


Endothelin receptor type A is involved in the development of oxaliplatin-induced mechanical allodynia and cold allodynia acting through spinal and peripheral mechanisms in rats

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Abstract

Oxaliplatin, a platinum-based chemotherapeutic agent, frequently causes severe neuropathic pain typically encompassing cold allodynia and long-lasting mechanical allodynia. Endothelin has been shown to modulate nociceptive transmission in a variety of pain disorders. However, the action of endothelin varies greatly depending on many variables, including pain causes, receptor types (endothelin type A (ET_A) and B (ET_B) receptors) and organs (periphery and spinal cord). Therefore, in this study, we investigated the role of endothelin in a Sprague–Dawley rat model of oxaliplatin-induced neuropathic pain. Intraperitoneal administration of bosentan, a dual ET_A/ET_B receptor antagonist, effectively blocked the development or prevented the onset of both cold allodynia and mechanical allodynia. The preventive effects were exclusively mediated by ET_A receptor antagonism. Intrathecal administration of an ET_A receptor antagonist prevented development of long-lasting mechanical allodynia but not cold allodynia. In marked contrast, an intraplantar ET_A receptor antagonist had a suppressive effect on cold allodynia but only had a partial and transient effect on mechanical allodynia. In conclusion, ET_A receptor antagonism effectively prevented long-lasting mechanical allodynia through spinal and peripheral actions, while cold allodynia was prevented through peripheral actions.

Keywords

ET-1, ET receptor, spinal cord, periphery, neuropathic pain

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Introduction

Oxaliplatin is a third-generation platinum-based chemotherapeutic agent and is a key drug used to treat advanced colorectal cancer. However, oxaliplatin causes peripheral neuropathy in approximately 80%–90% of patients.^{1,2} The most common symptom is cold allodynia in the hands and feet, which generally disappears within a few hours or days of treatment.³ In addition, approximately 10%–30% of patients suffer from long-lasting neuropathy such as pain and paresthesia,^{1,2,4} which are dose-limiting adverse effects that reduce the effect of anti-cancer therapy because of oxaliplatin dose reduction or cessation. In addition, long-lasting,

oxaliplatin-induced neuropathy may persist longer than 12 months, even with reduction or discontinuation of oxaliplatin treatment.^{1,2,5} However, existing analgesic drugs (e.g., duloxetine and pregabalin) and non-pharmacological treatments have limited effects against pain related to

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oxaliplatin-induced persistent neuropathy.^{6,7} Therefore, a therapeutic approach to prevent the development of oxaliplatin-induced neuropathy is expected to improve patient quality of life as well as cancer treatment.

Endothelin-1 (ET-1), a 21-amino acid peptide transmitter, is ubiquitously expressed and involved in a variety of physiological and pathological processes.^{8–10} ET-1 acts through two cognate receptors, endothelin type A (ET_A) and B (ET_B) receptors,⁸ both of which are involved in pain processing, apart from their vascular actions, in both the periphery and spinal cord.^{11,12} In the periphery, ET-1 has been repeatedly shown to cause mechanical allodynia observed in a variety of pain conditions through ET_A receptors.¹¹ However, the role of ET_B receptors in pain is more varied.^{13–15} In the spinal cord, the effect of ET-1 on nociceptive transmission is poorly understood. Intrathecal administration of an ET_A receptor antagonist has been shown to inhibit spinal nerve ligation (SNL)-induced neuropathic pain^{16,17} and spinal cord injury-induced mechanical allodynia.¹⁸ In contrast, intrathecal application of ET-1 induces ET_A receptor-mediated analgesia against incision-induced pain¹⁹ and neuropathic pain caused by sciatic nerve ligation.²⁰ Similarly, intrathecal administration of an ET_B receptor antagonist exerted an antinociceptive effect on SNL-induced mechanical allodynia²¹ but blocked the anti-nociceptive effect of spinal ET-1 on incision-induced pain¹⁹ and had no effect on spinal cord injury-induced mechanical allodynia.¹⁸ Thus, the function of ET-1 in the regulation of nociception seems dependent on the receptor type (ET_A and ET_B), pain condition and site of action (periphery or spinal cord), although ET-1 signaling is a plausible target to treat pain disorders. Interestingly, oxaliplatin was reported to increase ET_A receptor expression in the spinal cord and ET_A and ET_B receptor expression in the dorsal root ganglion (DRG).¹³ In addition, intraplantar administration of an ET_A or ET_B receptor antagonist partially suppressed mechanical allodynia. However, the role of ET_A and ET_B receptors in the spinal cord in oxaliplatin-induced neuropathic pain remains unknown. Therefore, in this study, we used endothelin receptor antagonists to investigate the role of ET-1 in the development of oxaliplatin-induced cold allodynia and long-lasting mechanical allodynia, especially focusing on the site of action and receptor types.

