Involvement of Substance P in Peripheral Neuropathy Induced by Paclitaxel but Not Oxaliplatin

Yoko Tatsushima, Nobuaki Egashira, Takehiro Kawashiri, Yuki Mihara, Takahisa Yano, Kazuto Mishima, and Ryozo Oishi

Department of Pharmacy, Kyushu University Hospital, Fukuoka, Japan

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ABSTRACT

The painful peripheral neuropathy occurring frequently during chemotherapy with paclitaxel or oxaliplatin is one of their dose-limiting factors. We reported previously that substance P is involved in the pathogenesis of pulmonary hypersensitivity reaction to paclitaxel in rats, and an antiallergic agent pemirolast reverses this reaction via the blockade of release of substance P. In the present study, we investigated the involvement of substance P in paclitaxel-induced peripheral neuropathy compared with that by oxaliplatin. In von Frey and acetone tests in rats repeated administration of paclitaxel (6 mg/kg i.p., once a week for 4 weeks) or oxaliplatin (4 mg/kg i.p., twice a week for 4 weeks) induced both mechanical allodynia and cold hyperalgesia. Paclitaxel-induced peripheral neuropathy was reversed primarily by the acute administration of pemirolast (0.1 and 1 mg/kg p.o.). Moreover, coadministration of the receptor antagonists neurokinin 1 [N-acetyl-L-tryptophan 3,5-bis(trifluoromethyl)benzylessar (L-732,138), 100 μg/body i.t.] and neurokinin 2 [5-fluoro-3-[2-[4-methoxy-4-[[[R]-phenylsulphonyl]methyl]-1-piperidiny]ethyl]-1H-indole (GR159897), 100 μg/body i.t.] strongly reversed paclitaxel-induced neuropathy. On the other hand, oxaliplatin-induced peripheral neuropathy was not reversed by pemirolast. In the in vitro study using cultured adult rat dorsal root ganglion neurons paclitaxel (1000 ng/ml) significantly increased the release of substance P, and pemirolast (100 and 1000 nM) significantly inhibited this increase of substance P release. Oxaliplatin, by contrast, did not increase the release of substance P. These results suggest that substance P is involved in paclitaxel-induced neuropathy, and the mechanism of its action is clearly different from that of oxaliplatin.

Introduction

Paclitaxel, an anticancer agent with a tubulin-stabilizing action, has been widely used for several malignancies, including ovarian and breast cancer, non–small-cell lung carcinoma, and stomach cancer. Oxaliplatin, a platinum-based chemotherapeutic agent, is very important in chemotherapy for metastatic colorectal cancer. Although the mechanisms of these antitumor effects are different, their uses are often limited because of the incidence of severe adverse reactions, including peripheral neuropathy.

Paclitaxel-induced peripheral neuropathy is characterized by frequently occurring sensory neuropathy, such as dysesthesia, numbness, pain and thermohyperesthesia in the feet and hands, and usually mild motor neuropathy (Lee and Swain, 2006). Oxaliplatin causes severe acute and chronic peripheral neuropathies (Gamelin et al., 2002). Acute neuropathy is peculiar to oxaliplatin and includes acral paresthesias enhanced by exposure to cold. On the other hand, chronic neuropathy is characterized by the loss of sensory and motor function after long-term treatment.

In clinical trials, amifostine, glutamine, acetyl L-carnitine, BNP7787 (disodium 2,2'-dithio-bis-ethanesulfonate), and vitamin E have been tested for paclitaxel-induced neuropathy (Takeda et al., 2002; Lorusso et al., 2003; Argyriou et al., 2005; Bianchi et al., 2005; Stubblefield et al., 2005). However, they are not commonly used in the clinical setting because of low effectiveness. Furthermore, calcium and magnesium infusions have been tried to reduce oxaliplatin-induced neuropathy (Gamelin et al., 2008). Gabapentin, an antiepileptic agent, has been reported to attenuate anticancer drug-induced peripheral neuropathy in rodents (Ling et al., 2007). This drug is recommended as first-line treatment for neuropathic pain (Dworkin et al., 2007). However, a phase III, randomized, double-blind trial failed to demonstrate any benefit to using gabapentin to treat symptoms of chemotherapy-induced peripheral neuropathy (Rao et al., 2007).
fore, new agents strongly reducing the symptoms of neuropathy are required.

