Effects of repeated milnacipran and fluvoxamine treatment on mechanical allodynia in a mouse paclitaxel-induced neuropathic pain model

Soh KATSUYAMA, Kazuma SATO, Tomomi YAGI, Yukinaga KISHIKAWA, and Hitoshi NAKAMURA

Department of Clinical Pharmaceutics, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558, Japan

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ABSTRACT

Paclitaxel is widely used in cancer chemotherapy for the treatment of solid tumors, but it frequently causes peripheral neuropathy. Milnacipran, a serotonin/noradrenaline reuptake inhibitor and fluvoxamine, a selective serotonin reuptake inhibitor, have shown efficacy against several chronic pain syndromes. In this study, we investigated the attenuation of paclitaxel-induced mechanical allodynia in mice by milnacipran and fluvoxamine. Paclitaxel was administered once per day (2 mg/kg, intraperitoneally (i.p.)) for 5 days to mice. Mechanical allodynia was evaluated by measuring the withdrawal response to stimulation with a von Frey filament. In paclitaxel-treated mice, mechanical allodynia was observed on days 3–15 of paclitaxel administration. A single administration of milnacipran (20 mg/kg, i.p.) or fluvoxamine (40 mg/kg, i.p.) had no effect on paclitaxel-induced mechanical allodynia. However, repeated administration of milnacipran (10, 20 mg/kg, once per day, i.p.) for 5 days significantly reduced paclitaxel-induced mechanical allodynia. In contrast, repeated fluvoxamine administration (40 mg/kg, once per day, i.p.) for 5 days resulted in a weak attenuation of paclitaxel-induced mechanical allodynia. These results suggest that chronic paclitaxel administration induces mechanical allodynia, and that repeated milnacipran administration may be an effective therapeutic approach for the treatment of neuropathic pain caused by paclitaxel treatment for cancer.

Peripheral neurotoxicity induced by antineoplastic drugs (taxanes, vinca-alkaloids and platin-based compounds) is a clinically significant complication that can be dose-limiting and can substantially diminish quality of life. Paclitaxel is commonly used for the treatment of solid tumors and ovarian and breast cancers. Paclitaxel induces antimitotic actions by impeding the cell cycle in the late phases of mitosis, stabilizing microtubule formation, and ultimately inducing apoptosis (26). However, paclitaxel-induced peripheral neuropathy is a side-effect of chemotherapeutic treatment which often manifests

as bilateral pain in the extremities in a stocking and glove-type distribution (6), sometimes greatly impairing the patients' quality of life and their ability to perform normal activities of daily living. To date, therapeutic drugs have been evaluated using various animal models of paclitaxel-induced allodynia. Ethosuximide (7), gabapentin (14), thalidomide and minocycline (3), neurotrophin (11) and acetyl-L-carnitine (8) have been examined against the paclitaxel-induced neuropathic pain model, although studies regarding their efficacy have not been conclusive.

Supplementary analgesics (antidepressants and anticonvulsants) have suppressive effects on opioid-resistant neuropathic pain (16, 24), and antidepressants are widely used for the treatment of neuropathic pain. Antidepressants, especially tricyclic antidepressants (TCAs), are regarded as first-line drugs for the treatment of neuropathic pain (16). Recently, more selective monoamine reuptake inhibitors, such as

Address correspondence to: Dr. Hitoshi Nakamura
Department of Clinical Pharmaceutics, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558, Japan
Tel: +81-22-727-0211, Fax: +81-22-727-0149
E-mail: nakamura@tohoku-pharm.ac.jp

serotonin (5-HT)/noradrenaline (NA) reuptake inhibitors (SNRI) and selective serotonin reuptake inhibitors (SSRI), have become available and are used clinically as neuropathic pain modalities (5). Newer antidepressants such as those in the SNRI and SSRI classes have been introduced, and their antidepressant function is better understood than that of TCAs.

The antinociceptive effects of SSRIs have been reported in a number of animal experiments (12, 13, 25). One typical SSRI, fluvoxamine, attenuated licking behavior in the late phase of the formalin test (22, 30) and also in the hot-plate test (27), but it failed to attenuate mechanical allodynia in a neuropathic pain model (chronic constriction injury model) (9). Moreover, fluvoxamine showed an antiallodynic effect in a streptozotocin-induced diabetic neuropathy model (9). Similar to the research on SSRIs, the analgesic effects of SNRIs have also been examined using a variety of animal models of pain (18). One typical SNRI, milnacipran, has been shown to be efficacious for the prevention and/or reversal of pain in several preclinical models of acute and chronic pain in rodents. Yokogawa *et al.* demonstrated that milnacipran significantly attenuated late phase paw licking behavior in the formalin test (30) and also reversed mechanical allodynia in the chronic constriction injury model or spinal nerve ligation model of neuropathic pain (9, 28). In addition, milnacipran had an antiallodynic effect on the streptozotocin-induced diabetic neuropathy model (9) and potentiated the antihyperalgesic effect of tramadol (opioid analgesic) (21). However, the antiallodynic effects of milnacipran and fluvoxamine on paclitaxel-induced mechanical allodynia remain unknown.

