**Full Paper**

**Gabapentin Prevents Oxaliplatin-Induced Mechanical Hyperalgesia in Mice**

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**Abstract.** Oxaliplatin, a platinum-based chemotherapy drug, frequently causes acute and chronic peripheral neuropathies including mechanical hyperalgesia. These adverse effects hinder anti-cancer therapy with the drug. In this study, we examined several drugs that might prevent oxaliplatin-induced peripheral neuropathy. Single intraperitoneal (i.p.) injection of oxaliplatin (10 mg/kg) induced cold allodynia (acetone test) and mechanical hyperalgesia (von Frey test). Gabapentin, but not simvastatin and atorvastatin, prevented oxaliplatin-induced mechanical hyperalgesia without affecting cold allodynia. Moreover, oxaliplatin caused phosphorylation of cofilin protein in the spinal cord, which has been shown to be involved in the neuropathic hyperalgesia. This increased phosphorylation of cofilin was also attenuated by gabapentin treatment. These results suggest that gabapentin is useful for relieving oxaliplatin-induced mechanical hyperalgesia and that the pathogenic mechanisms of cold allodynia and mechanical hyperalgesia differ.

**Keywords:** oxaliplatin, mechanical allodynia, gabapentin, neuropathic pain, cofilin

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**Introduction**

Oxaliplatin is commonly used for the treatment of various types of cancer. Since oxaliplatin is now approved in many countries for the treatment of colorectal cancer as initial therapy, it is used as a key drug in the folinic acid/fluorouracil/oxaliplatin (FOLFOX) regimen. On the other hand, the clinical value of oxaliplatin is reduced by acute and chronic forms of peripheral neuropathy that appear as side effects. Acutely, about 90% of patients exhibit muscle fasciculation (1, 2), sensory paresthesias, or occasional dysesthesias (3), all triggered by mild cold sensation (4 – 7). This oxaliplatin-induced peripheral neuropathy is the most frequent dose-limiting toxicity associated with therapy, and currently no treatment for it is available.

Although many investigations have attempted to clarify the pathophysiology of oxaliplatin-induced neuropathy and establish effective treatment for it, details of the mechanism responsible remain enigmatic. Oxaliplatin-induced acute neuropathy is caused by functional changes, rather than morphological damage, in sensory nerves (8, 9). Oxaliplatin increases the sodium current (10) and promotes lowering of potassium channel expression (11) in dorsal root ganglion neurons, leading to over-activation of sensory nerve fibers. Treatment with the anti-cancer drug paclitaxel increases the expression of the calcium channel \(\alpha_2\delta_1\) subunit in the spinal cord and dorsal root ganglion neurons (12). In addition, oxaliplatin also enhance the generation of sodium channel resurgent current, which is also responsible for peripheral neural excitation (13). Phosphorylation of the NR2B subunit of the \(N\)-methyl-D-aspartate (NMDA) receptor is also increased after repeated treatment with oxaliplatin (14).

Clinically, the calcium channel subunit \(\alpha_2\delta_1\) blocker gabapentin, which is used as an antiepileptic drug, is widely prescribed for treatment of chronic pain. Acute...
treatment with gabapentin inhibits nociceptive transmission at the supraspinal and spinal levels (15, 16). Repeated treatment with gabapentin attenuates mechanical allodynia and trafficking of the α2δ1 subunit to the plasma membrane in a neuropathic pain model involving spinal nerve ligation (17). These findings led us to investigate the effect of gabapentin on mechanical hyperalgesia and cold allodynia caused by oxaliplatin treatment.

It has been reported that lipid-lowering drugs exert antinociceptive and anti-hyperalgesic effects in mice and rats. Systemic treatment with the lipid-lowering drugs simvastatin and rosuvastatin attenuates thermal hyperalgesia and mechanical allodynia in nerve-ligated rats (18). We recently reported that intrathecal treatment with simvastatin attenuates the second, but not first, phase of the formalin-induced nociceptive response, suggesting inhibition of the sensitization of spinal nociceptive transmission (19). Therefore, these drugs are also expected to relieve the acute sensory paresthesias induced by oxaliplatin.

