

Article

Analgesic Effects of Bee Venom Derived Phospholipase A₂ in a Mouse Model of Oxaliplatin-Induced Neuropathic Pain

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Abstract: A single infusion of oxaliplatin, which is widely used to treat metastatic colorectal cancer, induces specific sensory neurotoxicity signs that are triggered or aggravated when exposed to cold or mechanical stimuli. Bee Venom (BV) has been traditionally used in Korea to treat various pain symptoms. Our recent study demonstrated that BV alleviates oxaliplatin-induced cold allodynia in rats, via noradrenergic and serotonergic analgesic pathways. In this study, we have further investigated whether BV derived phospholipase A₂ (bvPLA₂) attenuates oxaliplatin-induced cold and mechanical allodynia in mice and its mechanism. The behavioral signs of cold and mechanical allodynia were evaluated by acetone and a von Frey hair test on the hind paw, respectively. The significant allodynia signs were observed from one day after an oxaliplatin injection (6 mg/kg, i.p.). Daily administration of bvPLA₂ (0.2 mg/kg, i.p.) for five consecutive days markedly attenuated cold and mechanical allodynia, which was more potent than the effect of BV (1 mg/kg, i.p.). The depletion of noradrenaline by an injection of *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP4, 50 mg/kg, i.p.) blocked the analgesic effect of bvPLA₂, whereas the depletion of serotonin by injecting DL-*p*-chlorophenylalanine (PCPA, 150 mg/kg, i.p.) for three successive days did not. Furthermore, idazoxan (α 2-adrenergic receptor antagonist, 1 mg/kg, i.p.)

completely blocked bvPLA₂-induced anti-allodynic action, whereas prazosin (α 1-adrenergic antagonist, 10 mg/kg, i.p.) did not. These results suggest that bvPLA₂ treatment strongly alleviates oxaliplatin-induced acute cold and mechanical allodynia in mice through the activation of the noradrenergic system, via α 2-adrenergic receptors, but not via the serotonergic system.

Keywords: phospholipase A₂; Bee Venom; oxaliplatin; neuropathic pain; noradrenergic

1. Introduction

Oxaliplatin is an effective platinum derivative, which is widely used in the treatment of colorectal carcinoma [1,2], but causes neurotoxicity predominantly within the peripheral nervous system [3,4]. Two different types of oxaliplatin-induced peripheral neuropathy have been described hitherto, *i.e.*, cold and mechanical hypersensitivity [5,6]. However, effective treatment for oxaliplatin-induced cold and mechanical hypersensitivity still remains to be elucidated. Hence, it is required to discover therapeutic options for the management of oxaliplatin-induced neuropathic pain.

Bee Venom (BV) has been traditionally used in Korea to relieve pain and to treat chronic inflammatory diseases [7–12]. Previous studies have demonstrated that the analgesic effects of BV in various pain models are mediated mainly by activation of α 2-adrenergic and/or serotonergic receptors [12–16]. Rho and his colleagues have reported that subcutaneous injections of BV attenuated heat hyperalgesia and cold and mechanical allodynia in the rats with nerve injury-induced neuropathic pain through the activation of the endogenous noradrenergic system [17,18]. In a rat model of oxaliplatin-induced neuropathic pain, we found that the anti-allodynic effect of BV is at least partially mediated by the noradrenergic and serotonergic system, but not by the opioid system [9,16].

Phospholipase A₂ from BV (bvPLA₂), a prototypic group III enzyme that hydrolyzes fatty acids in membrane phospholipids, is one of the major active components of BV [19,20]. Several studies have shown that this bvPLA₂ prevents neuronal cell death and spinal cord injury [21,22]. Our previous study demonstrated that BV mitigates cisplatin-induced nephrotoxicity [23] and found that bvPLA₂ can reduce such nephrotoxicity more potently than BV [24]. However, the effect of PLA₂ on oxaliplatin-induced neuropathic pain and its mechanism have not been studied yet.

The aim of this study was to evaluate and compare the analgesic effect of BV and bvPLA₂ on oxaliplatin-induced cold and mechanical allodynia in mice. In addition, we examined whether the anti-allodynic effect of bvPLA₂ is mediated by the serotonergic or noradrenergic pain inhibitory system.

