

Gabapentin prevents oxaliplatin-induced central sensitization in the dorsal horn neurons in rats

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ABSTRACT

Objective(s): The present study aims to study the alteration of glutamatergic transmission in the dorsal horn neurons and the effect of gabapentin on oxaliplatin-induced neuropathic pain in rats.

Materials and Methods: Oxaliplatin (5 mg/kg) or saline was administered to adult male Sprague-Dawley rats. Gabapentin (60 mg/kg, IP) or vehicle was injected daily. Mechanical allodynia was assessed using a series of von Frey filaments. The expression of glutamate receptor subunits (NR2B and GluR1) and brain-derived neurotrophic factor (BDNF) was measured in the dorsal horn. The glutamatergic strength was recorded in the spinal cord slices.

Results: Administration of oxaliplatin induced significant hyperreactivity to mechanical stimuli in rats, which was attenuated by gabapentin. Significant increase in the expression of BDNF was found in the dorsal horn in rats receiving oxaliplatin, which was prevented by gabapentin. Further studies also observed a significant increase in the expression of GluR1 and NR2B, as well as enhanced glutamatergic transmission in the dorsal horn neurons in rats treated with oxaliplatin. The upregulation of glutamatergic transmission was significantly reversed by gabapentin.

Conclusion: These results illustrated an increased expression of BDNF and enhanced glutamatergic transmission in rats with oxaliplatin-induced neuropathic pain, which was markedly attenuated by gabapentin.

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Introduction

Oxaliplatin is a frequently used chemotherapeutic agent for solid tumors. However, treatment with chemotherapeutic agents including oxaliplatin often induces dose-limiting peripheral neuropathic pain with paresthesia and dysesthesia, which significantly hampers its optimistic clinical use (1). While previous studies reported that treatment with oxaliplatin induces pronounced loss of intraepidermal nerve fibers (2) and increased the responses of peripheral nociceptors to mechanical stimulation (3), the functional adaptation of glutamatergic transmission in the dorsal neurons had not been sufficiently elucidated in rats with oxaliplatin-induced neuropathic pain. Significantly increased glutamatergic strength was revealed in the spinal nociceptive neurons in rats with neuropathic pain induced by peripheral nerve injury (4). Treatment with chemotherapeutic agent paclitaxel induced decrease in glutamate release, thus leading to increased spontaneous activity of the dorsal horn neurons (5). Hence, the present study investigated the alteration of glutamatergic transmission in the

dorsal neurons in rats with oxaliplatin-induced neuropathic pain.

Recently, anticonvulsant agent, gabapentin has been commonly used to treat the neuropathic pain in rodent model and clinical patients (6). Gabapentin is a structural analogue of GABA with the ability to cross the blood-brain barrier. Gabapentin may bind to the accessory $\alpha 2\text{-}\delta\text{-}1$ subunit of voltage gated calcium channels (particularly the N- and L-types), and subsequently inhibit the membrane trafficking of calcium channels, thus decreasing the excitability of the peripheral nociceptors and dorsal horn neurons and attenuating the nociceptive sensation (7). Previous evidences also showed that gabapentin has the ability to directly inhibit NMDA receptors in *Xenopus* oocytes (8), suppress the activity of PKC- γ in the dorsal horn neurons (9), which may also be responsible for its anti-nociceptive activity. Series of preclinical and clinical studies demonstrated the anti-nociceptive effect of gabapentin in the neuropathic pain-induced peripheral nerve injury and diabetic neuropathy (6). The present study aims to investigate the effect of

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gabapentin on the glutamatergic transmission in the dorsal horn and the pain behaviors in rats with oxaliplatin-induced neuropathic pain.

Materials and Methods

Animal

Adult male Sprague-Dawley rats (weighing 180–220 g) were obtained from the Institutional Center of Experimental Animals and kept under standard lab conditions (22 ± 2 °C and 12:12 hr light:dark cycle) with food and water provided *ad libitum*. All animal protocols were approved by the Institutional Animal Care and Use Committee, and confronted with the guidelines of National Institution of Health.

Oxaliplatin (Sigma-Aldrich) was dissolved in 0.9% NaCl for intraperitoneal (IP) injections to rats. Oxaliplatin (5 mg/kg) or saline was administered on days 1, 4 and 7 at 10:00 AM. This dosage of oxaliplatin was in the range of those used in previous studies also aimed at inducing neuropathy in rodents (10). Injections of gabapentin (60 mg/kg, IP) or vehicle were done daily from day 1 to day 14. The dose of gabapentin was adopted from the previous reports, which effectively reversed central pain sensitization (11). The behavioral test was done on days 1, 4, 7, 10, 12 and 14 after the initial injection of oxaliplatin.