Several clinically applied agents to treat neuropathic pain have been shown to prevent the induction or maintenance of spinal long-term potentiation (LTP) that is mediated by the neural plasticity (20 – 22). Neural plasticity is dynamically modulated by the postsynaptic structure and function. The actin cytoskeleton is an important player for these changes. The dynamics of the actin cytoskeleton is regulated by ADF/cofilin that is mainly inactivated by phosphorylation (23, 24). Previous reports indicated that ADF/cofilin phosphorylation and dephosphorylation have been associated with spine growth and shrinkage during LTP and long-term depression (LTD), respectively (25 – 27). Therefore, a recent report provided the hypothesis that temporally regulated ADF/cofilin-mediated actin dynamics regulate several changes for synaptic potentiation (28).

Accordingly, in the present study using mice, we explored drugs that would be effective for ameliorating oxaliplatin-induced mechanical hyperalgesia and cold allodynia. We also examined the effect of oxaliplatin on phosphorylation of cofilin in the spinal cord.

Materials and Methods

All of the experimental protocols used in the present study were approved by the Animal Care and Use Committee of Nagoya City University and carried out in accordance with the guidelines of The Japanese Pharmaceutical Society.

Animals

Four- to five-week-old male ddY mice (SLC, Shizuoka) were used. The animals were housed 5 per cage in a room maintained at 23°C ± 2°C with an alternating 12-h light–dark cycle. Food and water were available ad libitum. Animals were used only once in all experiments.

Assessment of mechanical allodynia

Mice were placed in individual transparent Perspex cubicles with a wire mesh bottom, and a series of calibrated von Frey filaments (Semmes-Weinstein monofilaments; Stoelting, Wood Dale, IL, USA) was used to determine the 50% likelihood of a paw withdrawal response (50% threshold) employing the up–down method of Dixon (29). Eight von Frey filaments, with approximately equal logarithmic incremental bending forces, were chosen (0.02, 0.04, 0.07, 0.16, 0.4, 0.6, 1.0, 1.4 g). Testing was initiated with the 0.16-g hair, and each hair was applied perpendicularly to the plantar surface of the hindpaw, with sufficient force to bend the filament, for 3 – 4 s. Lifting of the paw indicated a positive response and prompted the use of the next weaker (i.e. lighter) filament. Absence of a paw withdrawal response prompted the use of the next stronger (i.e., heavier) filament. This paradigm was continued until 4 measurements had been obtained after an initial change in behavior or until 4 consecutive positive scores (score of 0.02 g) or 5 negative scores (score of 1.4 g) had been obtained. The resulting scores were used to calculate the 50% threshold (30).

Assessment of cold allodynia

The time course of cold allodynia was assessed using the acetone test (31). A drop (0.02 ml) of acetone was placed against the center of the plantar hind paw. Responses were graded with the following 4-point scale: 0 = no response; 1 = quick withdrawal, flick or stamp of the paw; 2 = prolonged withdrawal or repeated flicking; and 3 = repeated flicking of the paw with licking directed at the ventral side of the paw. Acetone was applied alternately 3 times to each paw and average scores were then generated from the 6 scores for each mouse.