The principal aim of this work was to test whether single or chronic administration of milnacipran and fluvoxamine would produce antinociception in the context of the paclitaxel-induced neuropathic pain model in mice.

MATERIALS AND METHODS

Animals. Male ddY-strain mice (Japan SLC, Hamamatsu, Japan) weighing an average of 23–25 g at the time of testing were used in these experiments. Mice were housed individually in a colony maintained in a controlled environment (12 h light/dark cycle, room temperature 23°C, 50–60% relative humidity). The animals had unlimited access to food pellets and water. All behavioral experiments took place during the light period between 10:00 and 16:00 in a quiet room. The animals belonging to the various treatment groups ($n = 10$ each group)

were tested in randomized order. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (32). Additionally, the study was approved by the Committees of Animal Care and Use of Tohoku Pharmaceutical University.

Experimental protocol. Paclitaxel (Bristol-Myers Squibb Company, Tokyo, Japan) was administered intraperitoneally (i.p.) to mice at doses of 0.5–2 mg/kg, once per day for 5 consecutive days. Paclitaxel dosing was determined based on previous reports that used mice (4, 19). Milnacipran hydrochloride or fluvoxamine maleate (Sigma Chemical Co., St. Louis, MO) was administered i.p. to mice. All drugs were dissolved in physiological saline, in an injection volume of 0.1 mL/10 g body weight. In the vehicle control group, physiological saline was administered by the same schedule. Mechanical allodynia of the hind paw was assessed using von Frey filaments with 0.4 g bending force. Briefly, mice were placed individually in a plastic cage with a wire mesh bottom. After mice had adapted to the testing environment for 60 min, a von Frey filament with 0.4 g bending force was pressed perpendicularly against the mid-plantar surface of the hind paw from below the mesh floor and held for 3–5 s with the filament slightly buckled. Lifting of the paw was recorded as a positive response. Stimulation of the same intensity (0.4 g filament) was applied to the point of bending ten times to the plantar surface of the hind paw of each mouse at intervals of 5 s. Behavioral testing was performed between 10:00 and 16:00 h. Testing was performed for two days before the start of the experiment to accustom the mice to the testing procedures.

Data analysis. Data are presented as means $\pm$ S.E.M. values. The statistical significance of the differences between groups was determined using the Student's *t*-test or one-way analysis of variance (ANOVA) followed by Dunnett's test, or two-way ANOVA followed by Bonferroni's test. The level of statistical significance was set at 5% ($P < 0.05$) in all experiments. Statistics were performed using GraphPad Prism software (version 3; Graph Pad Software, Inc, San Diego, CA).

RESULTS

Time course and dose-response of paclitaxel-induced mechanical allodynia

Paclitaxel or vehicle (saline) was administered to

mice once per day for 5 days (days 0–4). We evaluated withdrawal responses to mechanical stimulation applied to each hind paw using a von Frey filament (0.4 g) from day 0 to day 30 in ddY mice. Mechanical allodynia was not observed when paclitaxel was administered intraperitoneally at a dose of 0.5 mg/kg. When the dose was increased to 1 or 2 mg/kg, mechanical allodynia became marked (Fig. 1A). Thus, we subsequently used 2 mg/kg as the working dose of paclitaxel to induce mechanical allodynia. With repeated daily dosing of 1 or 2 mg/kg of paclitaxel, mechanical allodynia peaked 10 days after the start of administration, then decreased gradually, almost subsiding by day 20. In paclitaxel-treated mice, mechanical allodynia was significant on days 3–15, compared with the saline control group (Fig. 1B). In contrast to the repeated dosing regimen, mechanical allodynia was not observed after a single administration of paclitaxel (2 mg/kg; data not shown).

The effect of paclitaxel on the overall health of the mice appeared to be minimal. No mouse presented with weight loss or died within 30 days after administration.