Materials and methods

Experimental animals

Male Sprague–Dawley rats (6–7 weeks; 180–230 g; Sankyo Labo Service Corporation, Tokyo, Japan) were used. Rats were housed individually with lights on from 06:00 to 20:00 h at room temperature (23 ± 1°C). Animals had free access to water and food in their home cages. All experimental procedures were performed in agreement with the procedures set by the institution's Animal Experiments Ethical Review Committee and were previously approved by the President of Nippon Medical School (Approval number 27-037). To observe the effect of intrathecal atrasentan administration on

body temperature, the body temperature (rectal and surface of the hind paw pad) was measured using a digital thermometer or non-contact infrared thermometer before (day –1) and after (day 7) intrathecal atrasentan administration.

Drug administration

Oxaliplatin (Yakult Corporation, Tokyo, Japan) was diluted in 5% glucose solution (1 mg/mL) and intraperitoneally administered at a dose of 5 mg/kg at day 0.^{22,23} Bosentan, a dual ET_A/ET_B receptor antagonist (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), atrasentan, a selective ET_A receptor antagonist (Sigma-Aldrich, St Louis, MO, USA) and BQ-788, a selective ET_B receptor antagonist (Alomone Labs, Jerusalem, Israel) were dissolved in 60% dimethylsulfoxide and 40% propylene glycol. Bosentan was intraperitoneally administered for 8 consecutive days (5, 15, or 50 mg/kg per day from days –1 to 6).^{24,25} Atrasentan (1, 3, or 10 mg/kg per day) was intraperitoneally administered for 2 consecutive days (days –1 and 0).²⁶ BQ-788 was intraperitoneally administered at a dose of 1 mg/kg for 2 consecutive days (days –1 and 0).²⁷ On day 0, bosentan, atrasentan and BQ-788 were administered immediately after oxaliplatin treatment. For peripheral administration, atrasentan was injected into the hind paw at a dose of 40 µg in 40 µL of solution 30 min before oxaliplatin administration. An equivalent volume of vehicle was administered as a control.

An intrathecal catheter was inserted for drug administration in the rats 2–3 days before oxaliplatin administration, as previously described.²⁸ Briefly, a polyethylene catheter (PE-10) filled with saline was inserted into the spinal subarachnoid space to the level of the L4–5 enlargement of the spinal cord under 2–3% isoflurane anesthesia. Rats with neurological symptoms such as paralysis or dysesthesia of the hind legs after catheterization were excluded. Intrathecal administration of atrasentan or BQ-788 was delivered at a dose of 50 µg in 10 µL of solution, followed by injection of an additional 10 µL of saline, using a 10-µL Hamilton microsyringe, into the intrathecal catheter for 2 consecutive days from 1 day prior to oxaliplatin administration.

Evaluation of allodynia

We used a von Frey test to observe the response to mechanical stimuli. Rats were placed in a plastic box with a wire mesh floor and allowed to habituate for 15 min prior to testing. von Frey filaments (Muromachikikai, Tokyo, Japan) with bending forces ranging between 2 and 16 g were applied to the midplantar skin of the left hind paw from underneath the mesh floor, with each application held for 10 s. The test was started with the filament with the lightest bending force applied for five consecutive times, followed by stimulation with the next filament. The force of the first filament that elicited three positive paw withdrawal responses in five applications was recorded as the paw withdrawal threshold.

To observe the response to cold stimuli, an acetone test was performed. Rats were placed in a plastic box with a wire mesh floor and allowed to habituate for 15 min prior to testing. Using a 0.5 mL syringe with a 26 G needle, acetone was applied to the ventral side of one hind paw from underneath the mesh floor, and the rat's response was monitored, as previously described.²⁹ Briefly, rats were first evaluated for 20 s. If the rat did not respond within 20 s, the result was recorded as no response. However, if the rat responded within the initial 20 s, the rat was monitored for an additional 40 s (a total observation period of 1 min from the initial application). Acetone was applied alternately twice to each hind paw, with a 5-min interval between applications. Responses were graded according to a 4-point scale, as previously described³⁰: 0, no response; 1, quick withdrawal, flick or stamp of the paw; 2, prolonged withdrawal or repeated flicking of the paw; 3, repeated flicking of the paw with persistent licking directed at the ventral side of the paw. Cumulative scores were obtained by summing the points of four trials (two for each paw) with a minimum score of 0 (no response to any of the four trials) and a maximum score of 12 (repeated flicking and licking of paws in all four trials). The acetone and von Frey tests were performed on different animals. Behavioral tests were performed in a manner blinded to ET-1 antagonist treatment.

Open field test

An open field test was performed 7 days after intrathecal atrasentan administration. The open field consisted of an opaque black plastic board (100 cm × 100 cm) with walls (50 cm in height). Each rat was placed at the corner of the open field, and the behavior was recorded for 10 min using a CCD camera. The total distance travelled in the open field and the time spent in the center zone of the open field (defined as the central 60 cm × 60 cm area) were analyzed using ImageJ software (ImageJ OF; O'Hara and Co., Ltd., Tokyo, Japan).