Western blot analysis

The spinal cord was quickly removed following decapitation to evaluate protein expression. To measure the phosphorylation of cofilin protein, decapitation was performed 7 days after treatment with oxaliplatin (10 mg/kg, i.p.). The spinal cord was homogenized in ice-cold radio immunoprecipitation assay (RIPA) buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 250 μg of leupeptin per ml, 250 μg of aprotinin per ml, 1.0% sodium dodecyl sulphate (SDS), 0.5% sodium deoxycholic acid (DOC), and 1.0% Triton-X 100. The soluble fractions were obtained by centrifugation at 20,000 × g for 20 min.
at 4°C. The protein concentration was measured using a BCA assay (Thermo Fisher Scientific Inc., Rockford, IL, USA). A tissue sample aliquot was diluted with an equal volume of 2 × electrophoresis sample buffer containing 2% SDS and 10% glycerol with 0.2 M dithiothreitol. The supernatants (20 mg of protein) were separated by size on 4% – 20% SDS–polyacrylamide gradient gel using the buffer system and transferred to nitrocellulose membranes in Tris–glycine buffer containing 25 mM Tris and 192 mM glycine. For immunoblot detection, the membranes were blocked in Tris-buffered saline (TBS) containing 1% non-fat dried milk with 0.1% Tween 20 (Bio-Rad Laboratories, Hercules, CA, USA) for 1 h at room temperature with agitation. The membrane was immunoblotted overnight at 4°C with antibodies against phosphorylated cofilin (1:1000; Abcam, Cambridge, MA, USA) and cofilin (1:1000, Abcam). The membrane was washed in TBS containing 0.05% Tween 20 (TTBS) and then incubated for 2 h at room temperature with horseradish peroxidase–conjugated goat anti-rabbit IgG or rabbit anti-mouse IgG (Cell Signaling Technology Inc., Beverly, MA, USA) diluted 1:20,000 in TBS containing 0.5% non-fat dried milk with 0.1% Tween 20. The antigen–antibody peroxidase complex was finally detected by enhanced chemiluminescence (Pierce, Rockford, IL, USA) and visualized using a LAS-4000 system (GE Healthcare Asia Co., Tokyo). The intensity of the band was analyzed and semi-quantified by computer-assisted densitometry using the NIH imaging system. Each value was normalized by the respective value for β-actin as an internal control.

**Drugs**

The drugs used in this study were oxaliplatin (Tanaka Kikinzoku Kogyo, K.K., Tokyo), atorvastatin (Lipitor; Pfizer Japan Co., Ltd., Tokyo), simvastatin (Lipovas; Banyu Yakuhin Co., Ltd., Tokyo), and gabapentin (Toronto Research Chemicals Inc., Ontario, Canada). Other reagents used in the present study were all of molecular biology grade. Oxaliplatin was dissolved in 5% glucose solution. Atorvastatin and simvastatin were suspended in 0.5% sodium carboxymethyl cellulose (CMC) solution. Gabapentin was dissolved in distilled water. Oxaliplatin was administered intraperitoneally just after the nociceptive test on day 0. Atorvastatin, simvastatin, and gabapentin were administered by oral garbage just after the nociceptive test, from 1 day before oxaliplatin treatment, and 0, 1, and 2 days after oxaliplatin treatment.

**Statistical analyses**

The data were expressed as the mean ± S.E.M. The statistical significance of differences among multiple groups was assessed by a non-parametric multiple test with Bonferroni correction following the Kruskal-Wallis test. Differences at probability values of less than 0.05 ($P < 0.05$) were considered to be statistically significant.

**Results**

**Induction of mechanical hyperalgesia and cold allodynia in mice by intraperitoneal administration of oxaliplatin**

Intraperitoneal administration of oxaliplatin at a dose of 10 mg/kg decreased the mechanical threshold (baseline threshold, $0.68 \pm 0.03$ g; Fig. 1A), and the threshold remained lower for 7 days after the treatment ($0.34 \pm 0.06$ g; Fig. 1A). In addition, single intraperitoneal administration of oxaliplatin also produced hypersensitivity to cold stimulation 3 days after the treatment (Fig. 1B). This oxaliplatin-induced cold allodynia decreased gradually up to 7 days after the treatment (Fig. 1B). Treatment with vehicle alone had no effect on mechanical and cold sensitivity (Fig. 1: A and B).