The use of paclitaxel is also limited by several hypersensitivity reactions. We reported previously that paclitaxel markedly increases substance P in plasma and bronchoalveolar lavage fluid in rats and in plasma in patients with ovarian cancer (Itoh et al., 2004a; Sendo et al., 2004). Moreover, we have reported that pemirolast, an antiallergic agent, attenuates paclitaxel-induced pulmonary hypersensitivity reactions through inhibition of the release of sensory nerve peptides including substance P, neurokinin (NK) A, and calcitonin gene-related peptide (CGRP) in rat bronchoalveolar lavage fluid (Itoh et al., 2004b) and also prevents paclitaxel-induced hypersensitivity reactions in patients with ovarian cancer (Yahata et al., 2006). Thus, sensory nerve peptides such as substance P play an important role in paclitaxel-induced hypersensitivity reactions.

Substance P is well known to be a neurotransmitter related to pain (Harrison and Geppetti, 2001) and is synthesized in the dorsal root ganglion (DRG) (Vedder and Otten, 1991). Substance P is localized in high-threshold nociceptive C-fibers and released by noxious stimulus (Otsuka and Yoshioka, 1993), and it binds NK1 and NK2 receptors (Regoli et al., 1988). Substance P is involved in the regulation of nociceptive information at the first sensory synapse in the spinal cord (De Koninck and Henry, 1991). Moreover, substance P and NK1 receptors have been reported to be involved in neuropathic pain in traumatic rats (Goff et al., 1998; Gonzalez et al., 2000; Cahill and Codere, 2002; Vachon et al., 2004). NK1 and NK2 receptors have also been reported to be involved in neuropathic pain in diabetic rats (Coudérè-Civiale et al., 2000). It has been reported that paclitaxel evokes the release of substance P from cultured rat DRG cells (Miyano et al., 2009). However, the involvement of substance P in chemotherapy-induced peripheral neuropathy remains unknown. Therefore, we investigated the involvement of substance P in paclitaxel-induced peripheral neuropathy, compared with that of oxaliplatin. In this study, pemirolast, L-732,138 (N-acetyl-L-tryptophan 3,5-bis(trifluoromethyl)benzylester), a selective NK1 receptor antagonist, and GR159897 (5-fluoro-3-[2-[4-methoxy-4-[[R]-phenylsulphonyl]methyl]-1-piperidinyl]ethyl]-3H-indole), a selective NK2 receptor antagonist, were used to investigate the involvement of substance P.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing 200 to 250 g (Kyudo Co., Saga, Japan) were used in the present study. Animals were housed in groups of four to five per cage, with lights on from 8:00 AM to 8:00 PM. Animals had free access to food and water in their home cages. The experimental procedures were approved by the Committee for the Care and Use of Laboratory Animals at the Faculty of Medicine, Kyushu University.

Drugs

Paclitaxel (Taxol; 6 mg/ml in Cremophor EL/ethanol 1:1) and oxaliplatin (Erlapat) were obtained from Bristol-Myers Squibb (Tokyo, Japan) and Yakult Co., Ltd. (Tokyo, Japan), respectively. Pemirolast was a generous gift from Mitsubishi Tanabe Pharma Factory Ltd. (Osaka, Japan). L-732,138 and GR159897 were purchased from Enzo Life Sciences, Inc., (Farmington, NY) and Tocris Bioscience (Ellisville, MO), respectively. Pemirolast was dissolved in distilled water. Oxaliplatin was dissolved in 5% glucose solution. L-732,138 and GR159897 were dissolved in 100% dimethyl sulfoxide. The doses of these drugs were chosen based on previous reports (Cahill and Codere, 2002; Jamieson et al., 2005; Ling et al., 2007; Kawashiri et al., 2009).