Quantitative polymerase chain reaction

The L4 DRG and L5 dorsal spinal cord were removed 24 h after oxaliplatin administration, frozen in liquid nitrogen and stored at -80°C until RNA purification was performed. Total RNA was extracted using RNAiso plus (Takara Bio, Shiga, Japan). The total RNA (500 ng) was reverse-transcribed with random primers using an iScript Select cDNA Synthesis kit (Bio-Rad, Hercules, CA, USA). Quantitative PCR was performed using Power SYBR Green PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) on a QuantStudio 3 Real-time PCR System (Thermo Fisher Scientific). The PCR program was initiated at 95°C for 10 min, followed by 40 cycles consisting of 95°C for 15 s and 60°C for 1 min. Primer pairs for ET_A and ET_B receptors were designed using Primer blast (<https://www.nih.gov/>) as the following sequences: ET_A receptor (forward, 5'-AACCTGGCAACCATGAACCTCT-3' and reverse, 5'-GGACTGGTGACAACAGCAAC-3') and

ET_B receptor (forward, 5'-GAGTCCCGCCAAGATCCTTC-3' and reverse, 5'-TTCCCGATGATGCCTAGCAC-3'). All samples were measured in triplicate.

Statistical analysis

von Frey and acetone test data are presented as the mean ± SEM and as the median, minimum, maximum and interquartile range values in box plot graphs, respectively. Because normality of the data was not assumed by the Shapiro–Wilk test (SPSS software, version 25; IBM, Chicago, IL, USA), the Mann–Whitney *U*-test was used for statistical analysis using SPSS software. For dose–response effects, the Steel test was used for statistical analysis using KyPlot (version 6.0.2, KyenceLab, Tokyo, Japan). A *p* value of <0.05 was considered significant.

Results

Oxaliplatin induced long-lasting mechanical allodynia and cold allodynia

To clearly distinguish between long-lasting mechanical allodynia and cold allodynia, a single dose of oxaliplatin was given on day 0. The paw withdrawal threshold was significantly decreased from 2 h after oxaliplatin administration and persisted for at least 14 days (Figure 1(a)), indicating that mechanical allodynia persisted long after oxaliplatin administration. In contrast, the response score for acetone application was increased only for 4 days (Figure 1(b)), indicating cold allodynia.

Systemic administration of a dual ET_A/ET_B receptor antagonist prevented both mechanical allodynia and cold allodynia induced by oxaliplatin

To examine the preventive effect of an endothelin receptor antagonist on oxaliplatin-induced neuropathic pain, a dual ET_A/ET_B receptor antagonist, bosentan, was intraperitoneally administered once a day from 1 day before to 6 days after oxaliplatin injection (8 consecutive days). Pre-emptive systemic treatment with bosentan (50 mg/kg) blocked the decrease in the paw withdrawal threshold induced by oxaliplatin for at least 11 days compared with vehicle treatment (Figure 2(a)). The analgesic effect of bosentan at day 1 after oxaliplatin administration was dose-dependent (Figure 2(b)). The increase in the response score for acetone application was also blocked by intraperitoneal bosentan treatment, compared with the vehicle treatment (Figure 2(c)). Thus, endothelin receptor antagonism effectively prevented the development of mechanical allodynia and cold allodynia induced by oxaliplatin administration. Next, we examined ET_A and ET_B receptor expression in the DRG and dorsal spinal cord. The ET_A and ET_B receptor mRNA expression levels were unchanged in the L4 DRG and dorsal spinal cord 1 day after oxaliplatin administration (Supplemental Figure 1(a, b)). Additionally, pre-emptive systemic treatment with bosentan did not change the

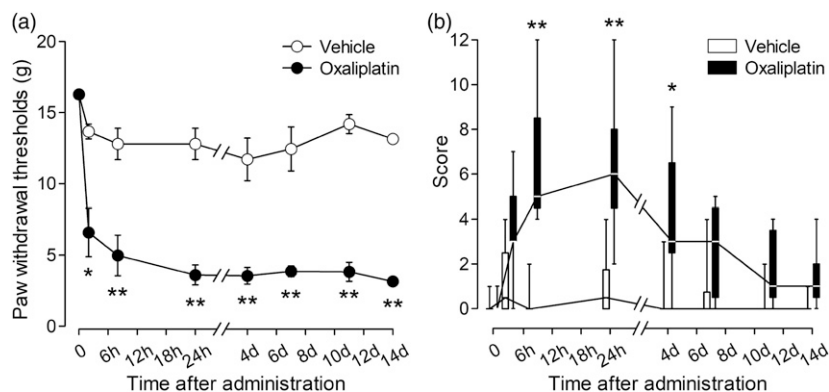


Figure 1. Administration of oxaliplatin produced long-lasting mechanical allodynia and transient cold allodynia: Oxaliplatin-induced mechanical allodynia (a) and cold allodynia (b) were examined using the von Frey test and acetone test, respectively, before and 2 h, 8 h, 24 h, 4 days, 7 days, 11 days, and 14 days after oxaliplatin administration. Oxaliplatin (5 mg/kg) was intraperitoneally administered in a single injection on day 0 ($n = 6-7$). * $p < 0.05$ and ** $p < 0.01$, compared with the vehicle group using the Mann–Whitney U -test.

