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Repetitive Acupuncture Point Treatment with Diluted Bee Venom Relieves Mechanical Allodynia and Restores Intraepidermal Nerve Fiber Loss in Oxaliplatin-Induced Neuropathic Mice

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Abstract: The chemotherapeutic agent, oxaliplatin, produces a robust painful neuropathy that results in the loss of intraepidermal nerve fibers (IENFs). We have previously reported that an acupuncture point (acupoint) injection of diluted bee venom (DBV) produces a temporary antiallodynic effect in oxaliplatin-induced neuropathic mice. Herein we show a significant long-lasting antinociceptive effect of repetitive DBV acupoint treatment on oxaliplatin-induced mechanical allodynia and a significant reduction in the loss of IENFs. DBV (0.1 mg/kg, subcutaneous) was administered once a day for 18 days beginning on day 15 after oxaliplatin injection. Immunohistochemistry for IENF was performed on the glabrous skin of the hind paw footpad using the pan-neuronal marker, protein gene product 9.5. A temporary increase in mechanical threshold was observed 60 minutes after a single DBV injection into the Zusanli acupoint, and this effect was enhanced over time with repetitive DBV treatments. The basal mechanical threshold before daily DBV injection also increased from day 7 after DBV injections, and peaked at day 14 after DBV treatment. Moreover, the oxaliplatin-induced loss of IENFs was significantly reduced in mice treated repetitively with DBV. Repetitive pretreatment with the α-2 adrenoceptor antagonist, yohimbine, (5 mg/kg, subcutaneous) completely prevented the antiallodynic effects and the increase in IENFs observed in mice treated repetitively with DBV.

***Perspective:** We showed that repetitive acupoint stimulation with DBV gradually and significantly reduced oxaliplatin-induced mechanical allodynia and restored the loss of IENFs in neuropathic mice via an α-2 adrenoceptor mechanism. Collectively, results of this study suggest that repetitive acupoint treatment with DBV can be a potential strategy for the management of chemotherapy-induced neuropathy.*

***Key words:** Bee venom, oxaliplatin, mechanical allodynia, intraepidermal nerve fibers, α-2 adrenoceptors.*

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Neuropathy is observed after application of various chemotherapy agents including paclitaxel, vincristine, and cisplatin,[6,38] and presents as a mildly disrupting tingling sensation to an extremely painful paresthesia.[39] The neuropathic side effects of chemotherapy treatment are much more disruptive and persistent, compared with other common side effects such as alopecia, emesis, diarrhea, and fatigue.[10] Oxaliplatin (OXA) is a third-generation platinum-based compound used as the primary therapy for metastatic colorectal cancer and other malignancies including lung, breast, and ovarian cancer.[11,13,40,50] OXA can also induce prominent neuropathic pain that is characterized by pronounced cold and mechanical hypersensitivity and spontaneous pain.[2,5,51] Several types of analgesics, anticonvulsants, and antioxidants that are approved for the treatment of other neuropathic pain states have shown little or no analgesic effect on chemotherapy-induced peripheral neuropathic pain in large randomized, placebo-controlled clinical trials.[1,48] Thus, it still remains a high priority to identify safe and effective approaches to prevent or ameliorate OXA-induced neuropathic pain.

It is well known that chemotherapy also results in the loss of nerve fibers that innervate the epidermis.[47] These intraepidermal nerve fibers (IENFs) are naked nerve endings that enter the epidermis as Aδ and C fibers across the dermal–epidermal junction. They are particularly important in transmitting noxious mechanical and thermal information,[33] and loss of IENFs is observed in a host of chronic painful neuropathic conditions, including diabetes,[18] complex regional pain syndrome,[35] and postherpetic neuralgia.[37] Although there is a paucity of research on whether IENF loss is related to functional impairment, several studies have found that IENF loss is most pronounced in areas specific to pain.[46,53] In this regard, the relationship between decreased IENFs and the generation of chronic pain has been proposed,[34] however, there are few studies on the potential effects of antinociceptive drugs or therapies with respect to the IENF changes related to chemotherapy-induced chronic 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 antinociceptive effect in human patients.[49] Our previous experimental studies have shown that DBV injection into the Zusanli acupoint (Stomach-36, ST-36) produced a prominent antinociceptive effect in several animal models of pain including the formalin test, the writhing test, a model of inflammatory pain, and in an arthritic model.[19,24,28] In addition, we have shown that the injection of DBV into the Zusanli acupoint alleviated thermal hyperalgesia and/or mechanical allodynia in the rat sciatic nerve chronic constrictive injury model of neuropathic pain.[17,42] We have further shown that these DBV-induced antinociceptive effects are mediated by the activation of descending coeruleospinal noradrenergic pathways, which in turn activate spinal α-2 adrenoceptors.[17,25,42] In this regard, we have recently shown that a single injection of DBV into the Zusanli acupoint, but not into a nonacupoint, also significantly reduced ipsilateral mechanical allodynia generated by OXA (10 mg/kg) in mice.[60] Moreover, this effect of DBV was also mediated by the activation of α-2 adrenoceptors, but not opioid receptors in the spinal cord.

The present study was designed to examine whether long-term repetitive injections of DBV into the Zusanli acupoint over an 18-day period produces an increase in the antiallodynic effects of DBV in OXA-induced neuropathic mice, and whether these repetitive acupoint treatments also affect the OXA-induced loss of IENFs in the glabrous skin of the hind paw footpad. In addition, we investigated whether the effects of DBV are associated with the activation of α-2 adrenoceptors.

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 (24 ± 2°C, 12/12-hour light/dark cycle with lights on at 07:00) for at least 1 week before 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 of Health guidelines (NIH publication No. 86-23, revised 1985). To examine the pain behaviors and the number and density of IENFs, animals (total N = 59) were randomly divided into 9 treatment groups as follows: vehicle (SHAM, n = 10), OXA (n = 11), OXA/saline into the acupoint (Saline, n = 6), OXA and DBV into the acupoint (DBV-AC, n = 6), OXA and DBV into a nonacupoint (DBV-NA, n = 5), OXA with yohimbine (YOH) and DBV into the acupoint (YOH-DBV, n = 5), OXA and YOH and saline into the acupoint (YOH-Saline, n = 5), OXA and distilled water (DW) and DBV into the acupoint (DW-DBV, n = 6), and OXA and DW and saline into the acupoint (DW-Saline, n = 5).

OXA Administration

OXA (Tocris Bioscience, Bristol, United Kingdom) 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 a dosage of 10 mg/kg.[60] Control animals received an equivalent volume of vehicle, which consisted of 5% dextrose and saline in the same final concentration as the OXA solution.

DBV and YOH Treatment

Bee venom from *Apis mellifera* (Sigma, St. Louis, MO) was dissolved in physiologic saline (20 μL) at a dose of 0.1 mg/kg (DBV).[41,60] In a previous study, we showed that adequate DBV (0.1 mg/kg) produces an antinociceptive effect without a significant induction of nociceptive behaviors in mice.[39] DBV was subcutaneously injected into the Zusanli acupoint (ST-36) of the right hind limb (DBV-AC) or into a nonacupoint (an arbitrary position on the back, DBV-NA) once a day for