2. Results

2.1. Effects of BV and bvPLA₂ on Oxaliplatin-Induced Cold and Mechanical Hypersensitivity

First, we investigated the effects of a single administration of oxaliplatin (6 mg/kg, i.p.) on behavioral sensitivity to cold and mechanical stimuli in mice (Figure 1). The administration significantly increased the frequency of licking and shaking of the hind paw in response to cold acetone stimuli. A significant cold allodynia was observed at day 1, peaked at day 3 and lasted for at least seven days after the

oxaliplatin administration, compared to the vehicle group (Figure 1a). Similarly, an administration of oxaliplatin significantly increased the withdrawal responses of the hind paw to von Frey filament applications (as expressed % of withdrawal response) at day 1, peaked at day 3–4 and maintained up to day 5 (Figure 1b).

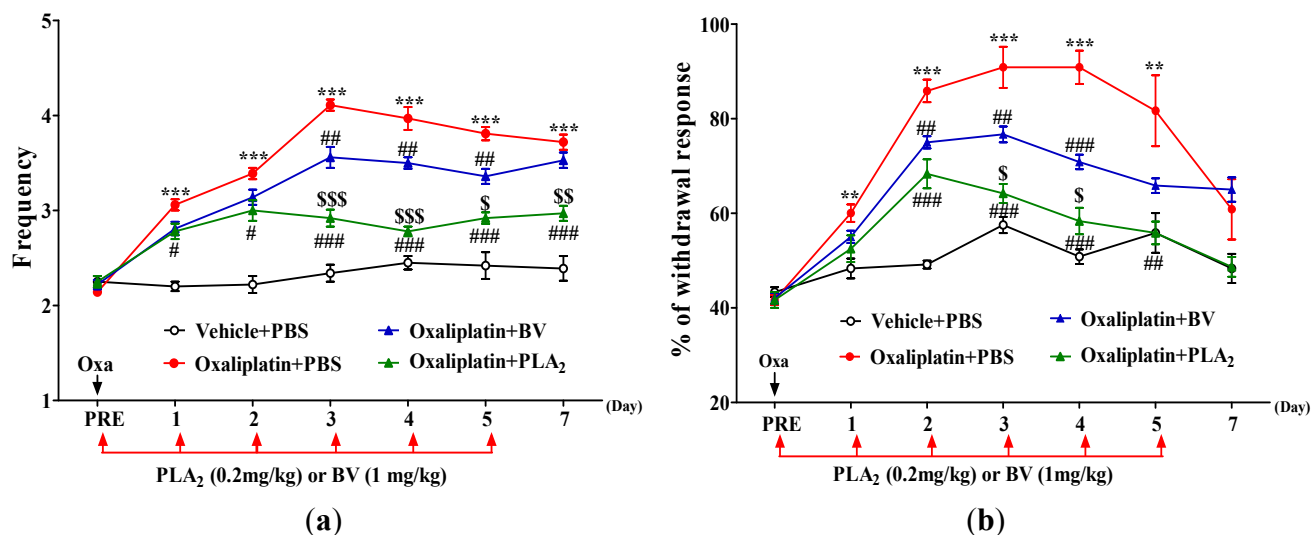


Figure 1. Effects of BV and bvPLA₂ on oxaliplatin-induced cold and mechanical allodynia in mice. The behavioral tests for cold (a) and mechanical (b) allodynia were performed before (PRE) and after the administration of oxaliplatin (6 mg/kg, i.p.). Vehicle (5% glucose) + PBS, Oxaliplatin + PBS, Oxaliplatin + BV, and Oxaliplatin + bvPLA₂ groups received daily injection of PBS, BV (1 mg/kg, i.p.) or bvPLA₂ (0.2 mg/kg, i.p.) for five days after an oxaliplatin or vehicle administration. Results are expressed as mean \pm SEM; $n = 6$ mice/group; The data was analyzed with one-way ANOVA followed by the Tukey's multiple comparison test. ** $p < 0.01$, *** $p < 0.001$, vs. Vehicle + PBS; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs. Oxaliplatin + PBS; \$ $p < 0.05$, \$\$ $p < 0.01$, \$\$\$ $p < 0.001$, vs. Oxaliplatin + BV.