Behavioral Studies

The procedure was a modification of that described previously (Kawashiri et al., 2009). Behavioral testing was performed blind with respect to drug administration.

Von Frey Test for Mechanical Allodynia. Rats were placed in a clear plastic box (20 × 17 × 13 cm) with a wire mesh floor and allowed to habituate for 30 min before testing. Von Frey filaments (The Touch Test Sensory Evaluator Set; Linton Instrumentation, Norfolk, UK) ranging from 1- to 15-g bending force were applied to the midplantar skin of each hind paw six times, with each application held for 6 s. Fifty percent paw withdrawal threshold was determined by the up-down method (Dixon 1980; Chaplan et al., 1994).

Acetone Test for Cold Hyperalgesia. The acetone test was performed according to the method described by Flatters and Bennett (2004). Rats were placed in a clear plastic box (20 × 17 × 13 cm) with a wire mesh floor and allowed to habituate for 30 min before testing. Fifty microliters of acetone (Wako Pure Chemicals, Osaka, Japan) was sprayed onto the plantar skin of each hind paw three times with a Micro Sprayer (Penn Century Inc., Philadelphia, PA), and the number of withdrawal responses was counted for 40 s from the start of the acetone spray. To investigate the effects of paclitaxel and oxaliplatin on the nociceptive behaviors, two independent experiments were carried out.

Effect of Paclitaxel on Mechanical and Cold Nociceptive Threshold

We investigated the effect of paclitaxel on mechanical and cold nociceptive threshold in the von Frey and acetone tests. Paclitaxel (6 mg/kg) or vehicle (Cremophor EL/ethanol 1:1) was injected intraperitoneally once a week for 4 weeks (on days 1, 8, 15, and 22). The von Frey test was performed before the first administration of paclitaxel (day 0) and on days 5, 12, 19, and 26. The acetone test was performed immediately after the von Frey test.

Effect of Oxaliplatin on Mechanical and Cold Nociceptive Threshold

We investigated the effect of oxaliplatin on the mechanical and cold nociceptive threshold in the von Frey and acetone tests. Oxaliplatin (4 mg/kg) or vehicle (5% glucose solution) was administered intraperitoneally twice a week for 4 weeks (on days 1, 2, 8, 9, 15, 16, 22, and 23). The von Frey test was performed before the first administration of oxaliplatin (day 0) and on days 3, 10, 17, and 24. The acetone test was performed immediately after the von Frey test.

Effect of Pemirolast on Paclitaxel- or Oxaliplatin-Induced Mechanical Allodynia and Cold Hyperalgesia

In the case of paclitaxel, we confirmed the incidence of peripheral neuropathy on day 28 and carried out the drug evaluation the next day. The von Frey test was performed immediately before (0 min) and 30, 60, 90, 120, and 180 min after oral administration of pemirolast. The acetone test was performed immediately after the von Frey test. In the case of oxaliplatin, we confirmed the incidence of mechanical allodynia and cold hyperalgesia on days 24 and 3, respectively. We carried out the drug evaluation the next day. The von Frey and acetone tests were performed immediately before (0 min) and 30, 60, 90, 120, and 180 min after oral administration of pemirolast.
Effects of L-732,138 and GR159897 on Paclitaxel-Induced Mechanical Allodynia and Cold Hyperalgesia

We confirmed the incidence of peripheral neuropathy on day 28 and carried out the drug evaluation the next day. L-732,138 and GR159897 were administered by intrathecal injection by direct lumbar puncture in a volume of 50 μl. The animals were anesthetized lightly with ether for intrathecal injection. Pain behavior was measured from 60 min after intrathecal injection when anesthesia had completely disappeared. The von Frey test was performed immediately before (0 min) and 60, 90, 120, and 180 min after administration of L-732,138 or GR159897. The acetone test was performed immediately after the von Frey test.