Effects of milnacipran or fluvoxamine treatment on paclitaxel-induced mechanical allodynia

A single administration of milnacipran (20 mg/kg, i.p.) or fluvoxamine (40 mg/kg, i.p.) had no effect on paclitaxel-induced mechanical allodynia on day 10 (Fig. 2A and 2B). However, repeated milnacipran administration (5–20 mg/kg, once per day, i.p.) for 5 days (days 0–4), in combination with paclitaxel, significantly attenuated paclitaxel-induced mechanical allodynia on day 5 in a dose-dependent manner (Fig. 3A). Moreover, repeated milnacipran administration (5–20 mg/kg, once per day, i.p.) on days 5–9 (after cessation of paclitaxel treatment) significantly attenuated paclitaxel-induced mechanical allodynia on day 10 in a dose-dependent manner (Fig. 3B). In contrast, repeated fluvoxamine administration (40 mg/kg, once per day, i.p.) for 5 days (days 0–4) or days 5–9, resulted in a weak attenuation of paclitaxel-induced mechanical allodynia on day 5 or 10 (Fig. 4A and 4B).

DISCUSSION

Neuropathic pain is defined as pain initiated or caused by a primary lesion or dysfunction in the nervous system (15). Neuropathic pain is often reported as having a lancinating or continuous burning character and is often associated with the appear-

ance of abnormal sensory signs, such as allodynia (pain as a result of a stimulus which does not normally induce pain) or hyperalgesia (an increased response to a stimulus which is normally painful) (29).

Fig. 1 Paclitaxel (0.5–2 mg/kg, i.p.) or saline was administered once per day for 5 days (days 0–4) to mice. **(A)** On day 10, the number of withdrawals to mechanical stimulation using a von Frey filament (0.4 g) was measured. **(B)** Time course of paclitaxel-induced mechanical allodynia. The number of withdrawals to mechanical stimulation using a von Frey filament (0.4 g) was measured on days 1, 2, 3, 5, 10, 15, 20, 30, prior to daily paclitaxel administration. Data were calculated as the mean $\pm$ S.E.M. values of 10 mice in each group. The statistical significance of the differences between the groups was assessed with one-way ANOVA or two-way ANOVA followed by Dunnett's test (A) or Bonferroni's test (B), respectively. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ when compared with the saline control group.

Fig. 2 Paclitaxel (PTX; 2 mg/kg, i.p.) or saline was administered once per day for 5 days (days 0–4) to mice. On day 10, the number of withdrawals to mechanical stimulation using a von Frey filament (0.4 g) was measured at 0 min (Pre) and 30 min after a single administration of milnacipran (20 mg/kg, i.p.) (A) or fluvoxamine (40 mg/kg, i.p.) (B). Data were calculated as the mean $\pm$ S.E.M. values of 10 mice in each group. The statistical significance of the differences between the groups was assessed with Student's *t*-test. *** $P < 0.001$ when compared with the saline control group, N.S.; not significant when compared with the paclitaxel pre-milnacipran group (PTX-Pre).

Fig. 3 (Top) Schedule of drug administration. (Bottom) Paclitaxel (PTX; 2 mg/kg, i.p.) and milnacipran (Mil; 5, 10, 20 mg/kg, i.p.) were co-administered once per day for 5 days (days 0–4) to mice (A), or milnacipran (Mil; 5, 10, 20 mg/kg, i.p.) was administered to mice once per day for 5 days (days 5–9) after cessation of PTX (2 mg/kg, i.p.) treatment (B). On day 5 (A) and day 10 (B), the number of withdrawals to mechanical stimulation using a von Frey filament (0.4 g) was measured. Data were calculated as the mean $\pm$ S.E.M. values of 10 mice in each group. The statistical significance of the differences between the groups was assessed with one-way ANOVA followed by Dunnett's test. *** $P < 0.001$ when compared with the saline control group, # $P < 0.05$, ### $P < 0.001$ when compared with the PTX-group.

In this study, we demonstrated that repeated but not acute administration of paclitaxel (1 or 2 mg/kg, i.p.) produced dose-dependent mechanical allodynia

in male ddY-strain mice. The mechanical allodynia was observed even after cessation of paclitaxel administration in this experimental model (Fig. 1A and

Fig. 4 (Top) Schedule of drug administration. (Bottom) Paclitaxel (PTX; 2 mg/kg, i.p.) and fluvoxamine (Flu; 10, 20, 40 mg/kg, i.p.) were co-administered once per day for 5 days (days 0–4) to mice (A) or fluvoxamine (Flu; 10, 20, 40 mg/kg, i.p.) was administered to mice once per day for 5 days (days 5–9) after cessation of PTX (2 mg/kg, i.p.) treatment (B). On day 5 (A) and day 10 (B), the number of withdrawals to mechanical stimulation using a von Frey filament (0.4 g) was measured. Data were calculated as the mean $\pm$ S.E.M. values of 10 mice in each group. The statistical significance of the differences between the groups was assessed with one-way ANOVA followed by Dunnett's test. *** P < 0.001 when compared with the saline control group, # P < 0.05 when compared with the PTX-group.