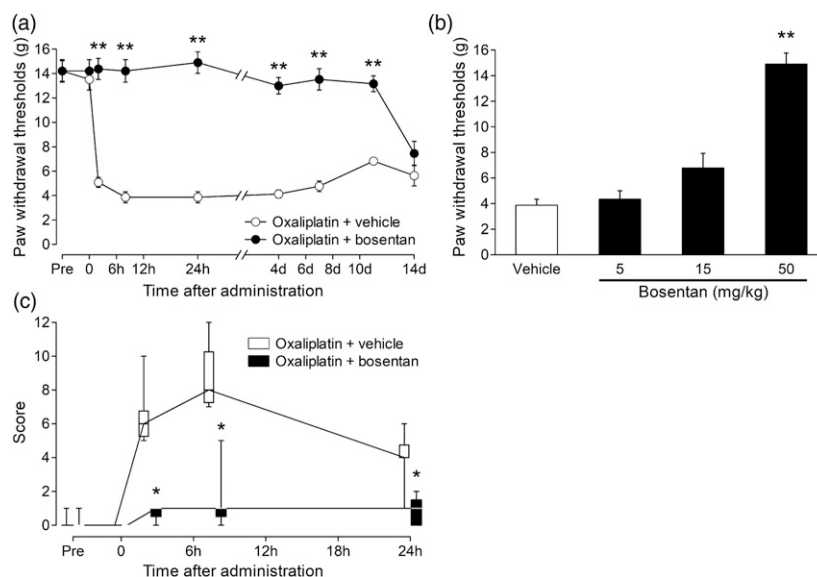


Figure 2. Systemic administration of a dual ET_A/ET_B receptor antagonist prevented oxaliplatin-induced mechanical allodynia and cold allodynia: Mechanical allodynia (a, b) and cold allodynia (c) were examined using the von Frey test and the acetone test, respectively. Bosentan (50 mg/kg) was intraperitoneally administered for 8 consecutive days (A; from days -1 to 6) and for 2 consecutive days (b and c; days -1 and 0) before intraperitoneal oxaliplatin administration ($n = 6$). (a) Mechanical allodynia was examined before each drug administration and 2 h, 8 h, 24 h, 4 days, 7 days, 11 days, and 14 days after oxaliplatin administration. (b) The dose–response relationship of bosentan was examined at day 1 after oxaliplatin administration. (c) Cold allodynia was performed before and 2 h, 8 h and 24 h after oxaliplatin administration. * $p < 0.05$ and ** $p < 0.01$, compared with the vehicle group using the Mann–Whitney U -test (a, c). *** $p < 0.01$, compared with the vehicle group using the Steel test (b). Pre: before administration of bosentan.

ET_A and ET_B receptor mRNA expression levels in the L4 DRG and dorsal spinal cord (Supplemental Figure 1(c, d)).

Inhibition of ET_A receptors, but not ET_B receptors, prevented oxaliplatin-induced mechanical allodynia and cold allodynia

To investigate whether the ET_A or ET_B receptor is involved in oxaliplatin-induced neuropathic pain, an ET_A receptor-selective antagonist, atrasentan, and an ET_B receptor-

selective antagonist, BQ-788, were intraperitoneally administered for 2 consecutive days from 1 day before oxaliplatin administration. Pre-emptive systemic atrasentan treatment blocked oxaliplatin-induced mechanical allodynia for 28 days compared with vehicle treatment (Figure 3(a)). The analgesic effect of atrasentan at day 1 after oxaliplatin administration was dose-dependent (Figure 3(b)). Cold allodynia was also prevented by systemic atrasentan administration (Figure 3(c)). In marked contrast, BQ-788 did not have any effect on mechanical allodynia or