Preparation of Rat DRG Cells for Assay of Substance P Release

L4-5 DRG cells were removed from male Sprague-Dawley rats (6 weeks old) anesthetized with sodium pentobarbital and cultured. Ganglia were incubated with 0.125% (w/v) collagenase type 1 (Worthington Biochemicals, Freehold, NJ) at 37°C for 90 min followed by 0.25% (w/v) trypsin-EDTA (Invitrogen, Carlsbad, CA) for 30 min. The DRG cells were grown in Dulbecco's modified Eagle's medium (MP Biomedicals, Solon, OH) supplemented with 100 unit/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine (Invitrogen), and 10% fetal bovine serum (Cell Culture Technology, Hannover, Germany). The cells (two DRGs/well) were maintained at 37°C in a water-saturated atmosphere with 5% CO2 for 10 days.

Measurement of Substance P Release from Cultured Rat DRG Neurons

DRG neurons were pretreated with or without pemrolast (10–1000 nM) in 1 ml of Hanks’ balanced salt solution (8.00 g/liter NaCl, 0.40 g/liter KCl, 0.14 g/liter CaCl2, 0.10 g/liter MgSO4·7H2O, 0.10 g/liter MgCl2·6H2O, 0.06 g/liter Na2HPO4·2H2O, 0.06 g/liter KH2PO4, 1.00 g/liter glucose, 0.35 g/liter NaHCO3, pH 7.4) with the peptidase inhibitor aprotinin (Wako Pure Chemicals) for 3 h at 37°C in a water-saturated atmosphere with 5% CO2. Then the medium was changed, and DRG neurons were treated with paclitaxel (1–1000 ng/ml) or oxaliplatin (0.01–100 μg/ml) with or without pemrolast (10–1000 nM) for 10 min at 37°C. The content of substance P in the culture medium was measured with an EIA kit (Cayman Chemical, Ann Arbor, MI).

Data Analysis

Values were expressed as means ± S.E.M and analyzed by Student’s t test or one-way or two-way (with repeated-measures) analysis of variance (ANOVA) followed by the Tukey-Kramer post hoc test (StatView; Abacus Concepts, Berkeley, CA) to determine differences among the groups. A probability level of p < 0.05 was accepted as statistically significant.

Results

Effects of Paclitaxel and Oxaliplatin in the Von Frey and Acetone Tests. Before the first drug administration, there were no significant differences in all groups in 50% paw withdrawal threshold in the von Frey test and in the number of withdrawal responses in the acetone test. In the von Frey test, numbers of animals are shown in parentheses. Values are expressed as the mean ± S.E.M on days 0, 5, 12, 19, and 26. **, p < 0.01 compared with day 0.

![Fig. 1](image-url). Mechanical alldynia in the von Frey test (A) and cold hyperalgesia in the acetone test (B) induced by paclitaxel or oxaliplatin in rats. Rats were treated with paclitaxel (6 mg/kg i.p.) once a week for 4 weeks. Numbers of animals are shown in parentheses. Values are expressed as the mean ± S.E.M on days 0, 5, 12, 19, and 26. **, p < 0.01 compared with day 0.

![Fig. 2](image-url). Mechanical alldynia in the von Frey test (A) and cold hyperalgesia in the acetone test (B) induced by oxaliplatin in rats. Rats were treated with oxaliplatin (4 mg/kg i.p.) twice a week for 4 weeks. Numbers of animals are shown in parentheses. Values are expressed as the mean ± S.E.M on days 0, 3, 10, 17, and 24. **, p < 0.01 compared with day 0.
test in paclitaxel-treated rats repeated-measures ANOVA revealed a significant time effect ($F_{4, 44} = 4.561, p < 0.01$) and a significant drug $\times$ time interaction ($F_{4, 44} = 4.199, p < 0.01$), but no significant drug effect. Paclitaxel (6 mg/kg i.p.) significantly reduced the 50% paw withdrawal threshold on days 19 and 26 compared with day 0 in the von Frey test ($p < 0.01$ by Tukey-Kramer post hoc test; Fig. 1A). In the acetone test repeated-measures ANOVA revealed a significant time effect ($F_{4, 44} = 9.940, p < 0.0001$) and a significant drug $\times$ time interaction ($F_{4, 44} = 3.172, p < 0.05$), but no significant drug effect. Paclitaxel at the same dose significantly increased the number of withdrawal responses in the acetone test on days 19 and 26 compared with day 0 ($p < 0.01$ by Tukey-Kramer post hoc test; Fig. 1B).