Daily BV treatment (1 mg/kg, i.p.) for five consecutive days significantly reduced the cold allodynia from three days after the oxaliplatin administration and such analgesic effect last up to day 5. In addition, bvPLA₂ treatment (0.2 mg/kg, i.p.) significantly attenuated the cold allodynia from day 1 after the oxaliplatin injection and such effect endured at least for the following six days (Figure 1a). BV treatment also significantly attenuated mechanical allodynia from day 2 after the oxaliplatin injection and this BV analgesia was continued up to day 4. Moreover, bvPLA₂ treatment showed a significant reduction in mechanical allodynia from day 2 after the oxaliplatin injection and such effect lasted up to day 5 (Figure 1b). The relieving effects of bvPLA₂ on oxaliplatin-induced cold and mechanical allodynia were significantly more potent than those of BV (Figure 1).

2.2. Effects of BvPLA₂ on Oxaliplatin-Induced Cold and Mechanical Allodynia in Serotonin Depleted Mice

We investigated the effects of bvPLA₂ on oxaliplatin-induced cold and mechanical allodynia in serotonin depleted mice by injecting DL-*p*-chlorophenylalanine (PCPA, 150 mg/kg, i.p.) for three successive days [25,26]. PCPA pretreatment itself did not affect the behavioral signs of cold and

mechanical allodynia induced by oxaliplatin ($p > 0.05$, Oxaliplatin + PBS + PCPA [$n = 6$] vs. Oxaliplatin + PBS + NS [$n = 4$], Cold (frequency): 1.83 ± 0.04 vs. 1.92 ± 0.05 at 0d, 4.61 ± 0.07 vs. 4.67 ± 0.07 at 3d, 3.83 ± 0.06 vs. 3.79 ± 0.08 at 5d, 3.25 ± 0.07 vs. 3.04 ± 0.10 at 7d; Mechanical (%): 30.00 ± 1.83 vs. 30.00 ± 0.00 at 0d, 94.17 ± 0.83 vs. 93.75 ± 1.25 at 3d, 72.03 ± 1.54 vs. 68.75 ± 1.25 at 5d, 50 ± 2.24 vs. 51.25 ± 1.25 at 7d). Thus, we pooled the data from the two groups as a control group (Oxaliplatin + PBS + PCPA/NS, Figure 2). Compared to this control group, bvPLA₂ treatment in mice without serotonin depletion (Oxaliplatin + PLA₂ + NS group) significantly attenuated the cold and mechanical hypersensitivity ($p < 0.01$ at days 3 and 5). Such anti-allodynic effects of bvPLA₂ were not blocked by PCPA pretreatment (Figure 2), indicating that the serotonergic mechanism is not involved in the analgesic effect of bvPLA₂ on oxaliplatin-induced neuropathic pain.

