Note

Milnacipran Inhibits Oxaliplatin-Induced Mechanical Allodynia through Spinal Action in Mice

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We investigated whether milnacipran, a serotonin–noradrenaline reuptake inhibitor, would have therapeutic effect on oxaliplatin-induced mechanical allodynia in mice. A single intraperitoneal injection of oxaliplatin (3 mg/kg) induced mechanical allodynia, which peaked on day 10 after injection and almost completely subsided by day 20. Ten days post-oxaliplatin injection, the intraperitoneal administration of milnacipran (3–30 mg/kg) significantly and dose-dependently inhibited the established mechanical allodynia. Intrathecal injections of milnacipran (2.1–21 μ g/site) also significantly and dose-dependently inhibited mechanical allodynia, but intracisternal and intracerebroventricular injections at the same doses did not. The present results suggest that milnacipran is effective against oxaliplatin-induced mechanical allodynia and that the antiallodynic effect is mainly mediated by actions on the spinal cord.

Key words milnacipran; oxaliplatin; allodynia; intrathecal injection; spinal cord

Oxaliplatin, a third-generation platinum-based chemotherapeutic agent, is a key drug for the treatment of colorectal cancer. However, oxaliplatin frequently causes dose-limiting adverse effects such as acute neurotoxicity and chronic peripheral neuropathy.¹⁾ The distressing symptoms of peripheral neuropathy include pain and dysesthesia.²⁾ Although the underlying mechanisms of oxaliplatin-induced peripheral neuropathy are not completely understood, it has been reported that oxalate, a metabolite of oxaliplatin, is involved in acute peripheral neurotoxicity, especially cold dysesthesia.^{3,4)} Several drugs, including calcium gluconate/magnesium sulfate, glutathione, carbamazepine, gabapentin, amifostine, acetyl-L-carnitine, and α -lipoic acid, have been used to manage oxaliplatin-induced peripheral neuropathy.^{4,5)} Although these drugs are effective against mild neuropathy, oxaliplatin-induced peripheral neuropathy is still difficult to treat.^{4,5)}

We have recently found that goshajinkigan, a traditional herbal medicine, suppresses oxaliplatin-induced mechanical allodynia and that this effect is, in part, mediated by both descending noradrenergic and serotonergic systems.⁶⁾ These findings imply that enhancement of descending monoaminergic systems can inhibit established mechanical allodynia. Although amitriptyline, which inhibits the reuptake of noradrenaline and serotonin,⁷⁾ has long been used for the treatment of neuropathic symptoms, especially in postherpetic neuralgia and diabetic neuropathy, it does not prevent chemotherapy-induced peripheral neuropathy in humans.⁸⁾ In contrast, prophylactic administration of venlafaxine, a serotonin–noradrenaline reuptake inhibitor (SNRI) with high potency in inhibiting serotonin reuptake,⁷⁾ has been reported to reduce oxaliplatin-induced acute neurotoxicity including spontaneous dysesthesia and daily pain in humans.⁹⁾ Prophylactic administration of duloxetine, a balanced SNRI,⁷⁾ has also been reported to reduce the severity of pain after 12 weeks of treatment.¹⁰⁾ In this study, we examined whether milnacipran, another balanced SNRI,⁷⁾ would inhibit established mechanical allodynia after oxaliplatin administration in mice. In rats with neuropathy induced by spinal nerve ligation, mechanical

allodynia has been reported to be inhibited by intrathecal, but not systemic, administration of milnacipran.¹¹⁾ Additionally, it has been reported that following spinal nerve ligation in mice, mechanical allodynia can be inhibited by systemic and intracerebroventricular injections of milnacipran.¹²⁾ Furthermore, it has been shown that systemic administration of milnacipran in mice does not affect allodynia induced by the chemotherapeutic agent paclitaxel.¹³⁾ Thus, the efficacy and mechanisms (including site of action) of the antiallodynic effect of milnacipran seem to differ depending on causative factors. Therefore, we examined the antiallodynic activity of milnacipran after systemic, intrathecal, intracisternal, and intracerebroventricular injections.

MATERIALS AND METHODS

Animals Male C57BL/6 mice (Japan SLC Inc., Hamamatsu) were used. Mice were 6 weeks of age and weighed 18 to 22 g at the start of the experiments. They were housed six per cage under controlled temperature (21–23°C) and humidity (45–65%). The room was lighted from 7:00 a.m. to 7:00 p.m. Food and water were available *ad libitum*. All procedures for animal experiments were approved by the committee for animal experiments at the University of Toyama (#S-2010 PHA-10).