In oxaliplatin-treated rats repeated-measures ANOVA revealed a significant drug effect ($F_{1, 11} = 12.121, p < 0.01$), a significant time effect ($F_{4, 44} = 2.605, p < 0.05$), and a significant drug $\times$ time interaction ($F_{4, 44} = 5.009, p < 0.01$) in the von Frey test. Oxaliplatin (4 mg/kg i.p.) significantly reduced the 50% paw withdrawal threshold on day 24 compared with day 0 in the von Frey test ($p < 0.05$ by Tukey-Kramer post hoc test; Fig. 2A). In the acetone test repeated-measures ANOVA revealed a significant drug effect ($F_{1, 11} = 30.967, p < 0.001$), a significant time effect ($F_{4, 44} = 8.521, p < 0.0001$), and a significant drug $\times$ time interaction ($F_{4, 44} = 4.946, p < 0.01$). Oxaliplatin at the same dose significantly increased the number of withdrawal responses in the acetone test on days 3, 10, 17, and 24 compared with day 0 ($p < 0.01$ by Tukey-Kramer post hoc test; Fig. 2B).

**Effect of Pemirolast on Paclitaxel- or Oxaliplatin-Induced Peripheral Neuropathy.** Paclitaxel (6 mg/kg i.p.) significantly reduced the 50% paw withdrawal threshold in the von Frey test and increased the number of withdrawal responses in the acetone test compared with vehicle on day 28 ($p < 0.01$ by Student’s $t$ test; Fig. 3). Before administration of pemirolast each group had equivalent 50% withdrawal threshold in the von Frey test and number of withdrawal responses in the acetone test. In the von Frey test repeated-measures ANOVA revealed a significant drug effect ($F_{3, 20} = 3.693, p < 0.01$), a significant time effect ($F_{5, 100} = 20.082, p < 0.01$), and a significant drug $\times$ time interaction ($F_{15, 100} = 4.776, p < 0.01$). Pemirolast (0.1 and 1 mg/kg p.o.) almost completely reversed the reduction of 50% paw withdrawal threshold by paclitaxel at 30 and 60 min after administration in the von Frey test ($p < 0.05$ or 0.01 by Tukey-Kramer post hoc test; Fig. 3A). At 120 min after administration this effect of pemirolast had disappeared. In the acetone test repeated-measures ANOVA revealed a significant time effect ($F_{5, 100} = 2.933, p < 0.05$), but no significant drug effect or drug $\times$ time interaction. Pemirolast (1 mg/kg p.o.) significantly reversed the increase of number of withdrawal responses by paclitaxel at 30 min after administration in the acetone test ($p < 0.05$ by Tukey-Kramer post hoc test; Fig. 3B). This effect of pemirolast disappeared by 120 min after administration.