![Fig. 1. Time course of oxaliplatin (L-OHP)-induced mechanical hyperalgesia (A) and cold allodynia (B). Oxaliplatin was administered intraperitoneally. Mechanical threshold and cold score were assessed by the von Frey test and acetone test, respectively. Each point represents the means ± S.E.M. of 8 – 10 mice. Asterisks (*) indicate data points for which there was a significant difference between the 5% glucose–treated and L-OHP-treated groups ($*P < 0.05$, $**P < 0.01$; non-parametric multiple comparisons with Bonferroni correction following the Kruskal-Wallis test, 2 comparisons among 3 groups).](image-url)
Effects of gabapentin, atorvastatin, and simvastatin on oxaliplatin-induced mechanical hyperalgesia and cold allodynia in mice

Oral administration of gabapentin at doses of 10, 30, and 100 mg/kg increased the lowered mechanical threshold in oxaliplatin-treated mice (Fig. 2A, left). This increase was evident at 1 day after the oxaliplatin treatment and persisted for 5 days after the treatment. The effect of gabapentin (30 mg/kg, p.o.) on cold allodynia was evident at 1 day after the oxaliplatin treatment (Fig. 2A, right). In contrast with mechanical threshold, oral administration of gabapentin at dose of 100 mg/kg did not affect the cold allodynia in oxaliplatin-treated mice (Fig. 2A, right). This lack of dose-dependency might be indicated that gabapentin could not affect the cold allodynia induced by oxaliplatin treatment.

Previous reports have indicated that statins, which are lipid-lowering agents, induce antihyperalgesic and antiallodynic effects in a neuropathic pain model (32). Here we examined the effects of atorvastatin and simvastatin on oxaliplatin-induced mechanical hyperalgesia and cold allodynia. However, neither atorvastatin nor simvastatin altered either of these effects of oxaliplatin (Fig. 2: B and C).
Effect of oxaliplatin on phosphorylation of cofilin in the spinal cord

Previous reports had indicated that oxaliplatin did not produce any morphological damage to the nervous system (8, 9). Therefore it is possible that oxaliplatin-induced acute neuropathy might result from functional changes in nociceptive transmission. Actin depolymerizing factor ADF/cofilin, whose activity is regulated by phosphorylation and dephosphorylation, is involved in hyperalgesia in the spinal cord (33). Therefore, we examined the effect of oxaliplatin on cofilin phosphorylation in the spinal cord. Single treatment with oxaliplatin led to increased cofilin phosphorylation in the spinal cord (Fig. 3: A and B). Treatment with gabapentin (100 mg/kg) attenuated the oxaliplatin-induced increase of cofilin phosphorylation in the spinal cord (Fig. 3: A and B).

Discussion

Our present study indicated that acute treatment with oxaliplatin produced long-lasting mechanical hyperalgesia and short-term cold allodynia in ddY mice. The onset of cold allodynia was more rapid than that of mechanical hyperalgesia. The oxaliplatin-induced mechanical hyperalgesia, but not cold allodynia, was attenuated by gabapentin, while simvastatin and atorvastatin had no effect. These results clearly indicate that gabapentin is a promising candidate drug for treatment of oxaliplatin-induced sensory paresthesia.

Our previous study had indicated that acute systemic treatment with gabapentin produced antihyperalgesic and antiallodynic effects (16). Repeated treatment with gabapentin also attenuated the allodynia and hyperalgesia in mice injected with herpes simplex virus type-1 (34). In the present study, gabapentin treatment applied from 1 day before until 2 days after oxaliplatin administration attenuated mechanical hyperalgesia during 5 days after oxaliplatin treatment. A previous report had indicated that chronic treatment with pregabalin, a gabapentin analog, attenuated mechanical hyperalgesia by inhibiting the sensitization of spinal nociceptive transmission (17). Therefore, it is possible that the effect of gabapentin on mechanical hyperalgesia induced by acute treatment with oxaliplatin might be mediated by inhibition of spinal nociceptive sensitization. On the other hand, cold hyperalgesia induced by oxaliplatin was not affected by the gabapentin treatment, suggesting that the mechanical allodynia and cold allodynia are caused by different mechanisms.