Materials Oxaliplatin (Sigma-Aldrich, St. Louis, MO, U.S.A.) was dissolved in 5% glucose and administered intraperitoneally at a dose of 3 mg/kg, which was selected from a published report¹⁴⁾ and according to its recommended clinical dose. Milnacipran hydrochloride (Sigma-Aldrich) was dissolved in physiological saline. Intraperitoneal injection of milnacipran was administered in a volume of 10 mL/kg. Intrathecal, intracisternal, and intracerebroventricular injections were administered to awake animals in a volume of 5 μ L.¹⁵⁾ Doses of milnacipran refer to the salt.

Behavioral Test Mechanical allodynia in the hind paw was evaluated using a fine von Frey filament (North Coast Medical Inc., Morgan Hill, CA, U.S.A.).¹⁴⁾ After an acclima-

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Fig. 1. Time Course of Mechanical Allodynia after a Single Injection of Oxaliplatin

Mice were given an intraperitoneal injection of oxaliplatin (3 mg/kg) or the vehicle on day 0. Mechanical allodynia was tested by punctate stimulation using a von Frey filament of 0.69-mN strength. Closed and open circles represent oxaliplatin and the vehicle, respectively. Data are presented as mean and standard error of the mean ($n=6$). Main effect of oxaliplatin treatment, $F_{1,10}=166.4$, $p<0.001$; interaction between oxaliplatin treatment and time, $F_{13,130}=19.5$, $p<0.001$ (two-way repeated measures ANOVA). * $p<0.05$ compared to vehicle (*post hoc* Holm–Šidák multiple comparisons).

tion period of at least 30 min, a von Frey filament with a bending force of 0.69 mN was applied perpendicularly against the central part of the plantar hind paw and was held for 1–3 s with it slightly bent. Responses to the stimuli were scored as follows: 0, no reaction; 1, lifting of the hind paw; 2, licking and flinching of the hind paw. A stimulus of the same intensity was applied three times alternately to each hind paw at intervals of several seconds, and the average response score served as the allodynia score (the maximum score was 2).¹⁴⁾

Data Processing All data are presented as means and standard error of the mean. Statistical significance was analyzed using two-way repeated measures ANOVA and *post hoc* Holm–Šidák multiple comparisons; $p<0.05$ was considered statistically significant.

RESULTS

A single intraperitoneal injection of oxaliplatin (3 mg/kg) caused mechanical allodynia, which became apparent on day 5 after injection, peaked on day 10, and almost subsided by day 20 (Fig. 1). The following experiments were performed on day 10. Intraperitoneal administration of milnacipran (3–30 mg/kg) produced a significant and dose-dependent inhibition of oxaliplatin-induced mechanical allodynia (Fig. 2A). This inhibition peaked 30–60 min after administration and subsided by 120 min. Intrathecal, but not intracisternal and intracerebroventricular, injection of milnacipran (2.1–21 μ g/site) produced a significant and dose-dependent inhibition of oxaliplatin-induced mechanical allodynia (Figs. 2B–D). The effect of intrathecal milnacipran peaked 30 min after administration and subsided by 120 min.

DISCUSSION

A single intraperitoneal injection of oxaliplatin (3 mg/kg) caused mechanical allodynia in mice, with the time course and intensity being similar to what has been previously reported.¹⁴⁾ We verified that systemic injection of milnacipran (3–30 mg/kg) suppressed the mechanical allodynia that had

Fig. 2. Effects of Systemic and Local Injections of Milnacipran on Oxaliplatin-Induced Mechanical Allodynia

Oxaliplatin (3 mg/kg) was injected intraperitoneally (i.p.) into mice, and 10 d later milnacipran (closed symbols) or vehicle (open circles) was injected i.p. (A), intrathecally (B), intracisternally (C), or intracerebroventricularly (D). (A) The doses of milnacipran were 3 (closed triangles), 10 (closed squares), and 30 mg/kg (closed circles). (B–D) The doses of milnacipran were 2.1 (closed triangles), 7.0 (closed squares), and 21 μ g/site (closed circles). Mechanical allodynia was examined before, 30, 60, and 120 min after milnacipran injection, as described in the legend for Fig. 1. Data are presented as mean and standard error of the mean ($n=6$). (A) Interaction between milnacipran treatment and time, $F_{9,60}=4.71$, $p<0.001$; (B) Interaction between milnacipran treatment and time, $F_{9,60}=3.78$, $p<0.001$ (two-way repeated measures ANOVA). * $p<0.05$ compared to vehicle (*post hoc* Holm–Šidák multiple comparisons).

been induced by oxaliplatin. Regarding the site of action, intrathecal injections of milnacipran (2.1–21 μ g/site) attenuated established allodynia. The degree of inhibition was similar between a systemic dose of 30 mg/kg and an intrathecal dose of 21 μ g/site. Given that the average body weight of the mice was 20 g, a local dosage of 21 μ g/site was equivalent to 1.05 mg/kg, which was much smaller than the intraperitoneal equipotent dose (30 mg/kg). Therefore, the spinal cord may be an important site in the antiallodynic effect of intraperitoneal milnacipran.