**Fig. 3.** Effect of pemirolast on mechanical allodynia in the von Frey test (A) and cold hyperalgesia in the acetone test (B) in paclitaxel-treated rats. Rats were treated with paclitaxel (6 mg/kg i.p.) once a week for 4 weeks. Pemirolast (0.01–1 mg/kg p.o.) was administered orally. Numbers of animals are shown in parentheses. Values are expressed as the mean $\pm$ S.E.M. ††, $p < 0.01$ compared with vehicle; †, $p < 0.05$, *, $p < 0.01$ compared with paclitaxel alone.
Oxaliplatin (4 mg/kg i.p.) significantly reduced the 50% paw withdrawal threshold in the von Frey test on day 24 ($p < 0.01$ by Student’s $t$ test; Fig. 4A) and increased the number of withdrawal responses in the acetone test compared with vehicle on days 3 and 24 ($p < 0.01$ by Student’s $t$ test; Fig. 4, B and C). Before administration of pemirolast each group had equivalent 50% withdrawal threshold in the von Frey test and number of withdrawal responses in the acetone test. In the von Frey test repeated-measures ANOVA revealed a significant time effect ($F_{5, 140} = 3.990$, $p < 0.01$), but no significant drug effect or drug × time interaction. Pemirolast had no effect on the reduction of 50% paw withdrawal threshold by oxaliplatin in the von Frey test (Fig. 4A). In the acetone test repeated-measures ANOVA revealed no significant time effect, drug effect, or drug × time interaction. Pemirolast did not affect the increase of number of withdrawal responses by oxaliplatin in the acetone test (Fig. 4, B and C).

**Effects of NK$_1$ and NK$_2$ Receptor Antagonists on Paclitaxel-Induced Peripheral Neuropathy.** In the von Frey test in rats treated with the selective NK$_1$ receptor antagonist pemirolast, there was a significant reduction in the 50% paw withdrawal threshold compared with vehicle (Fig. 4A). In the acetone test, repeated-measures ANOVA revealed no significant time effect, drug effect, or drug × time interaction. Pemirolast did not affect the increase of number of withdrawal responses by oxaliplatin in the acetone test (Fig. 4, B and C).

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**Fig. 4.** Effect of pemirolast on mechanical allodynia in the von Frey test (A) and cold hyperalgesia in the acetone test in the first (B) and fourth (C) week in oxaliplatin-treated rats. Rats were treated with oxaliplatin (4 mg/kg i.p.) twice a week for 4 weeks. Pemirolast (0.01–1 mg/kg) was administered orally. Numbers of animals are shown in parentheses. Values are expressed as the mean ± S.E.M. ††, $p < 0.01$ compared with vehicle.
antagonist L-732,138 repeated-measures ANOVA revealed a significant time effect \((F_{4, 92} = 9.675, p < 0.01)\) and a significant drug \times\ time interaction \((F_{12, 92} = 2.261, p < 0.05)\), but no significant drug effect. L-732,138 (100 \(\mu\)g/body i.t.) significantly reversed the reduction of 50% paw withdrawal threshold by paclitaxel at 60 min after administration in the von Frey test \((p < 0.05)\) (Tukey-Kramer post hoc; Fig. 5A). This effect of L-732,138 had disappeared at 120 min after administration. In the acetone test repeated-measures ANOVA revealed a significant time effect \((F_{4, 92} = 3.426, p < 0.01)\), but no significant drug effect or drug \times\ time interaction. L-732,138 at the same dose had no effect on the increase of number of withdrawal responses by paclitaxel in the acetone test (Fig. 5B).

In the von Frey test in rats treated with the selective NK\(_2\) receptor antagonist GR159897 repeated-measures ANOVA revealed a significant time effect \((F_{4, 92} = 3.543, p < 0.01)\) and a significant drug \times\ time interaction \((F_{12, 92} = 2.520, p < 0.01)\), but no significant drug effect. GR159897 (100 \(\mu\)g/body i.t.) significantly reversed the reduction of 50% paw withdrawal threshold by paclitaxel at 60 min after administration in the von Frey test \((p < 0.05)\) (Tukey-Kramer post hoc; Fig. 6A). This effect of GR159897 disappeared by 120 min after administration. In the acetone test repeated-measures ANOVA revealed a significant time effect \((F_{4, 92} = 4.456, p < 0.01)\) and a significant drug \times\ time interaction \((F_{12, 92} = 1.981, p < 0.05)\), but no significant drug effect. Coadministration of L-732,138 and GR159897 at the same dose significantly reversed the increase of the number of withdrawal responses by paclitaxel in the acetone test \((p < 0.05)\) (Tukey-Kramer post hoc; Fig. 7B). This effect had disappeared by 120 min after administration.