Behavioral test

Mechanical allodynia was assessed in rats to evaluate painful behavior induced by oxaliplatin. Mechanical sensitivity was assessed using a series of von Frey filaments with stimulation with bending forces ranging from 2 to 60 g (Stoelting Co., Wood Dale, IL) as previously described (12). Filaments were applied to the center region of plantar surface for about 6 sec in an ascending order. Hindpaw withdrawal due to normal locomotor behavior was ignored. The withdrawal latency was defined as the smallest filament size which evoked at least two withdrawal responses during three consecutive applications.

Immunoblotting

After behavioral test, rats were deeply anesthetized with pentobarbital sodium (50 mg/kg). The spinal cord was removed quickly and the lumbar segments of the dorsal horn (L4-L5) were punched (13) and homogenized in the lysis buffer. The total and nuclear protein were prepared and processed for immunoblotting. The following antibodies were used: polyclonal antibodies against NR2B (1:1000, Millipore), GluR1 (1:1000, Millipore), or monoclonal anti- β -actin antibody (1:1000; Santa Cruz Biotechnology), and the horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG secondary antibody (1:10,000; Jackson ImmunoResearch Laboratories). The immunoreactivity was detected using enhanced chemiluminescence (ECL Advance Kit, Amersham

Biosciences). The intensity of bands was analyzed quantitatively with ImageJ software.

ELISA

ELISA was performed to detect the concentration of BDNF in the spinal dorsal horn (L4-L5) as previously reported (14). The animals were deeply anesthetized and the spinal cord was quickly removed and cut into sections. The dorsal horn tissue (L4-L5) were punched and processed with commercial ELISA kit (R & D Systems) based on the instructions provided by the manufacturer.

Spinal cord slice preparation and electrophysiological recording

Rats were deeply anesthetized with inhalation of halothane and the lumbar segment of the spinal cord was removed. The spinal tissue was immediately placed in ice-cold artificial cerebrospinal fluid containing (in mM): sucrose 230, KCl 3.5, MgCl₂ 1.5, CaCl₂ 2.0, NaH₂PO₄ 1.2, glucose 12, and NaHCO₃ 25. Transverse spinal cord slices (400 μ m) were cut with a vibratome (Technical Products International, St. Louis, MO) and incubated in Krebs' solution (containing: 117 mM NaCl, 3.6 mM KCl, 1.2 mM MgCl₂, 2.5 mM CaCl₂, 1.2 mM NaH₂PO₄, 11 mM glucose, and 25 mM NaHCO₃, bubbled with 95% O₂ and 5% CO₂) at 35 °C for at least 1 hr before the recording was performed.

Whole-cell recording on the spinal cord slices (L4-L5) was performed as described previously (4). The neurons in lamina II of the dorsal horn were visualized using an upright microscope with infrared illumination. Whole-cell voltage-clamp recordings were performed using an Axopatch 200B amplifier with 3–5 M Ω glass electrodes containing the following internal solution (in mM): K-gluconate, 126; NaCl, 5; MgCl₂ 1.2; EGTA, 0.5; Mg-ATP, 2; Na₃GTP, 0.1; HEPES, 10; guanosine 5'-O-(2-thiodiphosphate) 1; lidocaine *N*-ethyl bromide (QX314), 10; pH 7.3; 290–300 mOsmol. A seal resistance of ≥ 2 G Ω and an access resistance of 15 – 20 M Ω were considered acceptable. The series resistance was optimally compensated by $\geq 70\%$ and constantly monitored throughout the experiments. The membrane potential was held at –60 mV throughout the experiment. Excitatory postsynaptic currents (EPSCs) in ipsilateral lamina II neurons were evoked by electrical stimulation (0.25 ms, 0.05 – 0.3 mA) of the dorsal root in the presence of strychnine (2 μ M) and bicuculline (10 μ M). The evoked EPSCs were filtered at 2 kHz, digitized at 10 kHz, and acquired and analyzed using pCLAMP 9.2 software.

Statistical analysis

All data are presented as means \pm SEM and were analyzed with student's *t*-test or ANOVA followed by *post-hoc* analysis. The criterion for statistical significance was $P < 0.05$.