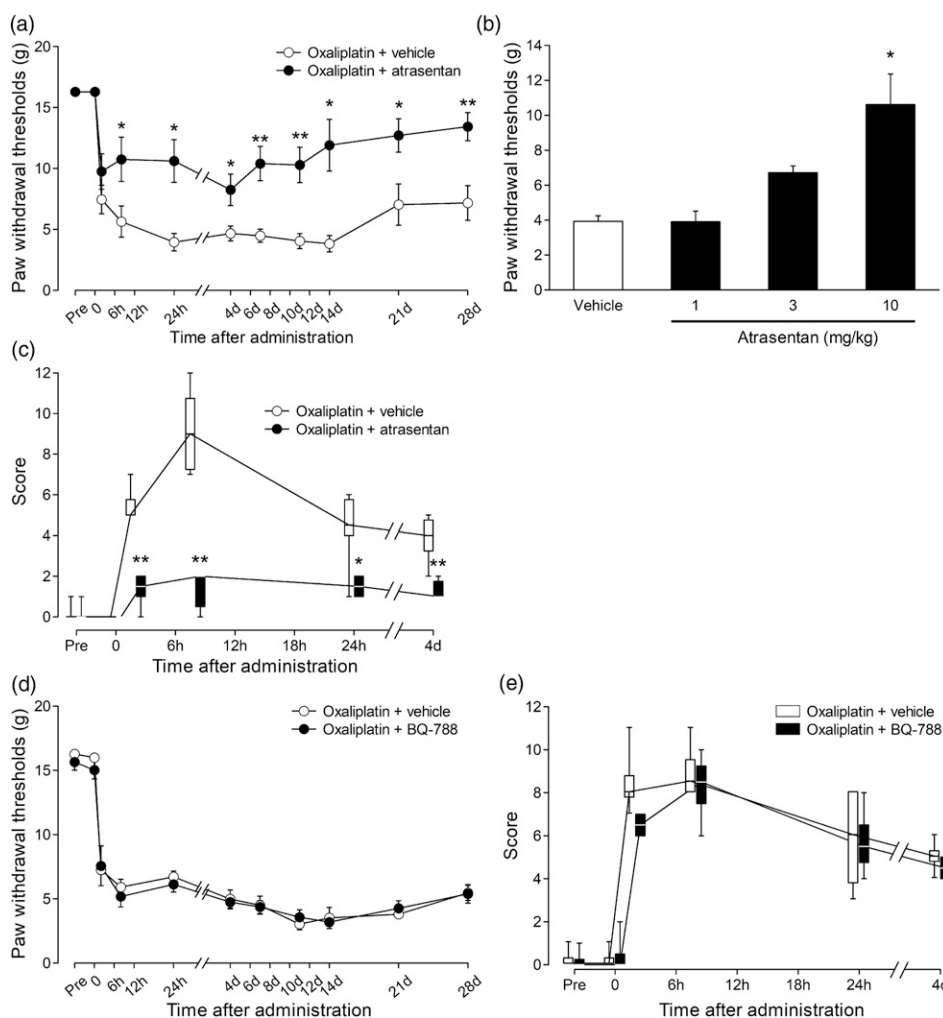


Figure 3. Systemic administration of an ET_A receptor antagonist, but not an ET_B receptor antagonist, prevented oxaliplatin-induced mechanical allodynia and cold allodynia: Mechanical allodynia (a, b, d) and cold allodynia (c, e) were examined using the von Frey test and the acetone test, respectively. An ET_A receptor antagonist, atrasentan (a–c), or an ET_B receptor antagonist, BQ-788 (d, e), was intraperitoneally administered for 2 consecutive days (days –1 and 0) before intraperitoneal oxaliplatin administration (5 mg/kg) on day 0 ($n = 5–8$). (a, d) Mechanical allodynia was examined before each drug administration and 2 h, 8 h, 24 h, 4 days, 7 days, 11 days, 14 days, 21 days, and 28 days after oxaliplatin administration. (c, e) Cold allodynia was examined before atrasentan, BQ-788 and oxaliplatin administration and 2 h, 8 h, 24 h and 4 days after oxaliplatin administration. * $p < 0.05$ and ** $p < 0.01$, compared with the vehicle group using the Mann–Whitney *U*-test. (b) The dose–response relationship of atrasentan was examined at day 1 after oxaliplatin administration. * $p < 0.05$, compared with the vehicle group using the Steel test. Pre: before administration of atrasentan or BQ-788.

cold allodynia throughout the observation period (Figure 3(d, e)).

Oxaliplatin-induced long-lasting mechanical allodynia, but not cold allodynia, was mediated by spinal ET_A receptors

Because ET_A receptor signaling has been shown to modulate nociceptive processing through both the spinal cord and periphery,^{11,17} we first investigated the involvement of spinal ET_A receptors in oxaliplatin-induced neuropathic pain. Intrathecal administration of atrasentan for 2 consecutive days from 1 day prior to oxaliplatin administration prevented the

development of mechanical allodynia for 28 days (Figure 4(a)). In marked contrast, cold allodynia was not prevented by intrathecal atrasentan administration (Figure 4(b)). Furthermore, intrathecal administration of BQ-788 for 2 consecutive days, from 1 day prior to oxaliplatin administration, did not have any effect on mechanical allodynia or cold allodynia throughout the observation period (Figure 4(c, d)). Intrathecal administration of atrasentan did not cause significant changes in locomotor activity and time spent in the center in the open field test on day 7 after oxaliplatin administration (Supplemental Figure 2(a), (b)). Intrathecal administration of an ET_A receptor antagonist before oxaliplatin administration also had no effect