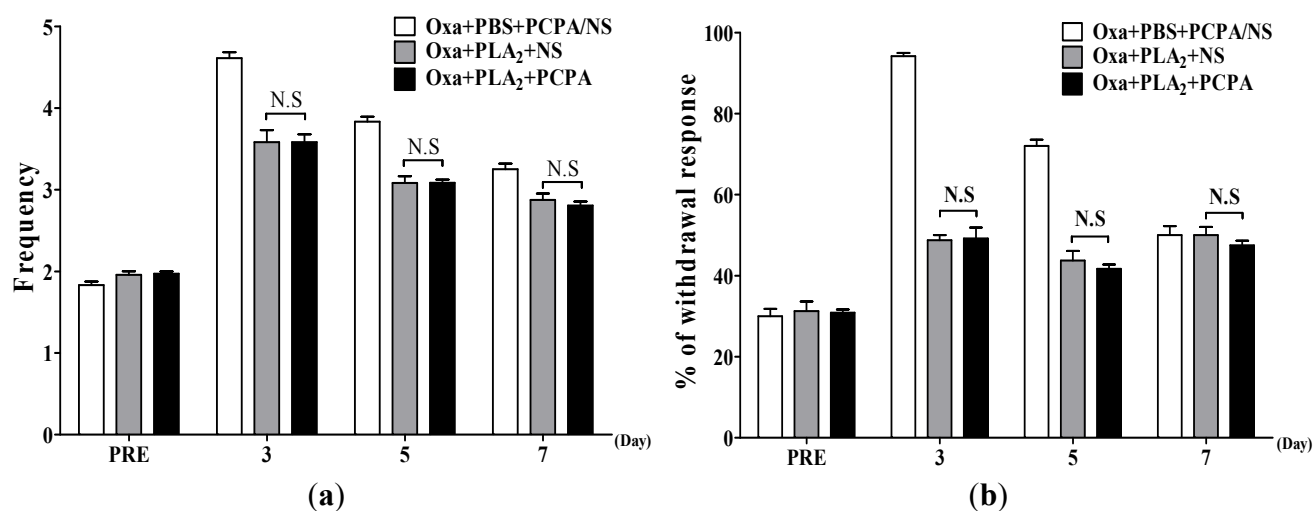


Figure 2. Effects of bvPLA₂ on oxaliplatin-induced cold and mechanical allodynia in serotonin depleted mice. The behavioral tests for cold (a) and mechanical (b) allodynia were performed before and after an administration of oxaliplatin (Oxa, 6 mg/kg, i.p.). Serotonin was depleted by daily injections of PCPA (150 mg/kg, i.p.) for three consecutive days prior to an oxaliplatin administration. Oxa + PBS + PCPA/NS, $n = 10$; Other groups, $n = 6$ mice/group; Results are expressed as mean \pm SEM; N.S, no significance ($p > 0.05$). The data were analyzed with one-way ANOVA followed by the Tukey's multiple comparison test.

2.3. Noradrenergic Mechanism of the Anti-Allodynic Effects of BvPLA₂ in Oxaliplatin-Administered Mice

We evaluated the effects of bvPLA₂ on oxaliplatin-induced allodynia in noradrenaline depleted mice by a pretreatment of DSP4 [27]. The anti-allodynic effects of bvPLA₂ (Oxa + PLA₂ + NS group, $p < 0.01$ vs. control Oxa + PBS + DSP4/NS group at days 3, 5 and 7) were significantly blocked by DSP4 pretreatment (Figure 3), unlike the aforementioned PCPA pretreatment. These results suggest that activation of the noradrenergic pain inhibitory pathway at least partially mediates the bvPLA₂-induced anti-allodynic action in oxaliplatin-administered mice.

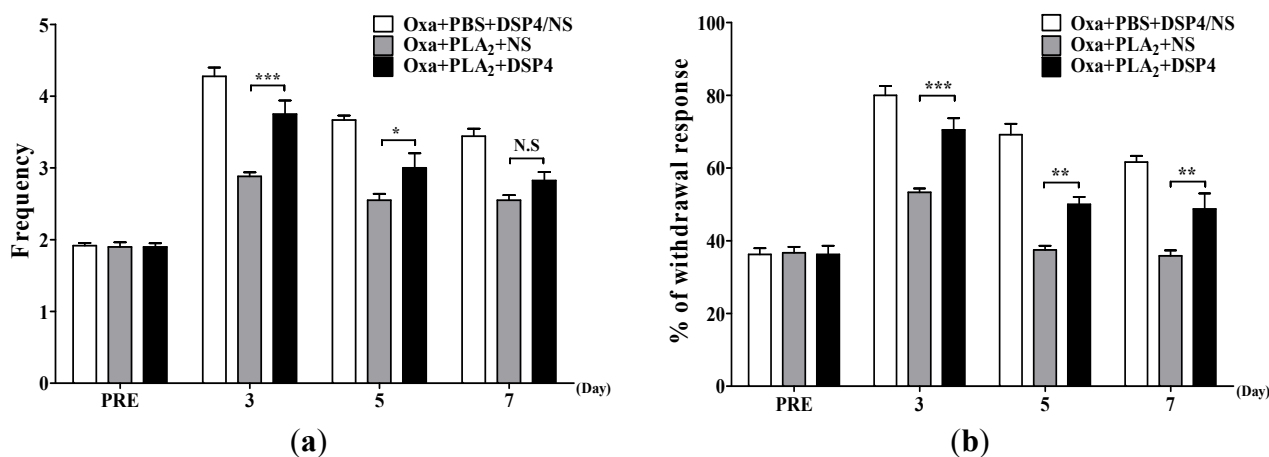


Figure 3. Effects of bvPLA₂ on oxaliplatin-induced cold and mechanical allodynia in noradrenaline depleted mice. The behavioral tests for cold (a) and mechanical (b) allodynia were performed before and after an administration of oxaliplatin (6 mg/kg, i.p.). Noradrenaline was depleted by an injection of DSP4 (50 mg/kg, i.p.) at a day before an oxaliplatin administration. Since DSP4 pretreatment itself did not affect the cold and mechanical allodynia signs induced by oxaliplatin ($p > 0.05$, Oxa + PBS + DSP4 [$n = 6$] vs. Oxa + PBS + NS [$n = 6$]), we pooled the data from the two groups as a control (Oxa + PBS + DSP4/NS, $n = 12$). Other groups, $n = 6$ mice/group; Results are expressed as mean \pm SEM; N.S, no significance ($p > 0.05$), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, The data was analyzed with one-way ANOVA followed by the Tukey’s multiple comparison test.