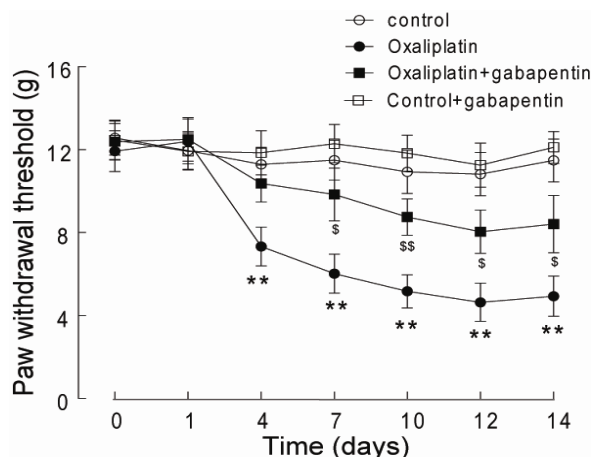


Figure 1. Administration of gabapentin (60 mg/kg) significantly attenuated mechanical allodynia in rats receiving oxaliplatin (5 mg/kg for 3 times). Significantly decreased threshold to mechanical stimuli was observed in rats receiving oxaliplatin which was recovered by the administration of gabapentin. N= 8-9 rats per group; compared to control group: *, $P < 0.05$; **, $P < 0.01$; compared to oxaliplatin group: \$, $P < 0.05$; \$\$, $P < 0.01$

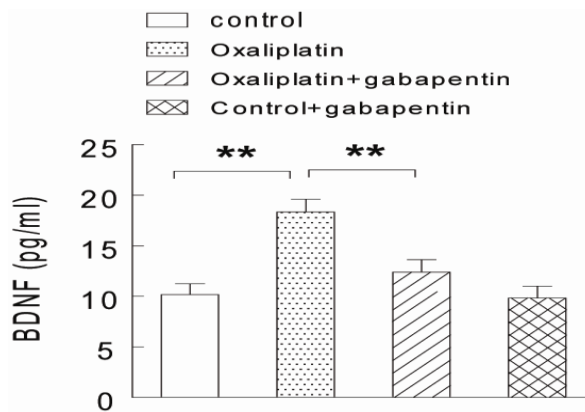


Figure 2. Significantly increased expression of BDNF was observed in the dorsal horn of rats receiving oxaliplatin, which was significantly attenuated by intrathecal administration of gabapentin. N=8 per group; *, $P < 0.05$; **, $P < 0.01$

Results

Gabapentin inhibited the mechanical allodynia

Administration of oxaliplatin (5 mg/kg for 3 times) significantly decreased the paw withdrawal threshold responsive to the mechanical stimuli (Figure 1A) which was remarkably detectable on day 4 and maintained until at least day 14 (the endpoint of the study). Administration of gabapentin (60 mg/kg for 14 days) significantly increased the paw withdrawal threshold responsive to the mechanical stimuli, while it had no significant effect in the control rats. These results demonstrated gabapentin-mediated anti-allodynia effect in rats with oxaliplatin-induced neuropathic pain.

Gabapentin inhibited the upregulation of BDNF in the dorsal horn

Then, we investigated the effect of intrathecal administration of gabapentin on expression of BDNF in the dorsal horn in rats treated with oxaliplatin. As shown in Figure 2, significantly increased expression of BDNF was observed in the dorsal horn of rats receiving oxaliplatin, which was significantly attenuated by the intrathecal administration of gabapentin. These results suggested that suppression of upsurge of BDNF in the dorsal horn potentially underlies gabapentin-induced anti-nociception effect in the rats receiving oxaliplatin.

Gabapentin inhibited the upregulation of GluR1 and NR2B in the dorsal horn

Here, significantly increased expression of GluR1 and NR2B was detected in the dorsal horn in rats receiving oxaliplatin which was significantly reduced by the administration of gabapentin (Figure 3, N=7-8 per group, $P < 0.01$). These results indicated that gabapentin attenuated the adaptation of glutamate receptor subunits in the dorsal horn in rats receiving oxaliplatin.