1B). These observations are consistent with those of other reports showing paclitaxel to induce mechanical allodynia in mice (4, 19). The present study further demonstrated a significant attenuation of paclitaxel-induced mechanical allodynia by repeated milnacipran or fluvoxamine treatment.

Milnacipran and fluvoxamine are used for the treatment of acute and neuropathic pain as a supplementary analgesic (16, 21). There have been several reports indicating that milnacipran and fluvoxamine have significant antinociceptive effects against nociceptive and inflammatory pain (20, 22, 27, 28, 30). Furthermore, milnacipran was more effective than SSRIs such as fluvoxamine, paroxetine, and citalopram in attenuating persistent pain (30) and neuropathic pain (9), and more effective than amitriptyline (TCAs) in attenuating cold allodynia (4°C cold plate stimuli) (1). However, the efficacy of milnacipran and fluvoxamine in attenuating paclitaxel-induced mechanical allodynia (tactile stimuli) is poorly understood. In this report, repeated but not acute administration of milnacipran significantly attenuated paclitaxel-induced mechanical allodynia. Repeated milnacipran administration was effective during both the development (days 0–4) and maintenance (days 5–9) of paclitaxel-induced mechanical allodynia. In

contrast, repeated but not acute fluvoxamine administration resulted in a weak attenuation of paclitaxel-induced mechanical allodynia.

Milnacipran shows equivalent inhibitory activity at both NA and 5-HT neuronal reuptake systems (18). Obata *et al.* reported the involvement of both NA and 5-HT in the antiallodynic effect of milnacipran in a rat neuropathic pain model (20). However, the antiallodynic and antihyperalgesic effects of milnacipran in neuropathic pain models were predominantly attenuated by the noradrenergic system rather than by the serotonergic system at the spinal level (9, 28). Moreover, modulation of the noradrenergic system is likely to be more effective than that of the serotonergic system for the control of pain (9, 22, 28, 30). We confirmed that when administered concurrently with paclitaxel or after cessation of paclitaxel treatment, repeated milnacipran significantly attenuated paclitaxel-induced mechanical allodynia. On the other hand, repeated administration of fluvoxamine resulted in weak attenuation of paclitaxel-induced mechanical allodynia. Therefore, the antiallodynic effect of milnacipran in the paclitaxel-induced neuropathic pain model might be mediated primarily by the noradrenergic system rather than by the serotonergic system.

Central glial cells such as astrocytes and microglia play a prominent role in pain modulation (17, 23). Spinal astrocytes and microglia have been found to play critical roles in facilitating central plasticity following both nerve injury and inflammation (17, 23). Indeed, spinal glial cells contribute to the development of paclitaxel-induced painful neuropathy in a rat model (2, 3). Recently, Zhang *et al.* reported that spinal astrocytes contribute to the pathogenesis of paclitaxel-induced painful neuropathy (31). The neuronal NA transporter is functionally expressed in cultured rat astrocytes and milnacipran potently inhibits NA uptake in these cells (10). The NA uptake system in astrocytes is sensitive to milnacipran, and the therapeutic effects of SNRIs may thus be mediated to some extent by their action on astrocytes. Together with these findings, our data suggest that chronic but not single milnacipran treatment attenuates paclitaxel-induced neuropathic pain that is, at least in part, through the inhibition of astrocyte activation.

In conclusion, chronic i.p. administration of paclitaxel at a once-daily dose of 1 or 2 mg/kg for 5 days induced mechanical allodynia which peaked on day 10, decreased gradually, and largely subsided by day 20. This model may prove useful for further study of paclitaxel-induced neuropathic pain. We demonstrate a significant attenuation of paclitaxel-induced neuropathic pain by repeated milnacipran treatment. Importantly, this effect was observed not only during the development (days 0–4) but also during maintenance (days 5–9) of paclitaxel-induced neuropathic pain. The present results indicate that milnacipran may be a promising supportive therapeutic approach for paclitaxel-induced neuropathic pain.

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