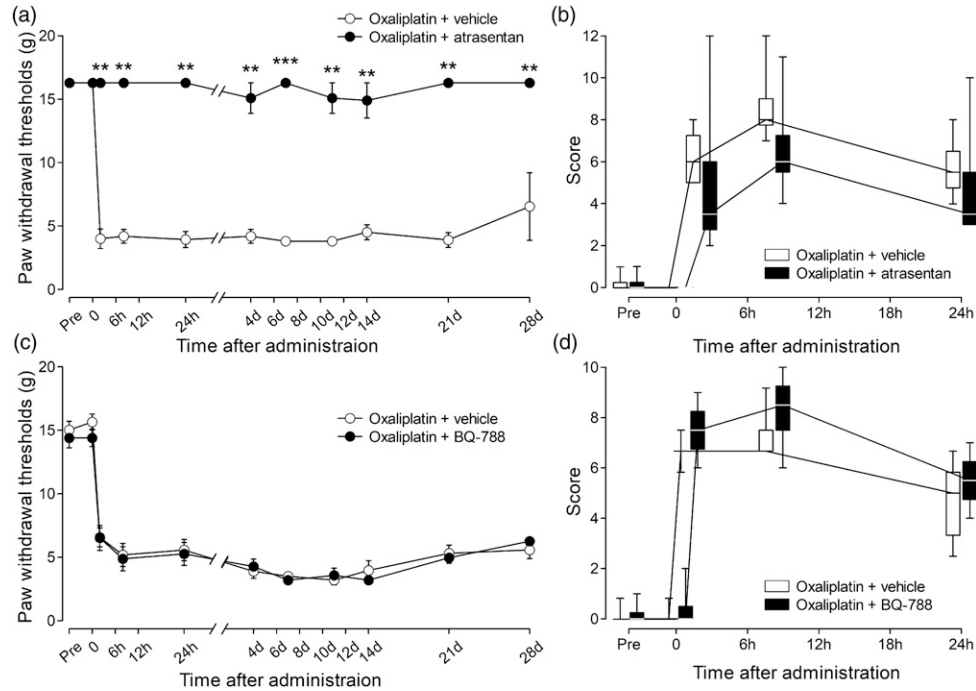


Figure 4. Intrathecal administration of an ET_A receptor antagonist prevented oxaliplatin-induced mechanical allodynia but not cold allodynia: Mechanical allodynia (a, c) and cold allodynia (b, d) were examined using the von Frey test and the acetone test, respectively. Atrasentan (50 μg) or BQ-788 (50 μg) was intrathecally administered for 2 consecutive days (days -1 and 0) before intraperitoneal oxaliplatin administration (5 mg/kg) on day 0 ($n = 5-6$). (a, c) Mechanical allodynia was examined before each drug administration and 2 h, 8 h, 24 h, 4 days, 7 days, 11 days, 14 days, 21 days, and 28 days after oxaliplatin administration. (b, d) Cold allodynia was examined before atrasentan, BQ-788 and oxaliplatin administration and 2 h, 8 h and 24 h after oxaliplatin administration. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$, compared with the vehicle group using the Mann-Whitney U -test. Pre: before administration of atrasentan or BQ-788.

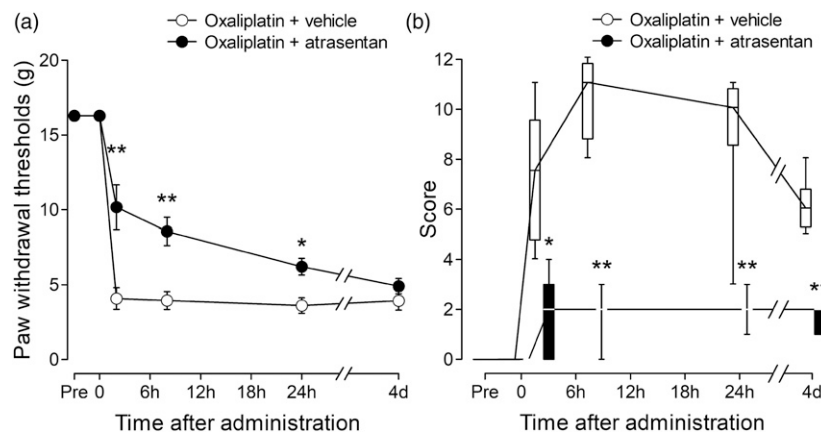


Figure 5. Intraplantar administration of an ET_A receptor antagonist prevented oxaliplatin-induced cold allodynia and temporarily suppressed mechanical allodynia: Mechanical allodynia (a) and cold allodynia (b) were examined using the von Frey test and the acetone test, respectively. Atrasentan was intraplantarly administered to the left midplantar paw (a) and to both midplantar paws (b) at 30 min before intraperitoneal oxaliplatin administration. In the control group, an equivalent volume of vehicle was intraplantarly administered ($n = 5-6$). Mechanical allodynia was examined before atrasentan and oxaliplatin administration and 2 h, 8 h, 24 h, and 4 days after oxaliplatin administration. Cold allodynia was examined before atrasentan and oxaliplatin administration and 2 h, 8 h, 24 h, and 4 days after oxaliplatin administration. * $p < 0.05$ and ** $p < 0.01$, compared with the vehicle group using the Mann-Whitney U -test. Pre: before administration of atrasentan.

on rectal or hind paw pad surface temperature on day 7 after oxaliplatin administration (Supplemental Figure 2(c, d)).