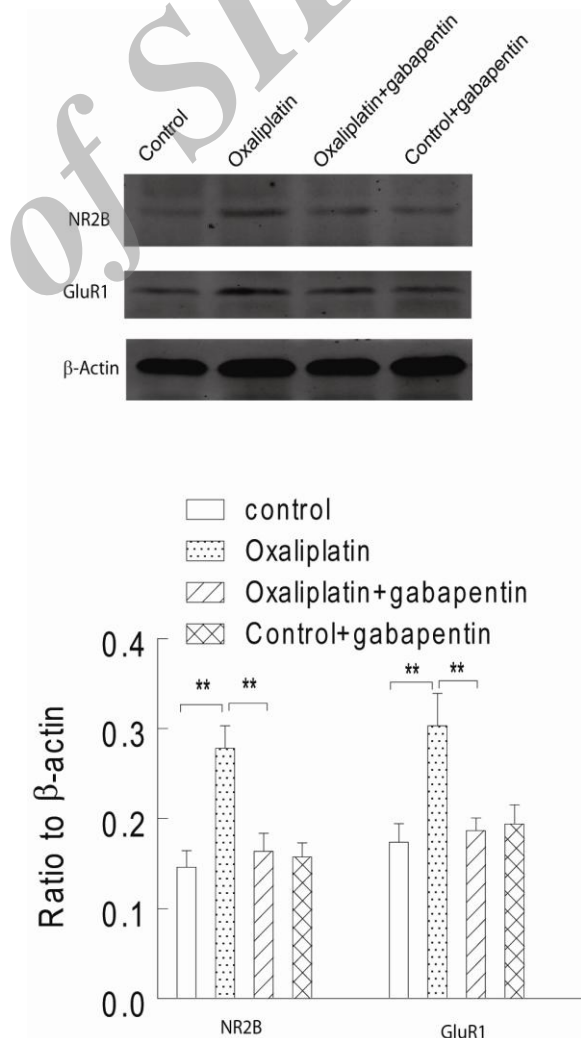


Figure 3. Significantly increased expression of GluR1 and NR2B was detected in the dorsal horn in rats receiving oxaliplatin which was significantly reduced by the administration of gabapentin. N=7-8 per group; *, $P < 0.05$; **, $P < 0.01$

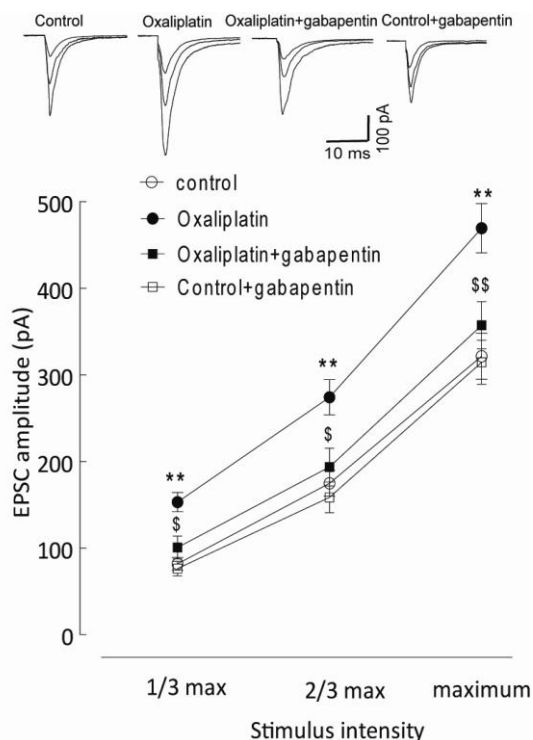


Figure 4. Significantly increased input - output response of evoked EPSC was observed in the dorsal horn neurons in the rats receiving oxaliplatin which was attenuated by the administration of gabapentin. N= 10-12 neurons per group; compared to control group: *, $P<0.05$; **, $P<0.01$; compared to oxaliplatin group: \$, $P<0.05$; \$\$, $P<0.01$

Gabapentin inhibited the enhancement of glutamatergic strength in the dorsal horn

Here, as shown in Figure 4, significantly increased input - output response of evoked EPSC was observed in the dorsal horn neurons in the rats receiving oxaliplatin, which indicates central sensitization induced by oxaliplatin. Notably, administration of gabapentin significantly reversed the upregulation of glutamatergic strength in the dorsal horn neurons in these rats. These results indicated that gabapentin mitigated the central sensitization induced by oxaliplatin.