**Effects of Paclitaxel and Oxaliplatin on Release of Substance P from Cultured Adult Rat DRG Neurons.** Paclitaxel (1000 ng/ml) significantly increased the release of substance P from cultured DRG neurons \((p < 0.01)\) (Tukey-Kramer post hoc). Moreover, pemrolast (100 ng/ml) significantly increased the release of substance P from cultured DRG neurons \((p < 0.01)\) (Tukey-Kramer post hoc).
and 1000 nM) significantly inhibited the increase of substance P release induced by paclitaxel ($p < 0.05$ by Tukey-Kramer post hoc test; Fig. 8B). On the other hand, oxaliplatin (0.01–100 μg/ml) did not increase the release of substance P (Fig. 8C).

**Discussion**

The present study showed that paclitaxel increases the release of substance P from cultured adult rat DRG neurons consistent with results described previously (Miyano et al., 2009). Apfel et al. (1991) reported that paclitaxel decreased the DRG cells content of substance P in mice. Paclitaxel has been reported to evoke the release of substance P from cultured DRG cells by extracellular Ca$^{2+}$ influx through transient receptor potential channels (Miyano et al., 2009). Oxaliplatin, in contrast, did not increase the release of substance P. These findings suggest that the effect of oxaliplatin may be different from that of paclitaxel, and oxaliplatin may have no effect on pathways mediating substance P release.

Ling et al. (2007) reported an increase of substance P expression in the dorsal horn spinal cord 24 h after a single administration of oxaliplatin (6 mg/kg i.p.). On the other hand, our data in this study revealed that oxaliplatin (4 mg/kg, i.p. twice a week for 4 weeks) did not increase the release of substance P from DRG neurons. This discrepancy might be caused by the difference of experimental methods such as administration schedule and measurement site.

We also found that pemirolast significantly inhibits this paclitaxel-induced increase of substance P release. Pemirolast inhibits not only the release of chemical mediators such as histamine and leukotriene but also the release of sensory nerve peptides including substance P, neurokinin A, and CGRP (Itoh et al., 2004b). Pemirolast also has inhibitory effects of inositol 1,4,5-trisphosphate production, Ca$^{2+}$ mobilization, and phosphodiesterase (Yanagihara et al., 1988; Fujimiya et al., 1994). Paclitaxel has been reported to evoke the release of substance P from cultured DRG cells by extracellular Ca$^{2+}$ influx through transient receptor potential channels (Miyano et al., 2009). Therefore, pemirolast might reduce the paclitaxel-induced increase of substance P release from cultured DRG neurons by inhibiting extracellular Ca$^{2+}$ influx.

