neuropathic mice.

Subsequently, we were to determine whether this analgesic action of DBV into acupoint was mediated by the peripheral neuronal activation in the treated region, or not. While the lidocaine itself did not affect the oxaliplatin-induced mechanical allodynia, the pretreatment of lidocaine into Zusanli acupoint totally blocked the anti-allodynic effect of DBV injection. On the other hand, YOH or NAL itself (YOH-saline, \(n=6\)) did not affect the oxaliplatin-induced mechanical allodynia in mice (Figs. 4A, B).

DISCUSSION

Chemotherapy treatment with oxaliplatin produces painful neuropathy characterized by reduced thresholds to mechanical stimuli. 14,17,20) In addition, this DBV injection produced a potent anti-allodynic effect via peripheral nerve activation at the site of injection, in which the capsaicin-insensitive fibers were maybe innervated abundantly.
side effects in both mice formalin test and rat sciatic nerve injury-induced neuropathic pain model.21) Similarly in the present study, we verified that i.t. pretreatment with YOH was able to completely block the anti-allodynic effect of DBV in oxaliplation-induced neuropathic mice, whereas i.t. NAL did not reverse the DBV-induced anti-allodynic effect. These findings suggested that DBV injection might serve as an effective therapeutic modality in oxaliplatin-induced pain management via the activation of spinal cord alpha-2 adrenergic receptors, but not opioid receptors. Moreover it is important to note that this study is the first document demonstrating that the modification of spinal cord alpha-2 adrenergic receptors can be a potential therapeutic target for oxaliplatin-induced mechanical allodynia.

In conclusion, the present study demonstrated that DBV injection into acupoint suppressed mechanical allodynia in ipsilateral hind paw to DBV injected site in oxaliplatin-induced neuropathic mice model; thus this might be an effective alternative therapy for mechanical allodynia originated from oxaliplatin-used chemotherapy. In addition, this anti-allodynic effect of DBV is mainly mediated by the peripheral nerve activation in injected site and the activation of spinal cord alpha-2 adrenergic receptors.

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REFERENCES