Discussion

A number of evidences establish that activity-dependent synthesis and secretion of BDNF from central neurons and astrocytes pivotally participates in the neuronal development and survival, synapse formation and maturation, and synaptic plasticity in the brain (15, 16). Upregulation of BDNF-mediated signaling also essentially contributes to the induction and maintenance of peripheral and central sensitization in the rodent models of neuropathic pain (17). Significantly increased expression of BDNF was detected in the dorsal horn in rats with neuropathic pain induced by spared nerve injury (18) or chronic constriction injury of sciatic nerve (19). Previous studies revealed that BDNF-mediated

signaling, involving PLC γ -dependent induction of CREB and CaMKIV phosphorylation, modulated the presynaptic transmitter release and postsynaptic glutamate receptor subunits trafficking, thus facilitating the induction and maintenance of long-term potentiation in the central neurons (20). It was found that inhibition of BDNF signaling significantly attenuated the enhanced glutamatergic strength in the dorsal horn neurons in rat with spared nerve injury (21). In the present study, a significant upregulation of BDNF was also observed in the dorsal horn in rats with oxaliplatin-induced neuropathic pain which indicates the potential role of BDNF in the induction of central sensitization in the rodent model of neuropathic pain induced by chemotherapy.

In the dorsal horn neurons, increased glutamatergic transmission was reported which critically contributes to the maintenance of painful behavior in the varieties of rodent models of neuropathic pain (22). Altered expression of glutamate receptor subunits, such as GluR1 and NR2B, was ever detected in the dorsal horn and contributed to the induction of central sensitization in varieties of rodent model of chronic pain. Significantly increased GluR2-lacking AMPA receptor which exhibits inward rectification and higher calcium permeability was observed in the dorsal horn neurons in rats with spinal nerve injury (22). It was reported that overproduction of cytokine IL-1 β mediated the enhanced glutamate release from the primary afferent terminals and non-NMDA glutamate receptor activities in postsynaptic neurons in the spinal dorsal horn in rats with spinal nerve injury (23). Substantial adaptation of glutamatergic transmission was consistently observed in the dorsal horn neurons in rats with diabetic neuropathic pain or bone cancer pain (24). In the present study, significantly increased expression of glutamate receptor subunits GluR1 and NR2B along with the enhanced glutamatergic strength was observed in the dorsal horn neurons in rats with oxaliplatin-induced neuropathic pain. This enhanced glutamatergic transmission in the dorsal horn neurons potentially contributed to maintenance of painful behavior induced by oxaliplatin.

Increasing evidences demonstrated the potential therapeutic effect of gabapentin to ameliorate the painful behavior in the rodent model and clinical patients with different types of neuropathic pain. It was reported that gabapentin may dose-dependently attenuated the increase of glutamatergic transmission and the synthesis of pro-inflammatory cytokines in superficial dorsal horn in the rodents with spinal nerve ligation (25, 26). The systemic or intrathecal administration of gabapentin has been shown to increase the paw withdrawal latency and produce anti-allodynia effects in a dose-dependent manner in the rodent models of neuropathic pain induced by partial

sciatic nerve ligation (27) or chronic constriction injury (28). Gabapentin also significantly reduced the hypersensitivity to the thermal stimuli in the rodents with peripheral nerve injury (29, 30). A systematic review reported that gabapentin had significant pain relief in the patients with post spinal cord injury neuropathic pain (31).

Emerging studies also suggested that administration of gabapentin exhibited beneficial effect in the rodent model of neuropathic pain induced by chemotherapeutic agents (6). Acute treatment with gabapentin significantly reduced oxaliplatin-induced hypersensitivity to mechanical stimuli in hindpaw in rodents (10). Repeated administration of gabapentin significantly reduced paclitaxel and vincristine-evoked mechano-allodynia and mechano-hyperalgesia in the rodents (32). A fixed low-dose of gabapentin led to significant relief of pain behaviors induced by chemotherapy in cancer patients (33). Currently the mechanisms underlying analgesic effect of gabapentin is not fully ascertained. Evidences implied that gabapentin may bind to $\alpha 2/\delta$ -1 subunit of the calcium channel, and subsequently inhibit calcium current (34) and calcium channel subunit trafficking (35). Gabapentin may also suppress the abnormal spontaneous activity and hyper-excitability of sensory neurons by blocking the sodium current (36) and opening potassium channels (37). In the present study, systemic administration of gabapentin significantly attenuated the increase of BDNF and the enhancement of glutamatergic transmission in dorsal horn and ameliorated the hypersensitivity to the mechanical stimuli in rats receiving oxaliplatin. These results suggested that administration of gabapentin mitigated the central sensitization in the rodents with oxaliplatin-induced neuropathic pain.

Conclusion

Taken together, the present study demonstrated an increased expression of BDNF and enhanced glutamatergic transmission in rats with oxaliplatin-induced neuropathic pain, which was significantly attenuated by the administration of gabapentin.

Conflict of interest

The authors declare no conflicts of interest.

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