In the present study, we observed mechanical allodynia (von Frey test) and cold hyperalgesia (acetone test) after paclitaxel or oxaliplatin administration, consistent with our previous reports (Kawashiri et al., 2009; Sakurai et al., 2009). Paclitaxel induced mechanical allodynia and cold hyperalgesia on days 19 and 26. We previously indicated that paclitaxel causes axonal degeneration of sciatic nerve on day 25 (Kawashiri et al., 2009). Clinical studies have shown that the axonal degeneration of nerves is caused by paclitaxel, as well as a reduction of myelinated fiber density and the loss of
large fibers (Lee and Swain, 2006). These neurodegenerations may contribute to paclitaxel-induced neuropathy symptoms such as mechanical allodynia and cold hyperalgesia. Oxaliplatin also caused mechanical allodynia on days 17 and 24 (at the same phase) in the present study. Oxaliplatin causes damage of the cell bodies, alterations in nucleus and nucleolus (Cavaletti et al., 2001; McKeage et al., 2001), and selective atrophy of subpopulations of DRG neurons (Jammieson et al., 2005) in animal models. Moreover, oxaliplatin induces the cell death and inhibition of neurite outgrowth (Luo et al., 1999; Ta et al., 2006) in neuronal cells. The neurotoxicity of oxaliplatin for the DRG neurons correlates with platinum-DNA bindings (Ta et al., 2006). In our previous study, dichloro(1,2-diaminocyclohexane)platinum, a metabolite of oxaliplatin, induced mechanical allodynia on days 17 and 24, but did not induce cold hyperalgesia/allodynia (Sakurai et al., 2009). These findings suggest that oxaliplatin-induced mechanical allodynia may be caused by the neurotoxicity of platinum. On the other hand, oxaliplatin caused cold hyperalgesia from day 3 (early phase). This phenomenon resembled the clinical symptom of oxaliplatin-induced acute neuropathy. We have indicated that oxalate, another metabolite of oxaliplatin, is involved in cold hyperalgesia but not mechanical allodynia in oxaliplatin-induced neuropathy, and that oxalate is the main causative agent in this cold hyperalgesia through chelating of Ca$^{2+}$ and Mg$^{2+}$ (Sakurai et al., 2009). Thus, the mechanism of its appearance seems to be clearly different from that of mechanical allodynia.

It is noteworthy that, in this study, pemirolast almost completely relieved the paclitaxel-induced mechanical allodynia and cold hyperalgesia, whereas pemirolast had no effect on the oxaliplatin-induced neuropathy. Paclitaxel, but not oxaliplatin, increased the release of substance P from cultured adult rat DRG neurons. Moreover, pemirolast inhibited this paclitaxel-induced increase of substance P release. The present results suggest that substance P may be involved in the neuropathy induced by paclitaxel but not by oxaliplatin, and that pemirolast may relieve paclitaxel-induced neuropathy by inhibiting the release of substance P. Thus, the difference in the role of substance P between paclitaxel and oxaliplatin might be caused by these drugs’ different effects on substance P release. In addition, pemirolast did not completely reverse the paclitaxel-induced increase of substance P release, although pemirolast almost completely relieved the paclitaxel-induced neuropathy, suggesting the possible involvement of other neuropeptides such as CGRP in the ameliorative effect of pemirolast.

In the present study, pemirolast was orally injected. Because pemirolast has poor blood-brain barrier permeability, the ameliorative effect of pemirolast is probably caused by the peripheral response. This idea is supported by our result that pemirolast inhibited the paclitaxel-induced increase of substance P release from DRG neurons. Moreover, intrathecal injection of NK1 and NK2 receptor antagonists strongly reversed paclitaxel-induced neuropathy, indicating that these antagonists inhibited the pain behavior by blocking these receptors in the spinal cord. These findings suggest...
that substance P released from periphery is involved in paclitaxel-induced neuropathy.


The present study revealed that substance P is involved in paclitaxel-induced neuropathy, and the mechanism of its action is clearly different from that of oxaliplatin. Finally, pemrolast is expected to be useful as a therapeutic drug for clinical paclitaxel-induced neuropathy.


drug for clinical paclitaxel-induced neuropathy. The mechanism of its action is clearly different from that of oxaliplatin. is involved in paclitaxel-induced peripheral neuropathy, and the pharmacological description of oxaliplatin-induced painful neuropathy in rat. J Pharmacol Exp Ther 294:443–450. Hara Partic in research design: Tatsushima, Egashira, Kawashiri, Mihara, Yano, Mishima, and Oishi. Performed data analysis: Tatsushima, Egashira, and Kawashiri. Wrote or contributed to the writing of the manuscript: Tatsushima, Egashira, Kawashiri, and Oishi.

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Address correspondence to: Dr. Nobuaki Egashira, Department of Pharmacy, Kyushu University Hospital, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. E-mail: n-egashi@pharm.med.kyushu-u.ac.jp