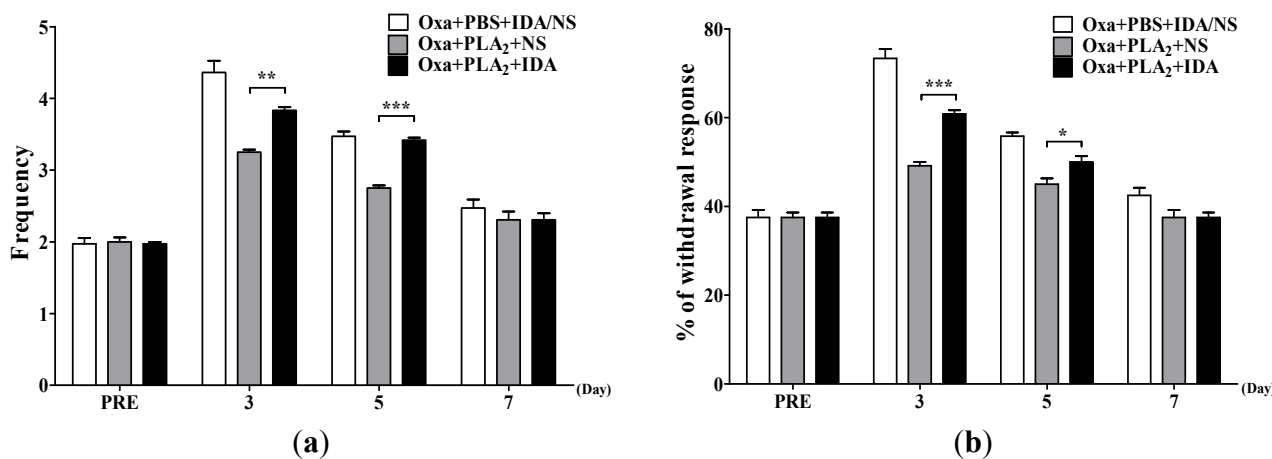


Figure 4. Effect of α_2 -adrenergic receptor antagonist, idazoxan, on bvPLA₂-induced anti-allodynic action in oxaliplatin-administered mice. The behavioral tests for cold (a) and mechanical (b) allodynia were performed before and after an administration of oxaliplatin (6 mg/kg, i.p.). Idazoxan (IDA, 1 mg/kg, i.p.) was administered 30 min prior to bvPLA₂ injection. Since IDA pretreatment itself did not affect the cold and mechanical allodynia signs induced by oxaliplatin ($p > 0.05$, Oxa + PBS + IDA [$n = 6$] vs. Oxa + PBS + NS [$n = 6$]), we pooled the data from the two groups as a control (Oxa + PBS + IDA/NS, $n = 12$). Other groups, $n = 6$ mice/group; Results are expressed as mean \pm SEM; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, The data was analyzed with one-way ANOVA followed by the Tukey’s multiple comparison test.

To identify which adrenergic receptor subtype mediates the analgesic effects of bvPLA₂ on oxaliplatin-induced neuropathic pain in mice, we examined the effect of prazosin (PRA, α 1-adrenergic receptor antagonist) or idazoxan (IDA, α 2-adrenergic receptor antagonist) on the bvPLA₂-induced anti-allodynic action. As shown in Figure 4, IDA (1 mg/kg, i.p.) significantly blocked the relieving effect of bvPLA₂ on oxaliplatin-induced cold and mechanical allodynia. However, PRA (10 mg/kg, i.p.) did not affect the bvPLA₂ effect, because there were no significant differences in cold and mechanical sensitivity between the Oxa + PLA₂ + NS and Oxa + PLA₂ + PRA groups ($p > 0.05$, Table 1). These results indicate that bvPLA₂ treatment alleviates oxaliplatin-induced acute cold and mechanical allodynia in mice via activation of α 2-adrenergic receptors, but not α 1-adrenergic receptors.

Table 1. Effect of α 1-adrenergic receptor antagonist, prazosin (PRA) on bvPLA₂-induced anti-allodynic action in oxaliplatin-administered mice.