Oxaliplatin-induced allodynia was significantly inhibited by milnacipran at an intrathecal dose of 21 μ g/site, and there was a decreased allodynic tendency at a dose of 7.0 μ g/site. In a previous report where mice that had undergone spinal nerve ligation, allodynia was inhibited by milnacipran at intrathecal doses of 7.0 and 21 μ g/site.¹²⁾ Thus, antiallodynic potency of intrathecal milnacipran was similar between allodynia induced by oxaliplatin and spinal nerve ligation. Intrathecal milnacipran has also been shown to inhibit mechanical allodynia in rats that had undergone spinal nerve ligation or muscle incision.^{11,16)} The most probable mechanism underlying the antiallodynic effect of milnacipran's spinal action is the inhibition

of noradrenaline and serotonin reuptake. Milnacipran is approximately twofold more potent in noradrenaline uptake inhibition than in serotonin uptake inhibition, although its binding affinity is higher to the serotonin transporter than the noradrenaline transporter.^{7,17)} When administered intravenously in naïve rats, milnacipran markedly increases extracellular noradrenalin concentration and to a lesser extent extracellular serotonin concentration.¹⁶⁾ Furthermore, the antiallodynic action of intrathecal milnacipran is markedly blocked by intrathecal injection of the α_2 -adrenoceptor antagonist idazoxan and partially by intrathecal injection of the 5-HT₂ receptor antagonist methysergide.¹⁶⁾ Taken together, these findings suggest that the descending noradrenergic and serotonergic pathways play key roles in the antiallodynic action of milnacipran.

Regarding intracerebroventricular administration in mice, milnacipran did not inhibit oxaliplatin-induced allodynia at doses of 21 μ g/site or lower (present study). In contrast, intracerebroventricular milnacipran has been shown to inhibit spinal nerve ligation-induced allodynia at 0.7 μ g/site or higher.¹²⁾ Although reason(s) for these differences remains unclear, it is possible that the antiallodynic mechanisms of milnacipran are somewhat different depending on the etiology of mechanical allodynia. In the current study, we observed that oxaliplatin-induced allodynia was not inhibited by intracisternal injections of milnacipran. The locus coeruleus in the pons is an important nucleus of origin of the descending noradrenergic system^{18,19)} and is easily affected by intracisternally injected agents.²⁰⁾ Spontaneous activity of neurons in the locus coeruleus is inhibited by systemic injections of venlafaxine,²¹⁾ duloxetine,²²⁾ and reboxetine (noradrenaline reuptake inhibitor).²³⁾ Furthermore, the activity of locus coeruleus neurons is also inhibited by local administration of citalopram (serotonin reuptake inhibitor) in the vicinity of the locus coeruleus.²⁴⁾ A decrease in neural activity in the locus coeruleus may cause the decrease of the activity of the descending noradrenergic system, leading to a decrease in noradrenaline release from its terminals in the dorsal horn. Thus, it may not produce antiallodynia but instead may counteract antiallodynic activity of the antidepressants.

Established mechanical allodynia 10d after oxaliplatin injection was inhibited by milnacipran at an intraperitoneal dose of 30 mg/kg (present study), while acute cold hypersensitivity 2h after oxaliplatin injection was shown not to be inhibited by milnacipran at the same intraperitoneal dose.²⁵⁾ Although reasons for these differences are unclear, it is possible that efficacy of milnacipran is different between oxaliplatin-induced cold hypersensitivity and chronic neuropathy. In humans treated with oxaliplatin, venlafaxine inhibits worst daily pain and pins-and-needles, but not cold-triggered pain.⁹⁾ In rats, daily administration of amitriptyline inhibits oxaliplatin-induced mechanical allodynia but not cold hypersensitivity.²⁶⁾ These findings raised the possibility that oxaliplatin-induced cold hypersensitivity is refractory to antidepressants including milnacipran.

In summary, the present results suggest that milnacipran is effective against established mechanical allodynia after oxaliplatin treatment. The inhibitory action of milnacipran on oxaliplatin-induced allodynia may be mainly mediated by actions on the spinal cord, but not the brain regions.

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Conflict of Interest The authors declare no conflict of interest.

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