Peripheral ET_A receptors mainly mediated oxaliplatin-induced cold allodynia

We further explored the involvement of peripheral ET_A receptors in oxaliplatin-induced neuropathic pain. To examine the effects on mechanical allodynia and cold allodynia, atrasentan was intraplantarly administered to the left midplantar paw and to both sides of the midplantar paws, respectively, 30 min before oxaliplatin administration. Intraplantar administration of the ET_A receptor antagonist partially inhibited mechanical allodynia only for 1 day after oxaliplatin administration (Figure 5(a)). However, cold allodynia induced by oxaliplatin was effectively blocked by intraplantar atrasentan administration (Figure 5(b)).

Discussion

We first showed that pre-emptive inhibition of ET_A receptors in the spinal cord effectively prevented the development of oxaliplatin-induced mechanical allodynia but not cold allodynia. In marked contrast, pre-emptive inhibition of ET_A receptors in the periphery blocked cold allodynia but only partially and transiently alleviated mechanical allodynia. Therefore, through multiple sites of action, the ET_A receptor antagonist effectively prevented the development of long-lasting mechanical allodynia and cold allodynia in oxaliplatin-induced neuropathic pain.

Spinal ET_A receptors are involved in the development of oxaliplatin-induced mechanical allodynia

ET-1/ET_A signaling in the spinal cord contributes to the development of oxaliplatin-induced long-lasting mechanical allodynia. Systemic and intrathecal administration of an ET_A receptor antagonist, atrasentan, had a long-lasting suppressive effect on mechanical allodynia. However, the suppressive effects of the dual ET_A/ET_B receptor antagonist bosentan on mechanical allodynia disappeared at day 14. The shortened effect of the dual ET_A/ET_B receptor antagonist may be caused by the interfering effect of ET_B receptor antagonism on the analgesic effect of the ET_A receptor antagonist.²⁶ In fact, inhibition of ET_B receptors has been shown to promote the effects of endothelin.^{11,13,14} Regarding the preventive effect of the ET_A receptor antagonist on mechanical allodynia in the long term, spinal ET_A signaling may be involved in the induction of long-lasting mechanical allodynia. In fact, central sensitization in the spinal cord is a well-known basis for chronic pain that continues even after the initial cause has dissipated.³¹ Spinal glutamatergic transmission and NMDA receptor function, which are critical mediators of central sensitization, were reportedly upregulated in oxaliplatin-

induced mechanical allodynia.^{32,33} Although ET_A receptor expression has been shown in spinal neurons¹³ and vascular smooth muscle cells,⁹ the mechanisms underlying the pronociceptive effect of ET_A receptor activation in these cells remain mostly unknown. However, ET-1 expression has been reported in various spinal cells, including neurons,¹⁷ astrocytes,³⁴ and microglia.¹⁷ Astrocytes^{35,36} and microglia³⁷ were shown to be activated in oxaliplatin-induced neuropathy, while it has also been reported that they were not activated by oxaliplatin.³⁸ Increased production of pro-inflammatory cytokines (tumor necrosis factor α and interleukin-1 β) in spinal astrocytes reportedly contributes to oxaliplatin-induced mechanical allodynia.^{35,36} However, penetration of systemically administered oxaliplatin into the central nervous system is limited because of its poor ability to cross the blood-brain barrier,³⁹ indicating that ET-1 signaling in the spinal dorsal horn may be mediated by indirect action of oxaliplatin on the spinal cord. In contrast, ET_A receptor expression was unchanged in both the DRG and dorsal spinal cord early after oxaliplatin administration, while it was increased in the DRG and dorsal spinal cord of mice 28 days after initiation of oxaliplatin administration twice a week.¹³ These results suggest that the role of ET-1 differs between the acute and chronic phases of oxaliplatin-induced neuropathic pain. Overall, ET-1/ET_A receptor-mediated central sensitization in the spinal dorsal horn may contribute to the development of oxaliplatin-induced mechanical allodynia. In contrast to oxaliplatin-induced mechanical allodynia, cold allodynia was not blocked by spinal inhibition of ET_A receptors. Considering that spinal processing of nociception is distinct among modalities of nociceptive stimuli,^{40,41} ET_A receptors in the spinal cord are thought to be specifically involved in the regulation of mechanical pain after oxaliplatin treatment.