Post-Oxaliplatin Day	D0	D3	D5	D7
Acetone test		Frequency		
Oxa + PBS + PRA/NS	2.1 ± 0.04	4.4 ± 0.08	3.6 ± 0.15	3.3 ± 0.09
Oxa + PLA ₂ + NS	2.0 ± 0.06	3.5 ± 0.15 ***	3.0 ± 0.10 **	2.6 ± 0.10 ***
Oxa + PLA ₂ + PRA	2.1 ± 0.06	3.1 ± 0.12 ***	3.1 ± 0.09 *	2.8 ± 0.07 **
von Frey test <0.4 g>		% withdrawal	response	
Oxa + PBS + PRA/NS	40.8 ± 0.83	76.7 ± 1.67	55.8 ± 1.54	44.2 ± 1.54
Oxa + PLA ₂ + NS	40.0 ± 1.83	59.2 ± 2.39 ***	48.1 ± 1.29 **	41.5 ± 1.12
Oxa + PLA ₂ + PRA	40.8 ± 2.01	57.5 ± 1.71 ***	47.5 ± 1.71 **	40.33 ± 1.67

Oxa + PBS + PRA/NS, $n = 12$; Other groups, $n = 6$ mice/group; Results are expressed as mean ± SEM; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, vs. Oxa + PBS + PRA/NS, one-way ANOVA followed by the Tukey's multiple comparison test.

3. Discussion

Oxaliplatin-induced neuropathic pain represents a major obstacle to successful cancer treatment as it restricts both individual and cumulative dosages. However, despite these limitations, it is widely used and many patients suffer from the development of long-lasting consequences (*i.e.*, peripheral neuropathy) [28,29]. Patients may also experience cold-induced paresthesias, throat and jaw tightness, and occasionally focal weakness [30]. Oxaliplatin is structurally similar to other platinum based chemotherapy drugs, such as cisplatin and carboplatin. They all have neurotoxicity, however oxaliplatin has little nephrotoxicity and hematotoxicity [31]. It has been shown that oxaliplatin-induced acute neuropathy is characterized by a specific somatosensory profile, *i.e.*, cold and mechanical hypersensitivity [32]. Therefore, cold and mechanical hypersensitivity is a hallmark of oxaliplatin-induced neuropathy. Indeed, in this study, a single intraperitoneal administration of oxaliplatin (6 mg/kg) significantly increased the cold and mechanical sensitivity in mice, corroborating the previous reports using rats [33,34]. This mouse model might help in exploring the molecular and genetic mechanism of oxaliplatin-induced neuropathic pain in the future studies, since transgenic and gene knock-out/knock-in animals have been developed primarily in the mouse [35–37].

There have been few reports about the effective treatment and prevention of oxaliplatin-induced neuropathic pain. However, our previous study has shown that BV has a beneficial role in relieving

oxaliplatin-induced neuropathic pain symptoms in rats, suggesting that BV could be an alternative therapeutic option for the management of oxaliplatin-induced peripheral neuropathy [9,16]. Interestingly, we recently found that bvPLA₂ mitigates cisplatin-induced nephrotoxicity and acetaminophen-induced hepatotoxicity, which was more potent than BV [24,38]. In the present study, we investigated for the first time whether bvPLA₂ has an analgesic effect on oxaliplatin-induced cold and mechanical allodynia. Our data showed that the treatment of bvPLA₂ significantly alleviated the allodynia in oxaliplatin-administered mice and such bvPLA₂ effect was superior to the BV effect. This powerful analgesic effect of bvPLA₂ led us to investigate the underlying mechanisms.

The extensive data support a role for the monoamine neurotransmitters (*i.e.*, serotonin and noradrenaline) and opioids, in the modulation of pain [39,40]. Serotonin and noradrenaline have been implicated as principal mediators of endogenous analgesic mechanisms in the descending pain pathways [39]. Our previous study suggested that the anti-allodynic effect of BV on oxaliplatin-induced cold allodynia in rats involves the noradrenergic, but not opioid, system [9]. In addition, spinal 5-HT₃ receptors play an important role in the BV-induced anti-allodynic action in oxaliplatin-injected rats [16]. In contrast, the present study clearly showed that serotonin depletion by pretreatment of PCPA did not significantly affect the anti-allodynic effects of bvPLA₂ in oxaliplatin-administered mice. This result suggests that the serotonergic inhibitory pathway is not involved in the analgesic effect of bvPLA₂ on oxaliplatin-induced cold and mechanical hypersensitivity, unlike the case of BV. Other active components of BV, such as apamin [41], might be responsible for the serotonergic mechanism of BV-induced analgesia. However, in noradrenaline-depleted mice, the suppressive effect of bvPLA₂ on oxaliplatin-induced cold and mechanical allodynia was significantly prevented. These results indicate that the noradrenergic analgesic system is at least partially involved in the analgesic effects of bvPLA₂ in oxaliplatin-administered mice.