Lipid-lowering drugs have been shown to produce antihyperalgesic and antiallodynic effects in several animal models. Systemic treatment with atorvastatin produced antinociceptive and antihyperalgesic effects in models of bacterial adjuvant-induced inflammatory pain (35) or lipopolysaccharide-induced pain (36). Repeated treatment with simvastatin and rosuvastatin also produced an antihyperalgesic effect in mice and rats with nerve injury-induced neuropathic pain (18). Recently, we also reported that intrathecal, but not intracerebroventricular, administration of simvastatin exerted an antinociceptive effect in formalin-induced nociception (19). In contrast to these studies, our present results obtained using an oxaliplatin-induced neuropathic pain model showed that neither simvastatin nor atorvastatin produced any antihyperalgesic or antiallodynic effects. Therefore, since simvastatin and atorvastatin elicit their antihyperalgesic, antiallodynic, and antinociceptive effects through inhibition of the inflammatory response, it is possible that the effects of oxaliplatin might not be produced through an inflammatory mechanism involving
microglial or astroglial activation in the nervous system.

In the present study, we found that phosphorylation of coflin in the spinal cord was increased in the oxaliplatin-treated group. Oxaliplatin modulates the activity of ionic channels, particularly voltage-gated sodium channels (37, 38). A previous study using hippocampal slices has shown that neurotransmission sensitization induced by theta burst stimulation (10 bursts of 4 pulses at 100 Hz with 200-ms interburst intervals) induces phosphorylation of coflin (39). Therefore, the increase of coflin phosphorylation in the spinal cord induced by oxaliplatin might be caused by sensory nerve activation. Phosphorylation inactivates coflin by preventing its binding to actin filaments (40), thus promoting actin polymerization and filament loading (41, 42). It is possible that increased phosphorylation of coflin may be involved in the sensitization of synaptic nociceptive transmission resulting from oxaliplatin treatment. It has been reported that prolonged activation (dephosphorylation) of coflin may negatively affect cell surface AMPA receptor levels in the spine (43), a parameter known to be involved in neuropathic pain (44). Therefore, oxaliplatin-induced long-term inhibition (phosphorylation) of coflin may be involved in spinal nociceptive sensitization.

Gabapentin exerts its antihyperalgesic properties through binding to calcium channel subunit α2δ1 (45). Previous reports have indicated that the effects of gabapentin appear acutely (15, 16). On the other hand, it has also been reported that gabapentin is ineffective when acutely administered after the induction of hyperalgesia by formalin administration (46). These results suggest that gabapentin prevents the initiation of a persistent pain state. In the present study, we found that gabapentin exerted antihyperalgesic and antiallodynic effects. Recently, chronic treatment with pregabalin has been shown to prevent neuropathic pain by inhibiting translocation of the α2δ subunit to presynaptic terminals (17). It has also been reported that expression of the α2δ1 subunit in the dorsal root ganglion is enhanced by paclitaxel treatment (12). Consequently, it is possible that the acute effect of gabapentin is mediated by inhibition of calcium channel activity and that the chronic effect results from inhibition of α2δ1 subunit expression.

In the present study, gabapentin prevented the oxaliplatin-induced increase of coflin phosphorylation in the spinal cord. Since the mechanisms responsible for oxaliplatin-induced phosphorylation of coflin are not known, the action sites of gabapentin remain unclear. Gabapentin inhibits calcium channel α2δ1 subunit, leading the inhibition of neurotransmission (47). In the spinal cord, glutamate is the key neurotransmitter in the nociceptive transmission. Glutamate activates NMDA receptors, which increase calcium influx in the post-synaptic neuron. Increased intracellular calcium stimulates signaling molecules and activates protein kinases. Since inhibition of calcium channel α2δ1 subunit attenuates the release of neurotransmitters from the presynaptic site, it is possible that gabapentin exerts its effect on oxaliplatin-induced spinal phosphorylation of coflin through the inhibition of glutamate release.

In conclusion, we have shown that gabapentin blocks mechanical hyperalgesia, but not cold allodynia, induced by acute oxaliplatin treatment. Gabapentin might inhibit the oxaliplatin-induced neuropathic pain that is involved in the increased phosphorylation of coflin in the spinal cord. Accordingly, gabapentin might be a promising candidate drug for treatment of acute sensory paresthesias.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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