ET_A receptors in the spinal cord may also be involved in the mechanical allodynia observed in other types of chemotherapy-induced neuropathy. Cisplatin, another platinum derivative, also induces mechanical allodynia, which is thought to be partly mediated by a shared mechanism with oxaliplatin.^{23,42} Because neuropathy induced by other chemotherapeutics, such as taxanes, also causes mechanical allodynia and spinal sensitization⁴² through shared and distinct mechanisms,⁴³ investigating the role of ET_A in other types of chemotherapy-induced neuropathic pain will provide further insight into the mechanisms of chemotherapy-induced peripheral neuropathy.

Peripheral ET_A receptors are critical for oxaliplatin-induced cold allodynia

ET-1/ET_A receptor signaling in the periphery was only partially involved in mechanical allodynia, consistent with a previous report.¹¹ However, we found that an ET_A receptor antagonist effectively suppressed oxaliplatin-induced cold allodynia. Thus, our findings suggest that mechanical

allodynia and cold allodynia are differentially mediated in the periphery by ET_A receptors, which is consistent with evidence that different oxaliplatin metabolites, oxalate and dichloro(1,2-diaminocyclohexane)platinum (Pt(dach)Cl₂), contribute to cold allodynia and mechanical allodynia, respectively.⁴⁴ ET-1 has been shown to be produced by vascular endothelial cells and keratinocytes in the periphery^{45–47} and to be released upon ultraviolet B irradiation-induced injury⁴⁶ and pro-inflammatory cytokine stimulation,⁴⁵ respectively. In fact, these non-neural cells were recently suggested to contribute to nociception, in addition to DRG neurons.^{48–51} ET-1 directly activates ET_A receptors in nociceptive DRG neurons to modulate nociception.⁵² For example, ET-1 was reported to enhance tetrodotoxin-resistant sodium currents,⁵³ sensitize TRPV1⁵⁴ and TRPA1⁵⁵ receptors to noxious thermal stimuli and induce release of neurotransmitters, glutamate,⁵⁶ substance P,⁵⁷ and CGRP.⁵⁸ Therefore, oxaliplatin may stimulate ET-1 release from non-neuronal cells in the periphery, leading to indirect ET_A receptor activation at the peripheral axon terminal of nociceptive DRG neurons to produce cold allodynia and mechanical allodynia.

Neither oxaliplatin-induced mechanical allodynia nor cold allodynia were suppressed by systemic administration of an ET_B receptor antagonist

In the present study, systemic administration of a selective ET_B receptor antagonist had no obvious effect on oxaliplatin-induced neuropathic pain in rats, although administration of an ET_B receptor antagonist in the peripheral tissue was previously shown to partially inhibit oxaliplatin-induced mechanical allodynia in mice.¹³ This discrepancy in ET_B receptor action may be caused by a difference of animal species. Alternatively, ET_B receptor inhibition at a site other than peripheral tissues may counteract the analgesic effect of peripheral ET_B receptor antagonism. In fact, ET_B receptor activation has both pronociceptive and anti-nociceptive effects in both the spinal cord and periphery.¹¹ Intrathecal administration of an ET_B receptor antagonist temporarily suppressed SNL-induced mechanical allodynia in rats²¹ while also suppressing the anti-nociceptive effect of ET-1 on postoperative pain in rats.¹⁹ Intraplantar administration of an ET_B receptor antagonist prevented oxaliplatin-induced¹³ and carrageenan/complete Freund's adjuvant-induced¹⁵ mechanical allodynia in mice. In contrast, intraplantar administration of an ET_B receptor antagonist enhanced flinching behavior induced by ET-1 in naive rats.¹⁴ Therefore, ET_B receptor-mediated modulation of nociception seems dependent on its site of action, the cause of pain or differences in experimental animal species. In addition to these causes of variation, sex differences in endothelin function may exist. The role of neuroendocrine stress axes in oxaliplatin-induced peripheral neuropathy was shown to be sexually dimorphic,⁵⁹ although the overall severity was not.¹ The effect of ET-1 was also reported to be sexually dimorphic in the control of

vascular tone⁶⁰ but not in the control of the renal microvasculature.⁶¹ Sex or sex hormones evoked different types of ET-1 control on vascular tone.⁵⁹

In conclusion, our present study showed that through peripheral and spinal effects, an ET_A receptor antagonist effectively prevented both cold allodynia and long-lasting mechanical allodynia, which is frequently observed as a dose-limiting adverse effect of oxaliplatin. These results may suggest that administration of an ET_A receptor antagonist prior to oxaliplatin administration may be useful to pre-empt the neuropathic effects of this chemotherapeutic agent. Therefore, elucidating the role of ET_A receptors in oxaliplatin-induced neuropathic pain is expected to lead to the development of new therapeutic strategies.

Author contributions

KM, AS, and HS designed the study. KM, AS, YM, and YW conducted the experiments. KM, AS, AS, and HS analyzed the data, prepared the figures, and wrote the manuscript. All authors have approved the final manuscript.

Declaration of conflicting interests

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Supplemental Material

Supplemental material for this article is available online.

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