α -adrenergic receptors have been demonstrated to play an important role in the modulation of cold allodynia via the noradrenergic pain inhibitory system [42]. Previous studies showed that either α 1- [43,44] or α 2-adrenergic receptors [45,46] are responsible for the adrenergic sensitivity of nerve injury-induced neuropathic rats. A dual contribution of α 1- and α 2-adrenergic receptors to neuropathic pain was also suggested [47,48]. Also other articles have elucidated that the analgesic effects of BV are mediated mainly by activation of α 2-adrenergic receptors in various pain models, such as nerve injury-induced neuropathic pain, acetic acid-induced visceral pain and inflammatory pain [12–15]. Our recent study also showed that α 2-adrenergic receptors mediate the anti-allodynic effect of BV in oxaliplatin-induced neuropathic pain in rats [9]. In the present study, we further examined which adrenergic receptor subtype mediates the effects of bvPLA₂ on cold and mechanical allodynia in oxaliplatin-administered mice. The current results showed that IDA (α 2-adrenergic antagonist) was able to completely block the anti-allodynic effect of bvPLA₂ on oxaliplatin-induced neuropathic pain in mice, whereas PRA (α 1-adrenergic antagonist) did not. These results indicate that the noradrenergic mechanism of the analgesic effect of bvPLA₂ on oxaliplatin-induced neuropathic pain is mediated by activation of α 2-adrenergic, but not α 1-adrenergic, receptors.

In this study, we have clearly shown that bvPLA₂ treatment strongly alleviates oxaliplatin-induced acute cold and mechanical allodynia in mice through the activation of the noradrenergic system, via α 2-adrenergic receptors. Besides such action through the neurochemical mechanism, bvPLA₂ is known to have a potent immune modulatory effect. The major pathway of the bvPLA₂-induced immune modulation is to increase peripheral regulatory T cells, which play a key role in the maintenance of

tolerance in the immune system. Our recent studies showed that such strategies using bvPLA₂ could be successful in the prevention of cisplatin-induced acute kidney and acetaminophen-induced acute liver injury, by suppressing immune response via the modulation of regulatory T cells [24,38]. Another previous study also demonstrated that regulatory T cells attenuate peripheral nerve injury-induced neuropathic pain in rats [49]. Thus, it would be of high interest to see if bvPLA₂ treatment before an oxaliplatin administration prevents the development of neuropathic cold and mechanical allodynia by regulating peripheral immune response. To our best knowledge, there are no clinical trials for bvPLA₂ treatment. Further research is required in this field. In addition, our current work is limited to behavioral and pharmacological approaches. Molecular and genetic studies using the advantage of the mouse model are now in progress to elucidate more detailed mechanism of bvPLA₂-induced analgesia.

4. Experimental Section

4.1. Animals

Male C57BL/6 mice (6–8 weeks old) were purchased from the Daehan Biolink (Chungbuk, Korea). They were kept under specific pathogen-free conditions with air conditioning and a 12 h light/dark cycle. The mice had free access to food and water during the experiments. The study was approved by the Kyung Hee University Animal Care and Use Committee (KHUASP(SE)-15-024).

4.2. Behavioral Tests

Behavioral tests representing different sensory components of neuropathic pain were conducted before and after an oxaliplatin administration. The mice were habituated to handling by investigators and to all testing procedures for a week before the start of the experiments. The experimenters were blind to oxaliplatin and any treatments.

Cold sensitivity was measured by an acetone test [50]. Mice were placed in a clear plastic box (12 × 8 × 6 cm) with a wire mesh floor and allowed to habituate for 30 min prior to the testing. Acetone (10 μL, Reagents Chemical Ltd., Gyeonggi-do, Korea) was sprayed onto the plantar skin of each hind paw 3 times and the frequency of licking and shaking of the affected paw was counted after the acetone spray for 30 s. The advantage of acetone test is that it is quite simple, economical, and that the assessment for multiple mice can be made in a short period of time. The disadvantage is that acetone can also induce some behavioral response in naïve mice. However, mice having allodynia (e.g., oxaliplatin-administered mice) show a significant increase in the level of response to acetone when compared to the control mice.

Mechanical sensitivity was measured by the von Frey hair test [51]. Mice were placed in a clear plastic box (12 × 8 × 6 cm) with a wire mesh floor and allowed to habituate for 30 min before the testing. A von Frey filament (Linton Instrumentation, Norfolk, UK) with a bending force of 0.4 g were applied to the midplantar skin (avoiding the base of the tori) of each hind paw 10 times, with each application held for 3 s [52]. The number of withdrawal responses to the von Frey filament applications from both hind paws were counted and then expressed as an overall percentage response.

4.3. Oxaliplatin Administration and BV/bvPLA₂ Treatment

Oxaliplatin (Sigma, St. Louis, MO, USA) was dissolved in a 5% glucose solution at a concentration of 2 mg/mL and was intraperitoneally (i.p.) injected at 6 mg/kg [9,33]. The vehicle control group received the same volume of a 5% glucose solution through the same injection route.

BV (1 mg/kg, i.p., Sigma) or bvPLA₂ (0.2 mg/kg, i.p., Sigma) [9,24,38] dissolved in phosphate buffered saline (PBS) was injected in oxaliplatin-administered mice for a continuous five days. Cold and mechanical sensitivity were measured by acetone test and von Frey hair test, respectively, before each bvPLA₂ or BV treatment. Control group was treated intraperitoneally with PBS.

4.4. Depletion of Serotonin or Noradrenaline

DL-*p*-chlorophenylalanine (PCPA, Sigma, an inhibitor of serotonin synthesis, 150 mg/kg/day) or vehicle (normal saline, NS) was injected intraperitoneally to mice prior to oxaliplatin administration for three days. The dosage and treatment course of PCPA have been widely used to deplete 5-HT stores [25,26]. After 5-HT depletion with PCPA, oxaliplatin and bvPLA₂ were administered as aforementioned.

N-(2-Chloroethyl)-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP4, TOCRIS, 50 mg/kg) or vehicle (NS) was injected intraperitoneally to mice a day before an oxaliplatin administration. DSP4 at a concentration of 50 mg/kg has been shown to be an effective dose for maximal NE depletion [53], with the advantage that mice did not require special care following injection as no adverse effects could be observed.

4.5. α 1- or α 2-Adrenergic Receptor Antagonist

To test which adrenergic receptor subtype mediates the anti-allodynic effects of bvPLA₂ in oxaliplatin-administered mice, specific antagonists were administered intraperitoneally 30 min prior to bvPLA₂ treatment for five days: α 1-adrenergic receptor antagonist (prazosin, 10 mg/kg, Sigma), α 2-adrenergic receptor antagonist (idazoxan, 1 mg/kg, Sigma). The dose of each antagonist was determined based on the previous studies showing the selective and effective antagonistic action against adrenergic receptor-mediated responses [54–56].

4.6. Statistical Analyses

The data are presented as mean \pm SEM and were analyzed by the unpaired t-test or one-way ANOVA followed by the Tukey's multiple comparison test to determine the statistical differences among the groups. $p < 0.05$ was considered as statistically significant.

5. Conclusions

In conclusion, our findings reveal that cold and mechanical sensitivity were significantly increased after a single injection of oxaliplatin in mice. BV and bvPLA₂ can exert significant relieving effects on oxaliplatin-induced cold and mechanical hypersensitivity, in which bvPLA₂ is more potent than BV. The serotonergic mechanism is not involved in the analgesic effect of bvPLA₂ on oxaliplatin-induced neuropathic pain, whereas the noradrenergic pain inhibitory system at least partially mediates the bvPLA₂ effect. Finally, we demonstrated that bvPLA₂ treatment alleviates oxaliplatin-induced acute cold

and mechanical allodynia in mice via activation of α 2-adrenergic receptors. These findings may provide clinically useful evidence for the application of bvPLA₂ in the management of peripheral neuropathic pain that occurs after the oxaliplatin administration.

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Author Contributions

Hyunsu Bae and Sun Kwang Kim conceived and designed the study. Dongxing Li and Younju Lee performed the experiments. Dongxing Li, Woojin Kim, Kyungjin Lee and Sun Kwang Kim analyzed the data. Dongxing Li, Younju Lee, Woojin Kim, Kyungjin Lee and Sun Kwang Kim wrote the manuscript. All authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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